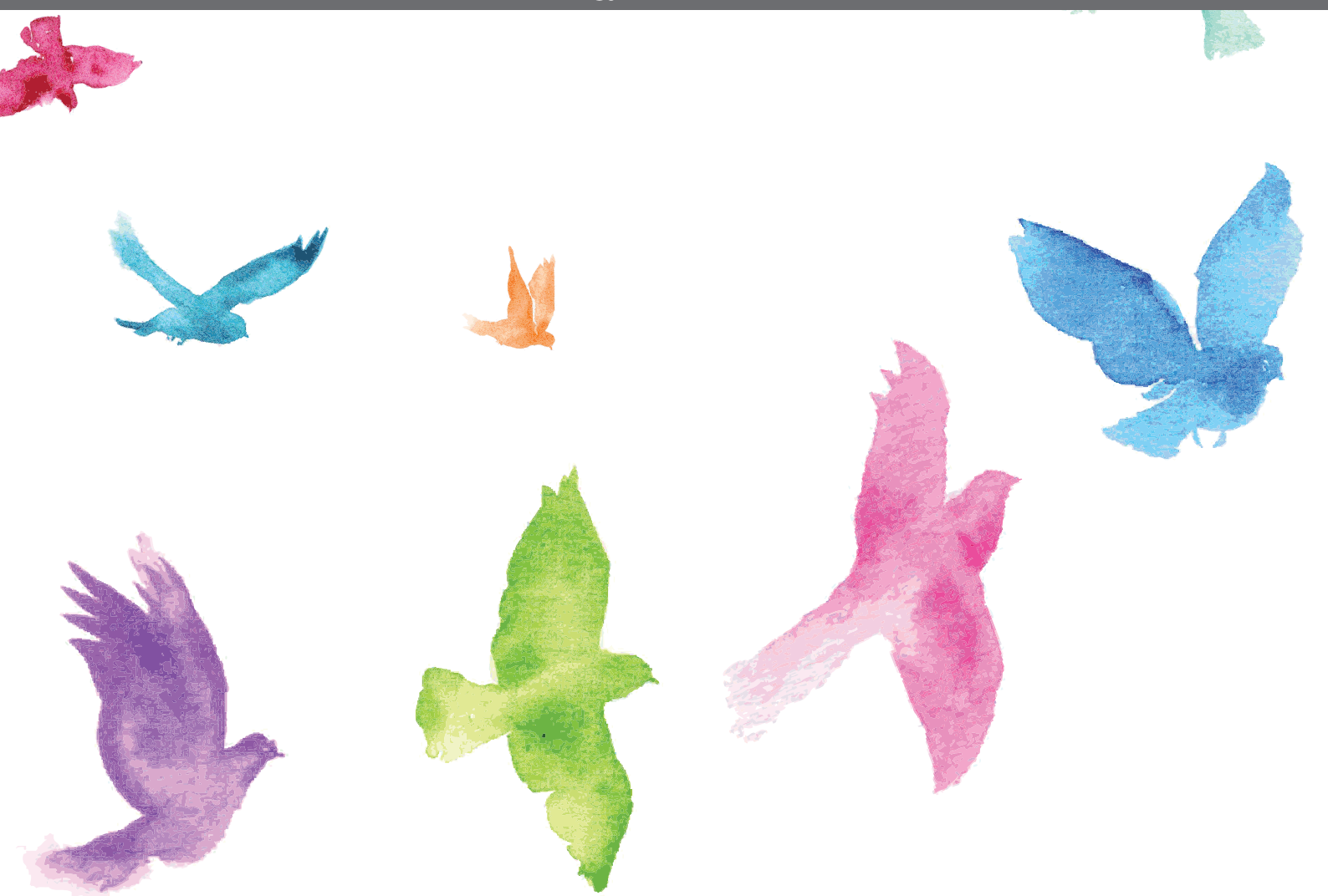
HORMONES AND LIFE HISTORY STRATEGIES

EDITED BY: Sarah Kelly McMenamin, Fedor N. Shkil, Takashi Koyama and Vincent Laudet

PUBLISHED IN: Frontiers in Ecology and Evolution



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88974-622-4
DOI 10.3389/978-2-88974-622-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

HORMONES AND LIFE HISTORY STRATEGIES

Topic Editors:
Sarah Kelly McMenamin, Boston College, United States
Fedor N. Shkil, Severtsov Institute of Ecology and Evolution (RAS), Russia
Takashi Koyama, University of Copenhagen, Denmark
Vincent Laudet, Okinawa Institute of Science and Technology Graduate University, Japan

Citation: McMenamin, S. K., Shkil, F. N., Koyama, T., Laudet, V., eds. (2022). Hormones and Life History Strategies. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88974-622-4

Table of Contents

frontiers in Ecology and Evolution

EDITORIAL
published: 11 February 2022
doi: 10.3389/fevo.2022.838555

Editorial: Hormones and Life History Strategies

Sarah McMenamin[1]*, *Fedor Shkil*[2] and *Takashi Koyama*[3]

[1] Biology Department, Boston College, Chestnut Hill, MA, United States, [2] AN Severtsov Institute of Ecology and Evolution, RAS, Moscow, Russia, [3] Section for Cell and Neurobiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark

Keywords: hormone, reproduction, metamorphosis, endocrine factors, thyroid hormone, serotonin, life history

Editorial on the Research Topic

Hormones and Life History Strategies

INTRODUCTION

Considering the hormonal contributions to life history strategies opens an exciting area of integrative research within evolutionary ecology. Through the course of its life, an organism must appropriately allocate resources between survival, growth, maturation and reproduction: these patterns are captured in the animal's *life history*. Life history encompasses timing of reproduction and number of offspring, and these characteristics ultimately determine a population's size and structure, shaping the ecology, and evolution of a species. *Hormones* are systemic signaling molecules that coordinate cellular activities between disparate organ systems. Endocrine factors mediate life history tradeoffs and transitions across diverse metazoan species. Indeed, hormones regulate the timing of hatching or birth, determine the tempo of development, trigger the onset of ecological transformations such as metamorphosis, and regulate reproductive cycles (see **Figure 1**). By coupling hormonal axes to the environment—including stimuli from social, habitat or resource conditions—many species have evolved plastic life history transitions that can adaptively adjust to match external conditions. Thus, research which integrates both endocrinology and life history holds considerable promise at the frontier of evolution and ecology.

In this special issue, researchers explore hormonal contributions to life history transitions and traits at different developmental stages (**Figure 1**; each citation is placed outside the life cycle at a stage relevant to the work). Reviews and original research from this issue focus on diverse animals across the metazoan phylogeny, from mollusks to mammals. Researchers have integrated perspectives and techniques from multiple biological disciplines which will be of interest to ecologists, evolutionary and organismal biologists, and comparative endocrinologists. In this editorial, we highlight some of the key interpretations and findings of these manuscripts, identify some of the common themes that emerge, and emphasize the promise of future research in this area.

HORMONAL REGULATION OF DIVERSE LIFE HISTORIES

Despite having vastly different life cycles, invertebrates as diverse as sea urchins, fruit flies and freshwater mollusks have each evolved hormonal triggers to regulate life history transformations; many of these stimulating endocrine triggers couple external environmental conditions with genomic and developmental responses. Voronezhskaya reviews the roles of a neuroendocrine factor—specifically, maternally-provided serotonin—in shaping the developmental and phenotypic plasticity of freshwater gastropod mollusks under seasonal environmental changes. Environmental cues, including temperature and daylight length, influence life history in insects through altering

Edited and reviewed by:
Mark A. Elgar,
The University of Melbourne, Australia

***Correspondence:**
Sarah McMenamin
mcmenams@bc.edu

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *17 December 2021*
Accepted: *19 January 2022*
Published: *11 February 2022*

Citation:
McMenamin S, Shkil F and Koyama T (2022) Editorial: Hormones and Life History Strategies. Front. Ecol. Evol. 10:838555. doi: 10.3389/fevo.2022.838555

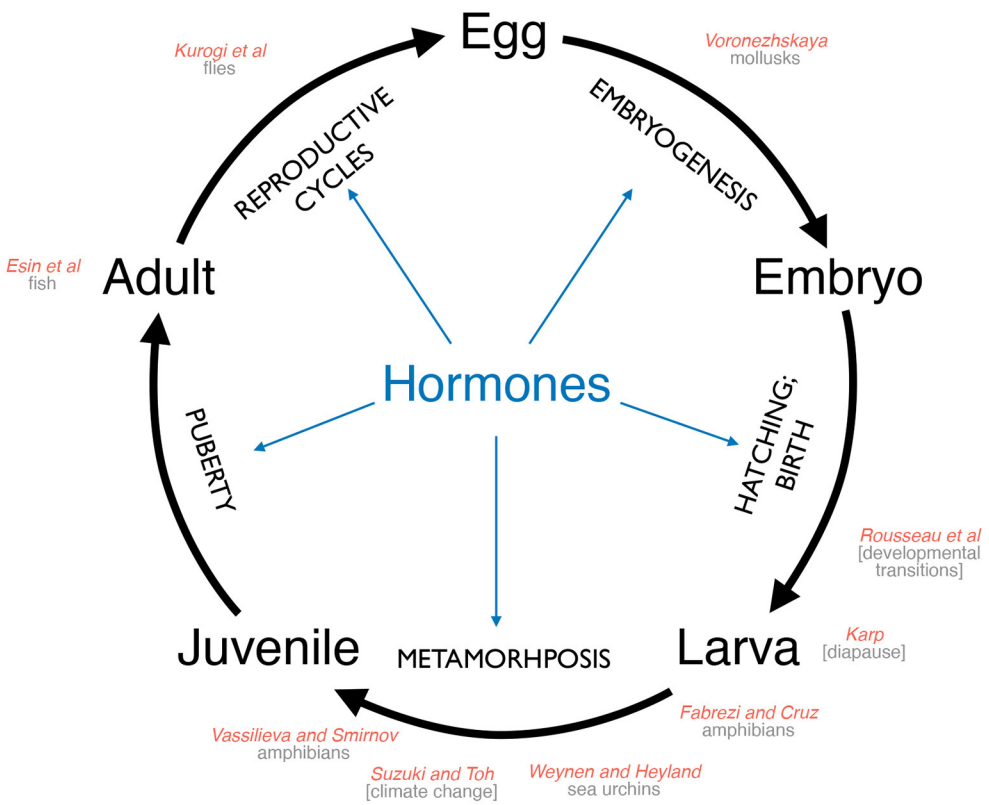


FIGURE 1 | A generalized metazoan life cycle. Transitions between each stage of life history require processes that involve hormones (shown in blue). The nine manuscripts in this special issue tackle different components of the metazoan life cycle in highly divergent organisms; each manuscript is cited (in red) near one of the life cycle stages or transitions on which the authors focus. The primary organism or broad subject of each manuscript is listed below in gray.

biosynthesis of juvenile hormone. Kurogi et al. review the roles of juvenile hormone as a mediator between environmental conditions and seasonal reproductive dormancy in *D. melanogaster*. Wynen and Heyland advance the idea that endocrine mechanisms regulate programmed cell death during the metamorphic transition of sea urchins, detailing the molecular basis of these processes and the relationships with environmental cues.

Since hormonal axes and the life history characteristics they control can be highly responsive to environmental conditions, changing climate should systematically (and catastrophically) alter life history patterns. Implications of global climate change on metamorphic organisms—both vertebrates and invertebrates—are explored in depth in this issue by Suzuki and Toh. These authors develop the concept of a "developmental goblet," which narrows not only at the phylotypic stage during embryogenesis, but again in post-embryonic development during the metamorphosis. The developmental and endocrine constraints of metamorphosis are predicted to be highly sensitive to changing ecological conditions, presenting both constraints, and opportunities for organismal adaptation (Suzuki and Toh).

Dormancy represents a discrete and dramatic type of plastic life history adaptation: under unfavorable environmental conditions, some organisms can enter a state of diapause, during which developmental, physiological, and reproductive functions are arrested. Karp surveys the endocrine mediators stimulating diapause across metazoan species, focusing specifically on nematodes, and certain specialized species of insects and fishes.

Original research in this issue tackles the question of how the endocrine factors influence life cycles and development in vertebrates. Many vertebrates—specifically amphibians and teleost fishes—undergo discrete metamorphic transitions stimulated by thyroid and corticosteroid hormonal axes. Moreover, egg hatching (in reptiles and birds) and birth (in mammals) represent profound developmental transitions that are similarly regulated by endocrine factors. Rousseau et al. compare and contrast these different types of vertebrate developmental transitions from a hormonal perspective. Esin et al. present evidence that thyroid hormone axis played a role in the ecological niche specialization and emergence of adaptive metabolic traits in three species of extremophile arctic salmonids. Fabrezi and Cruz experimentally altered thyroid production in an Argentinian frog, demonstrating that thyroid hormone modulates developmental rate and timing of metamorphosis in the species. Finally, Vassilieva and Smirnov ask whether amphibians show evolutionary trends in the hormonal controls of skeletal metamorphosis, highlighting the importance of endocrine factors in adaptation.

OUTLOOK

Although life history strategies are phenomenally diverse across metazoans, life cycles can be generalized into certain common stages, and transitions between these stages are all regulated by different hormonal axes (see **Figure 1**). Indeed, hormones influence nearly every aspect of organismal physiology across the life cycle. The collection of reviews and original research presented in this special issue emphasize the fact that numerous endocrine cascades regulate a variety of life history transitions (birth, metamorphosis, diapause) in myriad animal species. Many important questions remain: Are hormone-mediated life history transitions evolutionarily homologous between diverse species? What are the molecular mechanisms underlying ecologically-cued reproductive cycles? What are the hormonal contributions to subtle developmental transformations? How are hormonal cues interpreted into genomic and cellular activities? Such questions will provide rich areas for future investigation. Like the work presented in this special issue, the strongest future efforts will utilize and integrate the strengths of traditional model organisms (*Xenopus, Drosophila*) with the diversity and broad relevance of diverse non-model species. Emerging technologies continue to accelerate integrative and holistic research, and we anticipate that these types of interdisciplinary approaches will be essential in advancing a full understanding of developmental and ecological transitions, and the responsiveness of these transitions to a changing climate and ecosystem.

AUTHOR CONTRIBUTIONS

SM wrote and edited the manuscript. FS and TK edited the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank Dr. Vincent Laudet, the members of each of our labs and all the researchers who submitted their work to this special issue topic. We are also grateful for the helpful comments of our Review Editor.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

MINI REVIEW
published: 08 September 2021
doi: 10.3389/fevo.2021.739787

Maternal Serotonin: Shaping Developmental Patterns and Behavioral Strategy on Progeny in Molluscs

*Elena E. Voronezhskaya**

Laboratory of Comparative and Developmental Physiology Koltzov Institute of Developmental Biology Russian Academy of Sciences, Moscow, Russia

Serotonin is a well-known neurotransmitter and neurohormone regulating mood, sleep, feeding, and learning in high organisms. Serotonin also affects the embryonic events related to neurogenesis and maturation of hormonal systems, the underlying organism adaptation to a changing environment. Such serotonin-based mother-to-embryo signaling is realized via direct interactions in case of internal fertilization and embryonic development inside the mother body. However, the possibility of such signaling is less obvious in organisms with the ancestral type of embryogenesis and embryo development within the egg, outside the mother body. Our data, based on the investigation of freshwater gastropod molluscs (*Lymnaea* and *Helisoma*), demonstrated a correlation between seasonal variations of serotonin content within the female reproductive system, and developmental patterns and the behavioral characteristics of progeny. The direct action of serotonin via posttranslational protein modification—serotonylation—during early development, as well as classical receptor-mediated effects, underlies such serotonin-modulated developmental changes. In the present paper, I will shortly overview our results on freshwater molluscs and parallel the experimental data with the living strategy of these species occupying almost all Holarctic regions.

Keywords: adult-to-embryo chemical signaling, serotonylation, serotonin receptors, developmental dynamics, locomotion, oviposition activity

OPEN ACCESS

Edited by:
Takashi Koyama,
University of Copenhagen, Denmark

Reviewed by:
Jose Maria Martin-Duran,
Queen Mary University of London, United Kingdom
Patricia Johnston Moore,
University of Georgia, United States

***Correspondence:**
Elena E. Voronezhskaya
elena.voronezhskaya@idbras.ru

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *12 July 2021*
Accepted: *10 August 2021*
Published: *08 September 2021*

Citation:
Voronezhskaya EE (2021) Maternal Serotonin: Shaping Developmental Patterns and Behavioral Strategy on Progeny in Molluscs. Front. Ecol. Evol. 9:739787. doi: 10.3389/fevo.2021.739787

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine that can be found in most living organisms. It is a well-known neurotransmitter and neuromodulator in the nervous system of vertebrates and invertebrates, impacting such important aspects of life as learning and memory, aggression, sleep, arousal reaction, food uptake, and many others (Müller and Cunningham, 2020). It is also found in the peripheral tissues and organs where it serves as a neurohormone regulating blood pressure and platelets-dependent clotting, glucose metabolism and weight regulation in mammals (Muma and Mi, 2015; Pilowsky, 2018). The serotoninergic system includes 5-HT synthesis and degradation enzymes, receptors and coupled G-proteins, membrane, and vesicular transporters. Despite substantial variations existing across vertebrates and invertebrates, as well as

among different taxa and species (Schmidt-Rhaesa et al., 2016; Müller and Cunningham, 2020), some common organizational principles can be noted for the serotoninergic system. First, the 5-HT-containing neurons constitute a fairly small portion of neuronal elements. However, these elements provide widespread terminals contacting numerous cellular targets. Second, the serotonin receptors and transporters are present in the cell membrane of almost all tissues and organs and react to the surrounding serotonin as to hormone even far from the 5-HT released sites. Third, all components of the serotonergic systems appear to be highly plastic with both the anatomical organizations and biochemical pieces of machinery that can change at different periods of life, or under various environmental conditions. Such features of the serotonergic system allow us to speculate that 5-HT acts more as a basic modulator or integrating molecule (Sakharov, 1990; Moroz et al., 2021) at the level of the whole organism than just a local mediator transmitting a particular signal between certain cells and their targets. Moreover, serotonin has been found since the very early stage of animal development, in oocytes, zygotes, and cleaved blastomeres (Buznikov et al., 1964, 2001; Dubé and Amireault, 2007). Later in development serotonin affects the embryonic events related to neurogenesis and maturation of hormonal systems (Buznikov, 1991; Bonnin and Levitt, 2011; Bonnin et al., 2011; Vitalis et al., 2013). Serotonin appears evolutionarily before the formation of the first neurons and is a well-recognized component of ancient and archetypical signaling systems (Turlejski, 1996; Azmitia, 2010). Thus serotonin has a high potential to serve as a link between external signals, the physiological state of the maternal organism, and forming developmental and behavioral characteristics of progeny, which determine the life strategy of the generation.

To test the function of serotonin as such a broad regulating molecule we used freshwater snails: *Lymnaea stagnalis* and *Helisoma trivolvis* (Mollusca; Gastropoda), as experimental objects. These species are a popular model for neurobiology, physiology, and developmental biology. The morphology of the adult's and embryos nervous system, developmental patterns, reproduction, and behavior in normal conditions are documented in detail (Morrill, 1982; Meshcheryakov, 1990; Kemenes and Benjamin, 2009; Koene, 2010). The species are also subjects for numerous ecological and ecotoxicological studies (Morrison and Belden, 2016; Amorim et al., 2019; Fodor et al., 2020; Svigruha et al., 2020).

In this mini-review, I will compact our 20 years of research devoted to the delayed effects of serotonin shaping both the developmental patterns and behavior of progeny in gastropod molluscs. We demonstrated: (i) a correlation between season and the serotonin level in the local serotonergic network in the female reproductive system, (ii) increased intracellular serotonin during early cleavage, impacting embryonic development and juvenile behavior, (iii) the modulating role of serotonin produced by early embryonic neurons on developmental tempo and hatching. Two mechanisms underlie described long-lasting serotonin effects: (1) classical receptor-mediated regulations, and (2) the non-canonical intracellular action of serotonin as a chemical substance for transglutaminase-mediated posttranslational proteins modification (serotonylation). The combination of these mechanisms or the prevalence of one over the other varies stage-dependently at the course of embryogenesis. As a result, developing embryos demonstrate tune adaptations to the variable environmental challenges they will be faced with during their adult life, despite having no direct contact with the changing environment before birth or hatching. Such non-genetic transfer of a maternal serotonin-mediated signal provides the appropriate adaptive choice of the progeny life strategy, ensures population reproductive success and wide distribution of the species (**Figure 1**).

LOCAL SEROTONERGIC NETWORK IN THE FEMALE MOLLUSC REPRODUCTIVE SYSTEM AS THE LOCATION OF CLOSEST CONTACT BETWEEN MATERNAL TISSUES AND THE EARLY EMBRYO

One of the biggest questions is how environmental information or parents' behavioral experiences can be encoded and transmitted from the nervous system of adults to the gonads, and subsequently to progeny? Such maternal effects on offspring phenotype, particularly in how maternal experience can adaptively shape offspring behavior and the developmental state attracted the attention of evolutionary ecologists and evo-devo biologists for a long time. No single source can cover all aspects of the maternal effects. The principal topics, a number of general issues, updated coverage of problem agendas and perspectives mostly covered in comprehensive reviews (Bernardo, 1996; Rossiter, 1996; Mousseau and Fox, 1998; Bonduriansky and Day, 2009; Uller et al., 2009), with examples from mammals (Maestripieri and Mateo, 2009), maternal effects in marine environments (Marshall et al., 2008), some aspects of the evolution of maternal effects from a developmental perspective (Uller, 2012) and ecological and evolutionary implications, in particular, for plants (Sultan, 2015). In our work we concentrated in one possible particular underlying mechanism of adult-to-embryo signaling—the serotonin-mediated maternal effect—in a limited group of aquatic molluscs. We demonstrated that in freshwater gastropods' serotonin is a key player providing a link between generations by affecting the oocyte and fertilized zygote within the mother reproductive system.

In addition to neurons located within the central ganglia and rich peripheral innervation, the local network of 5-HT-containing cells located in the female part of the *Lymnaea* reproductive system. *L. stagnalis* is a hermaphroditic gastropod snail and the reproductive system contains both male and female parts. Oocytes start their way along the oviduct in response to the signals from the neuroendocrine cells. Ripe oocytes are fertilized in the fertilization pouch and supplied with perivitelline fluid secreted by the albumen gland. Then the zygote starts to move through the folded muscular uterus (pars contorta) and there it is enveloped in two membranes and forms the egg. The muciparous gland secretes mucus that fuses the eggs together, the oothecal gland surrounds the whole

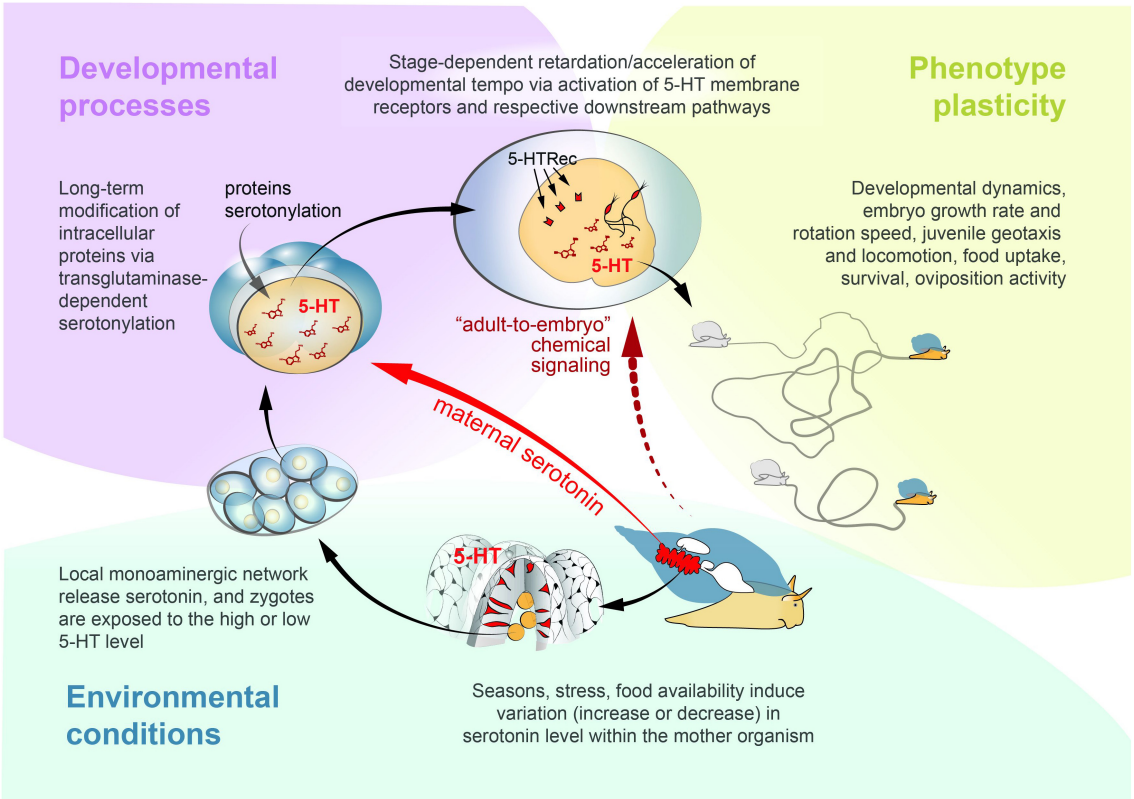


FIGURE 1 | Schematic representation of the tune adaptations in progeny live strategies based on serotonin-mediated regulatory mechanisms during the freshwater gastropod mollusc life cycle. The solid red arrow represents the direct effect of mother serotonin to the developing oocytes and zygotes. The dashed red arrow represents chemical water-born signaling from adults, which activates the serotonin release from specific embryonic sensory neurons. 5-HTRec—specific serotonin receptors in embryonic tissues. The female reproductive system represents a location of the closest contact between the mothers' serotonin releasing network with oocyte and zygote (maternal serotonin arrow). The seasonal and environmental factors lead to modulation of the serotonin level in the mother organism and subsequent changes within the developing embryo. High serotonin within cleaved blastomeres provides a substrate for the serotonylation of specific proteins, and appearance of the progeny with faster development and locomotion, raised survival and productivity. Embryonic apical neurons react to water-born chemical signals emitted by adults in unfavorable conditions ("adult-to-embryo" chemical signaling dashed arrow). Serotonin released from the apical neurons activate specific serotonin receptors located in embryonic cells and stage-dependently retard or accelerate the developmental tempo and juvenile behavior. Combinations of serotonin-dependent mechanisms in the course of development underlay the appropriate adaptive choice of the progeny life strategy, ensures the population reproductive success and the wide distribution of the species.

egg mass with tunica capsulis, and the complete egg mass leave the mother organism to the environment via female gonopore (Koene, 2010). Most aforementioned parts of the reproductive system are innervated by 5-HT-immunopositive fibers. However, only the uterus possesses the intensive local network of 5-HT-containing elements.

The dense network of 5-HT-immunoreactive cells and their processes in the epithelium, and in the muscular layer of the uterus, represent the key location where maternal serotonin contacts with zygotes (Ivashkin et al., 2017). Numerous multipolar 5-HT-containing cells are located between the epithelial cells in the folded part of the uterus (convoluted part of the pars contorta). Their thick bulb-shaped apical processes contact the inner lumen of the duct and the basal varicose fibers organize a basket-shape network on the surface of the folded epithelium. The morphology of multipolar 5-HT-containing cells suggested their exocrine function, and active release of their transmitter content –serotonin—into the reproductive tract lumen. The extensive folding of the uterus indicates that the fertilized egg spends a long time moving along this part of the reproductive system. And during all that time the zygote is exposed to serotonin which is released by the serotonergic cells of the mother's organism.

Direct measurements of 5-HT content in the *Lymnaea* confirmed the high level of 5-HT in the female part of the reproductive system, especially within the pars contorta region. The important fact is that serotonin level within this particular region of the reproductive system is season-dependent: 5-HT content within the uterus gradually increased from winter through spring to summer, then falls dramatically in the autumn (Ivashkin et al., 2015). Pharmacological experiments with the application of 5-HT immediate biochemical precursor—5-HTP—result in an enhanced serotonin level similar to in summertime while the application of chlorpromazine leads to serotonin depletion similar to the autumn condition. These pharmacological approaches allowed us to experimentally mimic

the natural seasons in the laboratory and follow the induced changes in progeny development and behavior under various conditions (see detailed description below).

SEASON-DEPENDENT TUNING OF PROGENY DEVELOPMENT AND BEHAVIOR AND THE UNDERLYING SEROTONIN-MEDIATED MECHANISM

As we demonstrated, the local serotonergic network within the female reproductive system represents the location of closest contact between maternal tissues and the zygote, and season-dependent serotonin production by a maternal organism, has likely mediated the transmission of 5-HT-based signals to progeny.

Indeed, summer and autumn embryos and juveniles varied in specific sets of characters. A *Lymnaea* embryo develops within the egg capsule and there it passes cleavage, gastrulation, premetamorphic larvae stages (trochophore, veliger, and hippo), and undergoes metamorphosis and hatches as a miniature adult-like snail (Meshcheryakov, 1990). The summer generation demonstrates the accelerated speed of embryo rotation within the egg capsule, developmental dynamics with faster premetamorphic and metamorphic phases, and hatch 1–2 days earlier than the representatives of the autumn generation. Juvenile *Lymnaea* snails leave the egg cocoon using intense terrestrial locomotion, and after hatching utilize both gliding and terrestrial locomotion to inspect their novel environment. Summer juveniles moved about two times faster than autumn individuals, prefer vertical surfaces, and often exhibit negative geotaxis, thus leaving the water and creeping onto the bank margin. They can survive drying and stay alive longer with low oxygen. On the contrary, autumn juveniles spend more time on horizontal surfaces underwater, and approach the water surface for respiration episodes only. Contrary to summer individuals they spend more time feeding and grow faster. Nevertheless, summer and autumn generations become reproductively active simultaneously. Moreover, summer individuals produce more eggs than autumn ones.

Such a combination of features makes the summer-born generation exceptionally efficient at dispersion. They preferentially search for new habitats and demonstrate the "migrants" complex of behavior features described above. In contrast, the snails that hatched during the autumn tend to remain in their local environment and demonstrate "resident" behavioral characters.

Previously, it has been shown that juveniles of gastropod snails may disperse under natural conditions to distant water habitats by riding on the feathers and inside the gut of waterfowl and similar birds (Kawakami et al., 2008; Boag, 1986; van Leeuwen et al., 2012). In such a case, the "migrant" strategy with a negative geotaxis, a tendency to crawl to the water surface, combined with the fast locomotion of summer-born juveniles, increases their chances to cling to the bird's feathers or be swallowed. Usually, birds do not migrate large distances during summer, so juvenile snails have a chance to be successfully transferred to new water habitats. On the contrary, spring and autumn are the times when birds migrate long distances to their final location. And in this case, the "resident" strategy of juvenile snails with more time deep in the water ensures their better survival in their original habitat.

Interestingly, the pharmacological treatment of the mother can switch the natural season-dependent phenotype of progeny. The application of 5-HTP to an autumn mother-snail (with low natural serotonin) enhanced serotonin levels within the local network and resulted in the "summer" phenotype appearance in the originally autumn generation. And vice versa, the depletion of serotonin in the mother organism during summer (the season with originally high serotonin) leads to loss of active summer phenotype and the appearance of progeny with autumn characteristics (Ivashkin et al., 2015).

It should be noted that the modulations of serotonin levels had an effect on the development and behavior of progeny only if that occurred early at embryogenesis: at zygote or during cleavage stages (Voronezhskaya et al., 2012). This fact indicates that possible mechanism(s) underlying the phenomenon of described long-term serotonin-mediated changes utilizes the non-canonical way of serotonin action.

Recently a novel mechanism explaining such prolonged serotonin actions has been found. It has been shown that in addition to the classical pathway via binding to membrane receptors, serotonin can modify the intracellular proteins. This process of covalent serotonin binding to glutamine residues of the target protein is mediated by transglutaminase (TGase) and has been termed "serotonylation" (Walther et al., 2003). Not only can serotonin be a substrate for TGase-mediated transamidation but also other monoamines (Hummerich et al., 2012). This novel posttranslational proteins modification has an impact on such important physiological processes as platelet activation, insulin release, smooth muscle contraction, and even the regulation of transcription (Muma and Mi, 2015; Bader, 2019; Farrelly et al., 2019).

In our experiments, we demonstrated that *L. stagnalis* zygote and cleaved blastomeres have all the necessary biochemical machinery to perform serotonylation. They can transport serotonin inside the cells using the membrane transporter SERT or synthesize it from the precursors (Voronezhskaya et al., 2012). The basic level of serotonylation occurs naturally for a specific set of proteins in cleaved blastomeres, and it is enhanced and modified in response to an increased serotonin level (Ivashkin et al., 2015). Notably, among these modified molecules some nuclear proteins serve as a substrate for transglutaminase-mediated serotonylation as well (Ivashkin et al., 2019). The specific pattern of serotonylated proteins can be modified by enhanced serotonin during the zygote and early cleavage stages, but not at veliger and post-metamorphic embryos. Accordingly, TGase inhibition prevents the formation of serotonylated proteins at the early developmental stages as well as negating the delayed effects of enhanced serotonin level on progeny development and behavior. That confirms the involvement of the serotonylation mechanism in the long-term effect of serotonin in the formation of behavioral characters in molluscan progeny.

Summarizing the role serotonin played during early *Lymnaea* development, we can conclude that oocyte, zygote, and cleaved blastomeres are the targets for serotonin released by the mother-snail local serotonergic networks. Serotonin deposited within the embryonic cells can modify specific sets of intracellular and nuclear proteins in differentiating blastomeres which give rise to numerous tissues and organs (Ivashkin et al., 2019). Finally, the modulation of early serotonin level leads to modification in developmental dynamics and behavior of progeny (Ivashkin et al., 2015).

The presence of 5-HT elements in gonads and the reproductive tract has been found in representatives of various molluscan species: in the bivalve *Patinopecten* (Matsutani and Nomura, 1986), in nudibranchs *Pleurobranchaea* and *Tritonia* (Moroz et al., 1997), in opisthobranch *Asperspina* (Delgado et al., 2012). In many bivalves, the application of 5-HT stimulates oocytes maturation, induces spawning, or stimulates parturition (Fong et al., 1994; Fong, 1998). Serotonin level in parent organisms is highly variable and reflects the animal particular physiological states and certain environmental conditions. The serotonin level is different in starved and satiated animals (Hernádi et al., 2004), changes after intense locomotion (Aonuma et al., 2020) or under anxiety and stress conditions (Fossat et al., 2014). Our experiments with molluscs clearly demonstrate that all these physiological states and changes may influence the future generation in case they are happening in a specific time window (early cleavage) during embryonic development.

The effect of maternally-derived serotonin on germ cells has been shown also for nematode *Caenorhabditis elegans.* Serotonin released by maternal neurons during stress acts through conserved signal transduction pathways and enables the transcription factor HSF1 to alter chromatin in soon-to-be fertilized germ cells. This mechanism ensures the viability and stress resilience of future offspring (Das et al., 2020).

We do not know yet all the players and certain pathways which link the modification of proteins within *L. stagnalis* blastomeres, and the formation of neuronal networks underlying juvenile molluscs' behavior. However, we clearly see the phenomenon of maternal serotonin-mediated phenotypic adjustments providing more efficient survival skills and effective dispersion of progeny (**Figure 1**).

PARENTAL CHEMICAL SIGNAL AND SEROTONIN-MEDIATED CHANGES IN DEVELOPMENTAL TEMPO AND JUVENILE BEHAVIORAL CHARACTERISTICS

Like many representatives of aquatic biosystems, freshwater gastropod molluscs have a biphasic life cycle with embryo and adult forms occupying greatly different ecological niches. Adult *Lymnaea* and *Helisoma* release egg cocoons to the external environment and then leave their progeny to develop. The embryonic snail passes larval stages and metamorphosis inside the egg capsule and then hatches as a young juvenile snail. So, in molluscan development, there are no further contacts with the mother organism and the embryo after the egg cocoon was formed. Maternal effects are known to be of particular importance for species in aquatic systems. They not only form a link between the phenotypes of different generations, but the biphasic life cycle of most marine organisms suggests that maternal effects also link the phenotypes of populations (Marshall et al., 2008). In our experiments, we revealed adult-to-embryo chemical signaling, which regulates larval development in freshwater gastropods. We also proved that serotonin is a molecule mediating the adult-derived chemical signal with embryonic developmental tempo and juvenile's behavioral characteristics (Voronezhskaya et al., 2004, 2007).

When the adult or young juvenile *Lymnae*a and *Helisoma* face unfavorable environmental conditions like starvation or crowding they start to release water-borne chemical cues (with a still unidentified chemical structure). In response to those signals, early embryos retard or even stop their development (Voronezhskaya et al., 2004), while metamorphic embryos accelerate developmental tempo, and hatchlings as well as young juveniles demonstrate more active locomotion, feeding and cardiac activity (Voronezhskaya et al., 2007; Glebov et al., 2014). Such an adaptive strategy allows the early embryos to leave its nutrients and wait out inside the egg until the external situation will be improved. On the contrary, the late embryo already used up the egg's nutrients supply and a more adaptive strategy will be to hatch as soon as possible and leave an unfavorable environment (**Figure 1**).

The chemical signal (which is certainly not serotonin itself) is emitted by adult snails under conditions of starvation or crowding, and is sensed by specific larval neurons. These neurons are two apical cells that appear early during development at the trochophore stage and contain serotonin in the case *of Helisoma,* and dopamine and serotonin in *Lymnaea*. These early cells are bipolar neurons bearing sensory cilia at their apical dendrite and emitting basal axons with numerous varicosities. The morphology and transmitter content of these cells indicate their homology with the apical sensory organ of other invertebrates (Voronezhskaya et al., 2004; Voronezhskaya and Croll, 2015). Upon sensing the water-born chemical signal from conspecific adults, apical cells activate their transmitter content (serotonin in case of *Helisoma*, and serotonin and dopamine in case of *Lymnaea*) production and release. Using *Helisoma trivolvis* embryo we demonstrated how one and the same transmitter—serotonin—can cause the manifestation of opposing adaptive programmes (retardation or acceleration of developmental tempo) stage-dependently, and prove involvement of various serotonin receptors in this regulation (Voronezhskaya et al., 2008; Glebov et al., 2014).

According to the classical view, the diverse physiological functions of serotonin are mediated via membrane serotonin receptors, coupled G-proteins and respective intracellular pathways. Serotonin receptors constitute the largest class of G protein-coupled receptors (GPCRs) which include 16 big families depending on the activation of $Na^+/K^+/Ca^{2+}$ ion channel, respective G-protein (G_s, $G_{i/o}$, $G_{q/11}$), activation or inhibition of adenylate cyclase (AC), and phospholipase C

(PLC) (Tierney, 2001). We found at least four types of 5-HT receptors in *Helisoma* embryos. Activation of 5-HT_1- and 5-HT_5-like receptors and respective coupled Gi-protein induce acceleration of development, while activation of 5-HT_4- and 5-HT_7-like and respective Gs-protein results in retardation. While all types of serotonin receptors are expressed during both the early and late stages of embryonic development, their proportions vary stage-dependently. The 5-HT receptors and respective G-proteins whose activation induces developmental retardation (5-HT_4-like, 5-HT_7-like, Gs) prevails at the early stages. Vice versa, that 5-HT receptors and G-proteins whose activation induces developmental acceleration (5-HT_1-like, 5-HT_5-like, Gi) preferentially expressed at late stages. Thus the serotonin released from the apical neurons in response to the water-born chemical cue (which is not serotonin but the chemical substance emitted by the adult snail in unfavorable conditions) retards developmental tempo at early stages and accelerated it at later stages, depending upon a certain combination of 5-HT receptors expressed at a particular developmental stage in the embryonic tissues (Glebov et al., 2014).

Our results demonstrated that adult and juvenile snails sufficiently inform their encapsulated larvae about the unfavorable environmental conditions they will be faced with after hatching. The embryo or metamorphic larvae sense the emitted chemical signal by apical neurons. Serotonin released from apical neurons modulates the embryo developmental tempo and juvenile behavior via activation of a certain poll of serotonin receptors located in the embryonic tissues at the current developmental stage. The opposite reactions of the molluscan embryo to the same water-born environmental cues at early and late developmental stages provide an adaptive response at the level of individual organism and better survival of the whole generation.

CONCLUSION AND FUTURE PERSPECTIVES

Gastropod molluscs are very successful species with incredible flexibility of life strategies that inhabit a wide variety of marine, freshwater, and terrestrial habitats distributed between the two poles, and ranging from alpine meadows down to the depths of the oceans. In this mini-review we just slightly disclosed one of the possible mechanisms underlying the prosperity of two freshwater gastropod species. We revealed that serotonin appears to be a key molecule playing both hormonal (during cleavage stages) and neurohormonal (during larval stages) roles during *Lymnaea* and *Helisoma* embryonic development. Classical receptor-mediated regulation and non-canonical protein modification (serotonylation) underlie serotonin effects. Via these pathways serotonin links the environmental signals received by adults and respective changes in progeny developmental tempo, hatching time, and behavioral characteristics of juveniles. The benefits of such maternal serotonin-driven progeny phenotypic adjustments includes more efficient dispersion, feeding, survival skills, and fertility rates. That features are tuned in to the next-generation according to the different environmental factors the parents experienced.

The possible directions of this field of investigation may be devoted to the following topics: (1) the distribution of serotonin-mediated shaping of life strategy in other species; (2) involvement of other monoamines in maternal regulation of progeny characteristics; (3) the detailed mechanism of serotonin-induced changes in development and behavior from the first step in oocyte till the differentiation of neuronal networks underlying behavior (including transcription regulation). Each of the mentioned tasks can include the field study of the behavior and developmental characteristics in different natural populations, as well as laboratory investigations of the underlying molecular mechanisms of the discovered phenomena in model animals.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

The works with long-lasting serotonin effects and serotonylated proteins were supported by the Russian Science Foundation (Grant No. 17-14-01353).

ACKNOWLEDGMENTS

I greatly acknowledged all co-authors and colleagues who contributed to the original works mentioned in the review. I thanks Olga Kharchenko for the art drawing illustrating the main ideas of this manuscript. The original researches were done using the equipment of the Core Centrum of IDB RAS. The work with serotonin receptors was partly conducted within a frame of IDB RAS Research program No. 0008-2021-0020. I thank the native speakers from the Flarus agency for the professional language proofreading.

REFERENCES

Amorim, J., Abreu, I., Rodrigues, P., Peixoto, D., Pinheiro, C., Saraiva, A., et al. (2019). *Lymnaea stagnalis* as a freshwater model invertebrate for ecotoxicological studies. *Sci. Total Environ.* 669, 11–28. doi: 10.1016/J.SCITOTENV.2019.03.035

Aonuma, H., Mezheritskiy, M., Boldyshev, B., Totani, Y., Vorontsov, D., Zakharov, I., et al. (2020). The role of serotonin in the influence of intense locomotion on the behavior under uncertainty in the mollusk *lymnaea stagnalis*. *Front. Physiol.* 11:221. doi: 10.3389/FPHYS.2020.00221

Azmitia, E. C. (2010). *Evolution of Serotonin: Sunlight to Suicide*. Amsterdam: Elsevier. doi: 10.1016/s1569-7339(10)70069-2

Bader, M. (2019). Serotonylation: serotonin signaling and epigenetics. *Front. Mol. Neurosci.* 12:288. doi: 10.3389/fnmol.2019.00288

Bernardo, J. (1996). Maternal effects in animal ecology. *Am. Zool.* 36, 83–105. doi: 10.1093/icb/36.2.83

Boag, D. A. (1986). Dispersal in pond snails: potential role of waterfowl. 64, 904–909. doi: 10.1139/Z86-136

Bonduriansky, R., and Day, T. (2009). Nongenetic inheritance and its evolutionary implications. *Annu. Rev. Ecol. Evol. Syst.* 40, 103–125. doi: 10.1146/annurev.ecolsys.39.110707.173441

Bonnin, A., Goeden, N., Chen, K., Wilson, M. L., King, J., Shih, J. C., et al. (2011). A transient placental source of serotonin for the fetal forebrain. *Nature* 472, 347–350. doi: 10.1038/nature09972

Bonnin, A., and Levitt, P. (2011). Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. *Neuroscience* 197, 1–7. doi: 10.1016/j.neuroscience.2011.10.005

Buznikov, G. A. (1991). The biogenic amines as regulators of early (pre-nervous) embryogenesis: new data. *Adv. Exp. Med. Biol.* 296, 33–48.

Buznikov, G. A., Chudakova, I. V., and Zvezdina, N. D. (1964). The role of neurohumours in early embryogenesis. I. Serotonin content of developing embryos of sea urchin and loach. *Embryol. Exp. Morph.* 12, 563–573.

Buznikov, G. A., Lambert, W. H., and Lauder, J. M. (2001). Serotonin and serotonin-like substances as regulators of early embryogenesis and morphogenesis. *Cell Tissue Res.* 305, 177–186. doi: 10.1007/s004410100408

Das, S., Ooi, F. K., Cruz Corchado, J., Fuller, L. C., Weiner, J. A., and Prahlad, V. (2020). Serotonin signaling by maternal neurons upon stress ensures progeny survival. *ELife* 9:e55246. doi: 10.7554/eLife.55246

Delgado, N., Vallejo, D., and Miller, M. W. (2012). Localization of serotonin in the nervous system of *Biomphalaria glabrata*, an intermediate host for schistosomiasis. *J. Comp. Neurol.* 520, 3236–3255. doi: 10.1002/cne.23095

Dubé, F., and Amireault, P. (2007). Local serotonergic signaling in mammalian follicles, oocytes and early embryos. *Life Sci.* 81, 1627–1637. doi: 10.1016/j.lfs.2007.09.034

Farrelly, L. A., Thompson, R. E., Zhao, S., Lepack, A. E., Lyu, Y., Bhanu, N. V., et al. (2019). Histone serotonylation is a permissive modification that enhances TFIID binding to H3K4me3. *Nature* 567, 535–539. doi: 10.1038/s41586-019-1024-7

Fodor, I., Hussein, A. A., Benjamin, P. R., Koene, J. M., and Pirger, Z. (2020). The unlimited potential of the great pond snail, *Lymnaea stagnalis*. *Elife* 9:e56962. doi: 10.7554/elife.56962

Fong, P. P. (1998). Zebra mussel spawning is induced in low concentrations of putative serotonin reuptake inhibitors. *Biol. Bull.* 194, 143–149. doi: 10.2307/1543044

Fong, P. P., Duncan, J., and Ram, J. L. (1994). Inhibition and sex specific induction of spawning by serotonergic ligands in the zebra mussel *Dreissena polymorpha* (Pallas). *Experientia* 50, 506–509. doi: 10.1007/BF01920759

Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Delbecque, J. P., and Cattaert, D. (2014). Anxiety-like behavior in crayfish is controlled by serotonin. *Science* 344, 1293–1297. doi: 10.1126/science.1248811

Glebov, K., Voronezhskaya, E. E., Khabarova, M. Y., Ivashkin, E., Nezlin, L. P., and Ponimaskin, E. G. (2014). Mechanisms underlying dual effects of serotonin during development of *Helisoma trivolvis* (Mollusca). *BMC Dev. Biol.* 14:1–19. doi: 10.1186/1471-213X-14-14

Hernádi, L., Hiripi, L., Dyakonova, V., Gyori, J., and Vehovszky, A. (2004). The effect of food intake on the central monoaminergic system in the snail, *Lymnaea stagnalis*. *Acta Biol. Hung.* 55, 185–194. doi: 10.1556/ABIOL.55.2004.1-4.23

Hummerich, R., Thumfart, J. O., Findeisen, P., Bartsch, D., and Schloss, P. (2012). Transglutaminase-mediated transamidation of serotonin, dopamine and noradrenaline to fibronectin: evidence for a general mechanism of monoaminylation. *FEBS Lett.* 586, 3421–3428. doi: 10.1016/j.febslet.2012.07.062

Ivashkin, E. G., Khabarova, M. Y., Melnikova, V. I., Kharchenko, O. A., and Voronezhskaya, E. E. (2017). Local serotonin-immunoreactive plexus in the female eproductive system of hermaphroditic gastropod mollusc *Lymnaea stagnalis*. *Invertebr. Zool.* 41, 134–139. doi: 10.15298/invertzool.14.2.06

Ivashkin, E., Khabarova, M. Y., Melnikova, V., Nezlin, L. P., Kharchenko, O., Voronezhskaya, E. E., et al. (2015). Serotonin mediates maternal effects and directs developmental and behavioral changes in the progeny of snails. *Cell Rep.* 12, 1144–1158. doi: 10.1016/j.celrep.2015.07.022

Ivashkin, E., Melnikova, V., Kurtova, A., Brun, N. R., Obukhova, A., Khabarova, M. Y., et al. (2019). Transglutaminase activity determines nuclear localization of serotonin immunoreactivity in the early embryos of invertebrates and vertebrates. *ACS Chem. Neurosci.* 10, 3888–3899. doi: 10.1021/acschemneuro.9b00346

Kawakami, K., Wada, S., and Chiba, S. (2008). Possible dispersal of land snails by birds. *Ornithol. Sci.* 7, 167–171. doi: 10.2326/1347-0558-7.2.167

Kemenes, G., and Benjamin, P. R. (2009). Lymnaea. *Curr. Biol* 19, R9–R11. doi: 10.1016/j.cub.2008.10.013

Koene, J. M. (2010). Neuro-endocrine control of reproduction in hermaphroditic freshwater snails: mechanisms and evolution. *Front. Behav. Neurosci.* 4:167. doi: 10.3389/fnbeh.2010.00167

Maestripieri, D., and Mateo, J. M. (2009). *Maternal Effects In Mammals*. Chicago, IL: Univ. of Chicago Press, doi: 10.7208/chicago/9780226501222.001.0001

Marshall, D. J., Allen, R. M., and Crean, A. J. (2008). "The ecological and evolutionary importance of maternal effects in the sea," in *Oceanography And Marine Biology: An Annual Review*, Vol. 46, eds R. N. Gibson, R. J. A. Atkinson, and J. D. M. Gordon (Enfield, NH: Science Publishers), 203–250.

Matsutani, T., and Nomura, T. (1986). Serotonin-like immunoreactivity in the central nervous system and gonad of the scallop, *Patinopecten yessoensis*. *Cell Tissue Res.* 2443, 515–517. doi: 10.1007/BF00212528

Meshcheryakov, V. N. (1990). The common pond snail, *Lymnaea stagnalis*. *Anim. Species Dev. Stud.* 69–132. doi: 10.1007/978-1-4613-0503-3_5

Moroz, L. L., Romanova, D. Y., and Kohn, A. B. (2021). Neural versus alternative integrative systems: molecular insights into origins of neurotransmitters. *Philos. Trans. R. Soc. B Biol. Sci.* 376, 20190762. doi: 10.1098/rstb.2019.0762

Moroz, L. L., Sudlow, L. C., Jing, J., and Gillette, R. (1997). . Serotonin-immunoreactivity in peripheral tissues of the opisthobranch molluscs *Pleurobranchaea californica* and *Tritonia diomedea*. *J. Comp. Neurol.* 382, 176–188. doi: 10.1002/(SICI)1096-9861(19970602)382:2<176::AID-CNE3<3.0.CO;2-0

Morrill, J. B. (1982). "Development of the pulmonate gastropod, *Lymnaea*," in *Developmental Biology Of The Freshwater Invertebrates*, eds F. W. Harrison and R. R. Cowden (New York, NY: Alan Liss), 399–483.

Morrison, S. A., and Belden, J. B. (2016). Development of *Helisoma trivolvis* pond snails as biological samplers for biomonitoring of current-use pesticides. *Environ. Toxicol. Chem.* 35, 2320–2329. doi: 10.1002/etc.3400

Mousseau, T. A., and Fox, C. W. (1998). *Maternal Effects as Adaptations*. New York, NY: Oxford Univ. Press.

Müller, C. P., and Cunningham, K. A. (2020). *Handbook of the Behavioral Neurobiology Of Serotonin*. Cambridge, MA: Academic Press.

Muma, N. A., and Mi, Z. (2015). Serotonylation and transamidation of other monoamines. *ACS Chem. Neurosci.* 6, 961–969. doi: 10.1021/cn500329r

Pilowsky, P. M. (2018). *Serotonin in Central Cardiovascular Regulation*. Amsterdam: Elsevier Inc, doi: 10.1016/B978-0-12-800050-2.00016-4

Rossiter, M. C. (1996). Incidence and consequences of inherited environmental effects. *Annu. Rev Ecol. Syst.* 27, 451–476. doi: 10.1146/annurev.ecolsys.27.1.451

Sakharov, D. (1990). "Integrative function of serotonin common to distantly related invertebrate animals," in *The Early Brain*, eds M. Gustafsson and M. Reuter (Abo: Abo Akademi Press), 73–88.

Schmidt-Rhaesa, A., Harzsch, S., and Purschke, G. (2016). Structure and Evolution of Invertebrate Nervous Systems. *Struct. Evol. Invertebr. Nerv. Syst*, 1 Edn. Oxford: Oxford University Press, 776. doi: 10.1093/acprof:oso/9780199682201.001.0001

Sultan, S. E. (2015). *Organism and environment. Ecological development, Niche Construction, And Adaptation*. New York, NY: Oxford Univ. Press, doi: 10.1093/acprof:oso/9780199587070.001.0001

Svigruha, R., Fodor, I., Padisak, J., and Pirger, Z. (2020). Progestogen-induced alterations and their ecological relevance in different embryonic and adult behaviours of an invertebrate model species, the great pond snail, *Lymnaea stagnalis*. *Environ. Sci. Pollut. Res. Int.* doi: 10.1007/S11356-020-12094-Z

Tierney, A. J. (2001). Structure and function of invertebrate 5-HT receptors: a review. *Comparat. Biochem. Physiol. Part A Mol. Integrat. Physiol.* 128, 791–804. doi: 10.1016/s1095-6433(00)00320-2

Turlejski, K. (1996). Evolutionary ancient roles of serotonin: long-lasting regulation of activity and development. *Acta Neurobiol. Exp.* 56, 619–636.

Uller, T. (2012). "Parental effects in development and evolution," in *The Evolution of Parental Care*, eds N. J. Royle, P. T. Smiseth, and M. Kölliker (Oxford: Oxford Univ. Press.), 247–266.

Uller, T., Wapstra, E., and Badyaev, A. V. (2009). Evolution of parental effects: conceptual issues and empirical patterns. *Philos. Trans. R. Soc. Lond. B* 365:1520.

van Leeuwen, C. H. A., van, Velde, G., van der, Lith, B., and van Klaassen, M. (2012). Experimental quantification of long distance dispersal potential of aquatic snails in the gut of migratory birds. *PLoS One* 7:e32292. doi: 10.1371/JOURNAL.PONE.0032292

Vitalis, T., Ansorge, M. S., and Dayer, A. G. (2013). Serotonin homeostasis and serotonin receptors as actors of cortical construction: special attention to the 5-HT3A and 5-HT6 receptor subtypes. *Front. Cell. Neurosci.* 7:93. doi: 10.3389/fncel.2013.00093

Voronezhskaya, E. E., and Croll, R. P. (2015). "Mollusca: gastropoda," in *Structure and Evolution Of Invertebrate Nervous Systems*, eds A. Schmidt-Rhaesa, S. Harzsch, and G. Purschke (Oxford: Oxford University Press), 196–221.

Voronezhskaya, E. E., Glebov, K. I., Khabarova, M. Y., Ponimaskin, E. G., and Nezlin, L. P. (2008). Adult-to-embryo chemical signaling in the regulation of larval development in trochophore animals: cellular and molecular mechanisms. *Acta Biol. Hung.* 59, 117–122. doi: 10.1556/ABiol.59.2008.Suppl.19

Voronezhskaya, E. E., Khabarova, M. Y., and Nezlin, L. P. (2004). Apical sensory neurones mediate developmental retardation induced by conspecific environmental stimuli in freshwater pulmonate snails. *Development* 131, 3671–3680. doi: 10.1242/dev.01237

Voronezhskaya, E. E., Khabarova, M. Y., Chaban, A. K., and Nezlin, L. P. (2007). Role of chemical signalling in release of motor programs during embryogenesis of freshwater snails *Lymnaea stagnalis* and *Helisoma trivolvis*. *Russ. J. Dev. Biol.* 38, 66–75. doi: 10.1134/S1062360407020038

Voronezhskaya, E. E., Khabarova, M. Y., Nezlin, L. P., and Ivashkin, E. G. (2012). Delayed action of serotonin in molluscan development. *Acta Biol. Hung.* 63(Suppl. 2), 210–216. doi: 10.1556/ABiol.63.2012.Suppl.2.28

Walther, D. J., Peter, J. U., Winter, S., Holtje, M., Paulmann, N., Grohmann, M., et al. (2003). Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell* 115, 851–862. doi: 10.1016/S0092-8674(03)01014-6

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

REVIEW
published: 08 September 2021
doi: 10.3389/fevo.2021.733787

Hormonal Regulation of Programmed Cell Death in Sea Urchin Metamorphosis

Hannah Wynen and Andreas Heyland**

Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

Programmed cell death (PCD) has been identified as a key process in the metamorphic transition of indirectly developing organisms such as frogs and insects. Many marine invertebrate species with indirect development and biphasic life cycles face the challenge of completing the metamorphic transition of the larval body into a juvenile when they settle into the benthic habitat. Some key characteristics stand out during this transition in comparison to frogs and insects: (1) the transition is often remarkably fast and (2) the larval body is largely abandoned and few structures transition into the juvenile stage. In sea urchins, a group with a drastic and fast metamorphosis, development and destruction of the larval body is regulated by endocrine signals. Here we provide a brief review of the basic regulatory mechanisms of PCD in animals. We then narrow our discussion to metamorphosis with a specific emphasis on sea urchins with indirect life histories and discuss the function of thyroid hormones and histamine in larval development, metamorphosis and settlement of the sea urchin *Strongylocentrotus purpuratus*. We were able to annotate the large majority of PCD related genes in the sea urchin *S. purpuratus* and ongoing studies on sea urchin metamorphosis will shed light on the regulatory architecture underlying this dramatic life history transition. While we find overwhelming evidence for hormonal regulation of PCD in animals, especially in the context of metamorphosis, the mechanisms in many marine invertebrate groups with indirect life histories requires more work. Hence, we propose that studies of PCD in animals requires functional studies in whole organisms rather than isolated cells. We predict that future work, targeting a broader array of organisms will not only help to reveal important new functions of PCD but provide a fundamentally new perspective on its use in a diversity of taxonomic, developmental, and ecological contexts.

Keywords: apoptosis, metamorphosis, PCD, hormones, settlement, life history, evolution

OPEN ACCESS

Edited by:
Sarah Kelly McMenamin,
Boston College, United States

Reviewed by:
Yun-Bo Shi,
National Institutes of Health (NIH), United States
Markus Friedrich,
Wayne State University, United States

****Correspondence:***
Hannah Wynen
hwynen@uoguelph.ca
Andreas Heyland
aheyland@uoguelph.ca

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *30 June 2021*
Accepted: *06 August 2021*
Published: *08 September 2021*

Citation:
Wynen H and Heyland A (2021) Hormonal Regulation of Programmed Cell Death in Sea Urchin Metamorphosis. Front. Ecol. Evol. 9:733787. doi: 10.3389/fevo.2021.733787

REVIEW OF CELL DEATH MECHANISMS

Cell death, whether active (apoptosis) or passive (necrosis), effectively results in the removal of cells from organisms and therefore stands in contrast to cell proliferation which results from cell division. Apoptosis and other forms of programmed cell death (PCD) occur in response to internal (cellular environment) signals and external environment cues, including stressors, and involve a broad array of now well established molecular and cellular responses, ultimately packaging the

cellular material for removal (Kerr et al., 1972; Green and Fitzgerald, 2016). PCD plays an extremely important role in development and the molecular and genetic processes are tightly regulated. Still, many diseases have been shown to emerge due to dysregulation of cell suicide mechanisms and it is therefore assumed that over evolutionary time multiple complementary and redundant regulatory mechanisms have emerged in organisms. Furthermore, many of these processes and pathways also perform other functions in development and physiology (Amiesen, 2002).

Traditionally three upstream apoptotic pathways and one downstream apoptotic pathway are distinguished in the literature, however their functions are deeply intertwined (**Figure 1**). The *extrinsic pathway* involves death receptors of the tumor necrosis factor (TNF) receptor gene superfamily. Extracellular cell death signals trigger the binding of homologous trimeric ligands such as FasLG to death effector domain (DED)-containing receptors like Fas to activate the extrinsic pathway (**Figure 1**; Fulda and Debatin, 2006; Elmore, 2007). These complexes then bind to cytoplasmic adaptor proteins like FADD. After binding the ligands either FADD or TRADD with the assistance of RIPK1 and FADD associate with procaspase-8 to form the death-inducing signaling complex (DISC). DISC is responsible for the activation of CASP8 which then cleaves CASP3 to start the execution pathway (Elmore, 2007).

The *intrinsic signaling pathway* initiates apoptosis through non-receptor mediated stimuli, acting either directly on pro-apoptotic targets upstream of the pro-apoptotic B-cell lymphoma 2 (Bcl-2) family, or within the cell by inhibiting anti-apoptosis targets (**Figure 1**). This signaling pathway is triggered by environmental stressors such as radiation, toxins, hypoxia, viral infections, and others. The intrinsic pathway is often also referred to as the mitochondrial pathway due to the role that the permeabilization of the mitochondrial outer membrane plays in the activation of CASP9. It responds to intracellular signals produced by non-receptor mediated stimuli. These lead to the activation of Bcl-2 family members such as the Bcl-2 effector proteins BAX and BAK1 (**Figure 1**), which in turn are responsible for mitochondrial outer membrane permeabilization (Degterev and Yuan, 2008; Kalkavan and Green, 2018). Mitochondrial outer membrane permeabilization can be prevented by anti-apoptotic members of the Bcl-2 family like Bcl-2 and BCL2L1 (Elmore, 2007; reviewed in Daniel et al., 2003). For apoptosis to proceed, anti-apoptotic members must be blocked by proteins such as BAG1 and Bik-like killer protein (BLK) (**Figure 1** and **Table 1**; Hegde et al., 1998; Götz et al., 2005; Degterev and Yuan, 2008). Upon mitochondrial outer membrane permeabilization completion, several proteins are released from the outer mitochondrial membrane (i.e., cycs, HtrA2, DIABLO, AIFM1, and DFFB). One of the proteins released is cycs which forms the apoptosome with APAF1 and procaspase-9. Apoptosome formation is blocked by AVEN which binds BCL2L1 and APAF1 to prevent activation of procaspase-9 by the apoptosome. If the apoptosome can form, procaspase-9 is cleaved turning into activated CASP9, which can then trigger the execution pathway (**Figure 1**). Even once the apoptosome has cleaved CASP9 there are still a few ways the execution phase can be blocked. Inhibitor of apoptosis proteins (IAPs) can bind CASP9, CASP3, and CASP7 in order to prevent the start of the execution pathway (Obexer and Ausserlechner, 2014). Prior to this, DIABLO and HtrA2 can inhibit IAPs and encourage apoptosis (**Figure 1**; Du et al., 2000; Suzuki et al., 2001; Elmore, 2007). After IAPs have bound to caspases, BCL10 can cause dissociation of those caspases (Yui et al., 2001). The protein AIFM1, which is released from the outer mitochondrial membrane plays a role triggering DNA fragmentation (Elmore, 2007). Despite being independent processes, the extrinsic and intrinsic pathways share many of the same molecules and a molecule from one pathway has the potential to influence the other.

The third, and least well-known pathway is the *perforin/granzyme pathway* which induces apoptosis through proteins secreted into the cell by Cytotoxic T-lymphocytes. This pathway is initiated when cytotoxic t-lymphocytes detect tumor or virus-infected cells and begin to secrete perforin in order to trigger pore-formation in the plasma membrane (**Figure 1**). Next, GZMA and GZMB are secreted into the cell where they begin to cleave other proteins. GZMA cleaves the 270–420 kDa endoplasmic reticulum-associated complex (SET complex) and GZMB begins cleaving CASP10 and DFFA. Once the SET complex is cleaved, NME1 is released and begins to cleave DNA (Elmore, 2007). CASP10 then triggers the execution pathway by cleaving CASP3 and DFFB is released from DFFA. There is also a significant overlap between the perforin/granzyme pathway and the intrinsic and extrinsic pathways due to the ability of GZMB to cleave proteins associated with mitochondrial permeability.

Finally, all three of these pathways meet up at the *execution pathway* which is activated with the cleavage of CASP3 and is responsible for the dismantling of the cell. When CASP3 is cleaved it begins to activate endonucleases and proteases in the cytoplasm that then degrade the nucleus and cytoskeleton (Coleman et al., 2001; Andrade et al., 2010). The other two effector caspases, CASP7, and CASP6 play a very similar role and can be activated by initiator caspases or CASP3. An important part of the execution pathway that requires more information is the role that cleavage of certain proteins such as PARP1 and SPTAN1 play int his process. Currently it is thought that cleavage of PARP1 and SPTAN1 may help facilitate cellular disassembly (Oliver et al., 1998; Williams et al., 2003).

Autophagic cell death (autophagic type II cell death) may serve as an alternate regulated mechanism of killing cells when apoptosis is blocked (Fuchs and Steller, 2011). It can be characterized by the cytoplasmic vacuolization, autophagosome formation and clearance of material via the lysosome. Autophagy (macroautophagy) fulfills an important function in cell metabolism as it blocks the buildup of toxic protein aggregates. It may also drive other forms of cell death, block anti-apoptotic factors, and play a role in ensuring proper engulfment of dead cells to minimize inflammation. Furthermore, it may also be involved in dying cells switching between different forms of cell death (Doherty and Baehrecke, 2018). Autophagic cell death and autophagy both serve as a

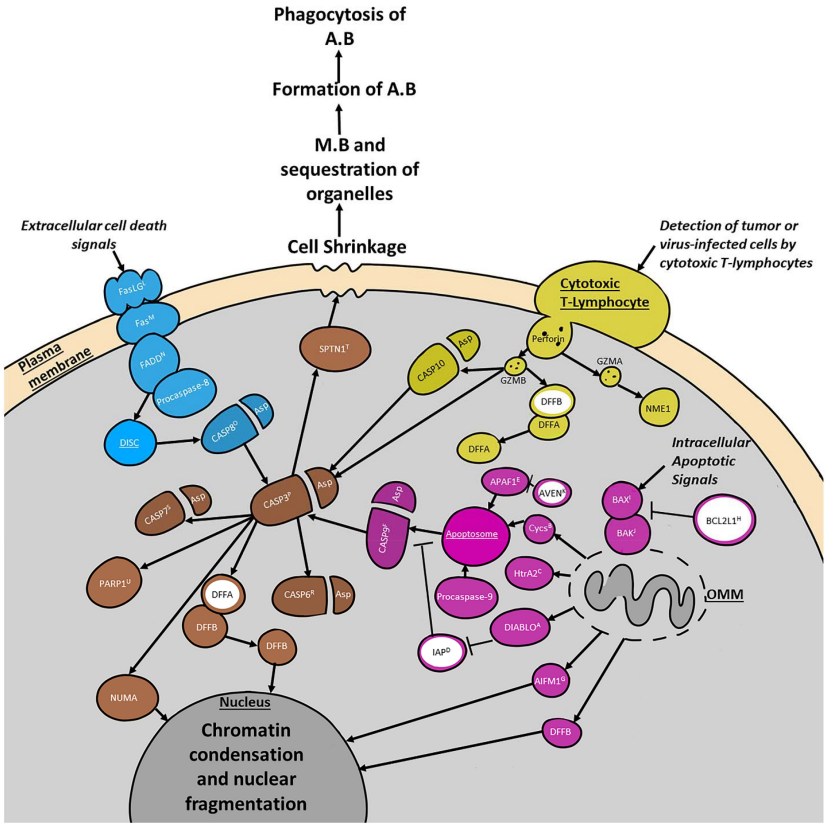


FIGURE 1 | Summary of four major programmed cell death (PCD) pathways discussed in the text. Selected cell organelles are marked in dark gray. Proteins involved in the *extrinsic pathway* are blue. Proteins involved in the *intrinsic pathway* are purple. Proteins involved in the *granzyme/perforin* pathway are indicated in yellow. All three pathways converge at the *execution pathway* which is colored brown. Major inhibitory proteins are filled in white with a ring colored according to the pathway they are primarily involved in. Major complexes such as the Apoptosome and DISC are underlined. The intrinsic pathway is activated by intracellular apoptotic signals that either suppress anti-apoptotic proteins or increase pro-apoptotic proteins. This leads to the activation of pro-apoptotic BCL-2 family proteins such as BAK1 and BAX which triggers the mitochondrial aspect of the pathway by increasing the permeability of the outer mitochondrial membrane (OMM). The granzyme/perforin pathway is activated by the detection of tumor/virus-infected cells by cytotoxic t-cells. These cells secrete perforin which creates pores in the plasma membrane into which GZMA and GZMB can be secreted. The extrinsic pathway is activated by extracellular cell death signals that trigger the binding of homologous trimeric ligands like FasLG to DED-containing receptors such as Fas. Next Cytoplasmic adaptor proteins like FADD are recruited to the complex, along with procaspase-8. Together these proteins form the DISC. All three pathways meet at the execution pathway which is responsible for triggering chromatin condensation and nuclear fragmentation, as well as cell shrinkage. This shrinking leads to membrane blebbing (M.B) and sequestration of the organelles prior to the formation of the apoptotic bodies (A.B). The A.B are then phagocytosed and the chromatin fragments and organelles within them are degraded or incorporated into other cells. Protein superscripts indicate sea urchin (S. purpuratus) orthologous genes from echinobase. A, DIABLO (LOC574919); B, Cycs (LOC575421); C, HtrA2 (LOC115918650); D, IAP (LOC581540); E, APAF1 (LOC591503); F, CASP9 (LOC115924698); G, AIFM1 (LOC578253); H, BCL2L1 (LOC100890351); I, BAX (LOC586236); J, BAK1 (LOC115917937); K, AVEN (LOC762362); L, FasLG (LOC582167); M, Fas (LOC586563); N, FADD (LOC587131); O, CASP8 (LOC585496); P, CASP3 (LOC115918952); R, CASP6 (LOC584221); S, CASP7 (LOC580916); T, SPTAN1 (LOC580822); U, PARP1 (LOC752216).

good reminder that PCD is a highly regulated, conserved, and important process for development and homeostasis.

While apoptosis and autophagic cell death are the most well-known forms of programmed cell death, other types have recently been observed and characterized like pyroptosis, ferroptosis and oxeiptosis. Pyroptosis is a form of programmed necrosis in response to a strong inflammatory response caused by pathogenic infections (Hanson, 2016). Like apoptosis, pyroptosis requires caspase activation however, the caspases involved are completely different from those involved in apoptosis which cleave the inflammatory proteins pro-IL-1β and pro-IL-18 (Hanson, 2016). These pro-inflammatory cytokines then burst from the dying cell (Hanson, 2016). While little is known about the existence of pyroptosis outside of vertebrates, there is some evidence of a pyroptosis induction pathway in bivalves that is regulated by caspase-3 and gasdermin E-like proteins (Vogeler et al., 2021). Ferroptosis is another non-apoptotic form of programmed cell death which is executed by excessive production of reactive oxygen species (Hanson, 2016). Unlike pyroptosis which appears very apoptosis-like, ferroptosis appears very necrosis-like but is still the product of the activation of a unique pathway (Hanson, 2016). Ferroptosis is characterized by an overwhelming and iron-dependent accumulation of lipid lethal ROS (Dixon et al., 2012). Another type of non-apoptotic programmed cell death is oxeiptosis which is triggered by oxidative stress

(Holze et al., 2018). Oxeiptosis is caspase-independent and, similar to ferroptosis, is activated by harmful amounts of ROS (Holze et al., 2018).

Still, not all cell death unfolds via a regulated mechanism. *Necrosis* is a passive, uncontrolled, toxic process that follows an energy-independent mode of death. Necrosis often also affects vast areas of cells where apoptosis usually affects individual cells or small clusters of cells (Elmore, 2007). It typically leads to organelle swelling, lysis, release of intracellular contents, and inflammation (Doherty and Baehrecke, 2018).

TABLE 1 | Summary table listing proteins often involved in apoptosis and which pathway they are involved in, how they are referenced in the text, their LOC identification in echinobase, and their primary function in apoptosis.

Pathway	Name (Human ref)	Reference in text	Echinobase LOC	Role
Intrinsic	Second mitochondrial activator of caspases/direct IAP binding protein with low PI (DBLOH_HUMAN)	DIABLO	LOC574919	Inhibits IAP
	Cytochrome c (CYC_HUMAN)	cycs	LOC575421	Binds APAF1 and procaspase-9
	High-temperature requirement (HTRA2_HUMAN)	HtrA2	LOC115918650	Inhibits IAP
	Inhibitor of apoptosis proteins (XIAP_HUMAN)	IAP	LOC581540	Binds casp-9, casp-3 and casp-7
	Apoptotic protease activating factor (APAF_HUMAN)	APAF1	LOC591503	Binds cycs and procaspase-9
	Cysteinyl aspartic acid-protease-9 (CASP9_HUMAN)	CASP9	LOC115924698 (Predicted)	Cleaves CASP3
	Apoptosis inducing factor (AIFM1_HUMAN)	AIFM1	LOC578253	Triggers DNA fragmentation
	BCL2 like 1 (B2CL1_HUMAN)	BCL2L1	LOC100890351	Inhibits MOMP
	BCL2 associated anathanogene (BAG1_HUMAN)	BAG1	LOC579477	Interacts with anti-apoptotic Bcl-2 family members
	BCL2 associated X protein (BAX_HUMAN)	BAX	LOC586236	Triggers MOMP
	BCL2 antagonist killer 1 (BAK_HUMAN)	BAK1	LOC115917937	Triggers MOMP
	Bik-like killer protein (BLK_HUMAN)	BLK	*SFK7*	Prevents Bcl-2 and BCL2L1 from blocking apoptosome formation
	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein (1433E_HUMAN)	14-3-3	LOC581376	Phosphorylates and sequesters Bad
	Cell death regulator Aven (AVEN_HUMAN)	AVEN	LOC762362	Binds BCL2L1 and APAF1 to block activation of procaspase-9
	Oncogene Myc (MYC_HUMAN)	Myc	LOC373385	Plays a role in both p53-dependent and independent apoptosis
Extrinsic	Tumor necrosis factor receptor 1 (TNR1A_HUMAN)	TNFRSF1A	LOC105446527	Binds ligands to recruit TRADD
	Fas antigen ligand (TNFL6_HUMAN)	FasLG	LOC582167	Binds with Fas
	Apoptosis-mediating surface antigen FAS (TNR6_HUMAN)	Fas	LOC586563 (Predicted)	Binds with ligands to recruit FADD
	Apo3 Ligand (TNF12_HUMAN)	TNFSF12	LOC100890814 (Predicted)	Binds with TNFRSF25 to recruit CAPs
	Apo2 ligand (TNF10_HUMAN)	TNFSF10	LOC100891737	Binds with either TNFRSF10A or TNFRSF10B to CAPs
	Fas-associated death domain (FADD_HUMAN)	FADD	LOC587131	Associates with CASP8 to form the DISC
	Receptor interacting protein (RIPK1_HUMAN)	RIPK1	LOC105436416	Assists FADD in binding TRADD to TNF/TNFRSF1A
	Caspase-8 (CASP8_HUMAN)	CASP8	LOC585496	Cleaves effector caspases
Granzyme	Granzyme B (GRAB_HUMAN)	GZMB	*bf* (Predicted)	Cleaves proteins leading to activation of CASP10 and CASP3 and release of DFFB
	Caspase-10 (CASPA_HUMAN)	CASP10	LOC587820 (Predicted)	Cleaves CASP3
	Granzyme A (GRAA_HUMAN)	GZMA	LOC115929050	Cleaves SET complex to release NME1
	NME1 (NDKA_HUMAN)	NME1	LOC594617	Causes DNA nicks

(Continued)

TABLE 1 | Continued

Pathway	Name (Human ref)	Reference in text	Echinobase LOC	Role
Execution	Cysteinyl aspartic acid-protease-3 (CASP3_HUMAN)	CASP3	LOC115918952 (Predicted)	Cleaves enzymes leading to nuclear and cytoskeletal degradation
	Cysteinyl aspartic acid-protease-6 (CASP6_HUMAN)	CASP6	LOC584221	Cleaves enzymes leading to nuclear and cytoskeletal degradation
	Cysteinyl aspartic acid-protease-7 (CASP7_HUMAN)	CASP7	LOC580916 (Predicted)	Cleaves enzymes leading to nuclear and cytoskeletal degradation
	Poly (ADP-ribose) polymerase (PARP1_HUMAN)	PARP1	LOC752216	Cleavage of PARP1 facilitates cellular disassembly
	Spectrin alpha chain (SPTN1_HUMAN)	SPTAN1	LOC580822	Cleavage of SPTAN1 leads to breakdown of the cellular membrane and formation of the apoptotic bodies

MOMP, mitochondrial outer membrane permeabilization; CAPs, cytoplasmic adaptor proteins. Only proteins found in S. purpuratus are listed in this table. CD, programmed cell death; DISC, death inducing signaling complex; Bcl-2, B-cell lymphoma 2; IAPs, Inhibitor of apoptosis proteins; THs, thyroid hormones; BH, Bcl-2 homology; CARD, caspase recruitment domain.

Since necrosis can occur independently, sequentially, or simultaneously to PCD it can be difficult to distinguish these processes. Furthermore, there are forms of cell death that share morphological features of both necrosis and apoptosis called aponecrosis (Elmore, 2007). There is also necroptosis, which functions as a unique signaling pathway that is characterized by 2 major necroptotic death effector complexes, caspase inactivity and a series of morphological changes (Hanson, 2016). The necrosome and ripoptosome are the two major necroptotic death effector complexes and are induced by TNFR1 and toll like receptor 3 (TLR3) signaling (Hanson, 2016). During this process the cell undergoes several morphological and internal changes such as cellular rounding, an increase in cytosolic calcium ions and reactive oxygen species, depletion of ATP, intracellular acidification, cellular swelling and finally plasma membrane rupture, and the release of damage-associated molecular patterns (Hanson, 2016). Many of the proteins important for necroptosis are found across Deuterostomia however, it is unclear if the actual process of necroptosis occurs in some of the non-vertebrate deuterostomes (Dondelinger et al., 2016).

Traditionally, a large part of mechanistic understanding of cell death originates from cell culture experiments and less so from whole organism studies. Still, in recent years, new data on cell death regulation and function has emerged from a diversity of taxa and this data suggests that PCD mechanisms are used in a range of morphological, physiological, and developmental contexts. For example, animals with an indirect life-history, characterized by a drastic morphological and physiological transition (Tata, 1993; Balon, 1999; Bishop et al., 2006; Heyland and Moroz, 2006; Ishizuya-Oka et al., 2010; Heyland et al., 2018; Tettamanti and Casartelli, 2019) have been repeatedly shown to employ PCD for the removal of cells and the transformation of the larval body plan into a juvenile (Heyland and Moroz, 2006; Hodin et al., 2016; Lutek et al., 2018). Here we integrate available mechanistic information on PCD from whole organism studies with our understanding of the regulatory mechanisms of metamorphosis with an emphasis on endocrine regulation. We propose, that the metamorphic transition in animals provides an excellent model system to study the role of PCD in post-embryonic development and therefore provide critical functional background for PCD from an ecological, physiological, and developmental perspective.

PCD IN METAMORPHIC DEVELOPMENT AND ITS REGULATION BY HORMONES

The importance of PCD in development is exemplified during the metamorphic transition of animals. This drastic and often rapid change in morphology, physiology and ecology of an organism is typically associated with substantial cellular remodeling, differentiation, and the removal of cells and tissues. Historically, frogs and insects have been extensively studied in this context and a significant amount of information on PCD regulation and functions exists in these groups (Tata, 1993; Bishop et al., 2006; Heyland and Moroz, 2006; Ishizuya-Oka et al., 2010; Heyland et al., 2018; Tettamanti and Casartelli, 2019). Even though metamorphosis evolved independently in insects and vertebrates (Hadfield, 2000; Heyland and Moroz, 2006; Hodin, 2006; Heyland et al., 2018), similar, convergent mechanisms are used in its regulation. Notably, hormones have emerged as a key regulator of cell death and these endocrine mechanisms continue to be investigated (Nakajima et al., 2005; Heyland and Moroz, 2006; Mané-Padrós et al., 2010; Heyland et al., 2018). In contrast, marine invertebrate species with indirect life histories undergo equally dramatic transitions but the cellular and molecular processes (including PCD) have received little attention, considering invertebrate

diversity (but see Wray and Raff, 1990; Degnan, 2001; Davidson and Swalla, 2002; Bishop and Brandhorst, 2003; Bishop and Anderson, 2005; Heyland and Moroz, 2006; Hodin, 2006; Paris et al., 2008, 2010; Nakayama-Ishimura et al., 2009; Heyland et al., 2018; Wong et al., 2019). Here we briefly review the function of PCD in metamorphosis and settlement among animals to create a foundation for our discussion of PCD in sea urchin embryonic and post-embryonic development in the next section.

Frog tadpoles develop specific structures during the metamorphic transition that allow them to live a semi-aquatic life in the adult stage. This transformation impacts most physiological and morphological systems (**Figure 2**). Apoptosis has been studied in some detail during frog tail resorption, the rewiring of the nervous system, the digestive system, and gill remodeling (Ishizuya-Oka et al., 2010). As tadpoles transition to live on land, the aquatic tail of the tadpole is no longer required and rapidly disintegrates. The process of tail resorption is tightly regulated and can be roughly divided into three stages. First, the dorsal fins are resorbed, then the muscle fibers are fragmented and finally the notochord lamella is dissolved (Das et al., 2002; Brown et al., 2005). In addition to these morphological changes, there are several important cellular events that make up this process. In response to THs, tail muscle cells up-regulate and activate CASP3 and a high level of pro-apoptotic BAX expression in the tail during its resorption has been described in Xenopus laevis (Das et al., 2002, 2006). This is supported by the fact that overexpression of the anti-apoptotic Bcl-2 in tail muscle inhibits TH-induced cell death. Similarly, downstream of BAX, pro-apoptotic CASP9 is also upregulated in the brain, intestine and tail during metamorphic climax (Das et al., 2006). Still, the tadpole tail is not the only structure that is removed or modified by apoptosis. The gills also degenerate almost completely, and the intestine and pancreas are drastically remodeled to accommodate the change in diet from the herbivorous tadpole stage to the carnivorous adult stage (Sun et al., 2014). A similar remodeling must occur in the organs of the immune system like the spleen as the larval immune system is not equipped to defend against the same pathogens as an adult frog immune system (Ishizuya-Oka et al., 2010). Just like the tail resorption, regulation of these processes is under the control of thyroid hormones (THs) (Ishizuya-Oka et al., 2010). The thyroid hormones T3 and T4 are secreted by the developing tadpole's thyroid gland and, in the case of T4, is converted to T3 by type I and II deiodinases in the target tissue. TH actions are mediated via thyroid hormone receptors, a type of nuclear hormone receptor that is necessary for mediating the metamorphic effects of TH in tadpoles. In frogs two thyroid hormone receptors have been identified and characterized, Thra and Thrb (Ishizuya-Oka et al., 2010; Faunes and Larraín, 2016). Apoptosis in larval tissue is primarily mediated by Thrb (Ishizuya-Oka et al., 2010). While it was previously thought that TH was both necessary and sufficient for metamorphosis, recent research has indicated that steroid hormones such as glucocorticoids may play an important role in the progression of tail resorption as well (Sachs and Buccholz, 2019).

In insects, proliferation and apoptosis are tightly linked during the pupal stage, where many steps of differentiation occur (Eroglu and Derry, 2016). Apoptosis contributes to the patterning and development of almost all adult structures in the imaginal disks of the larva (**Figure 2**; Fuchs and Steller, 2011). Furthermore, epithelial cells in the larval skin and the abdominal region are removed through apoptosis and are replaced by proliferating and migrating neuroblasts – stem cell-like founder cells (Parasathy and Palli, 2008; Ninov and Martín-Blanco, 2009). In Drosophila, group apoptosis is observed in the posterior wing imaginal discs, haltere and leg discs (Eroglu and Derry, 2016). Group apoptosis occurs as dying cells emulate signals that are partially sufficient in stimulating apoptosis in their neighbors. Additionally, overexpression of both the anti-apoptotic Cdk5α and pro-apoptotic hid genes can trigger neighboring cells into undergoing apoptosis. This coordinated apoptosis likely plays an important role in regulating proper tissue growth and preventing group crowding during metamorphosis (Eroglu and Derry, 2016; Kawamoto et al., 2016). The insect steroid hormone ecdysone plays a fundamental role in the regulation of development, being responsible for cell proliferation during larval molts and the pupal transition. Specifically, ecdysone activates transcriptional cascades that lead to the expression of Reaper, Hid Grim (RHG) genes and active caspases in insects. Conversely, in adult neurons, ecdysone acts as a pro-survival factor (Fuchs and Steller, 2011).

In many marine invertebrate groups, the drastic transition from the planktonic larva a free living benthic juvenile body that occurs during settlement involves PCD (Leise et al., 2004; Gifondorwa and Leise, 2006; Hadfield, 2011; Heyland et al., 2011, 2018; Lutek et al., 2018; Hadfield et al., 2021). In indirectly developing mollusks, metamorphosis, and settlement is characterized by substantial tissue remodeling. For example, veliger larvae of bivalves, scaphopods and gastropods lose their velar lobes during this process and build the adult ganglionic nervous system (Vogeler et al., 2021). Meanwhile, the central component of the larval nervous system, the apical organ, degenerates likely through apoptosis (Kiss, 2010; Heyland et al., 2011). Cell death within this organ has been shown to be initiated by the neurotransmitter serotonin and inhibited by nitric oxide (NO) (Bishop and Brandhorst, 2001; Kiss, 2010). In fact, using nitric oxide to inhibit apoptosis in some mollusc species may inhibit metamorphosis itself in competent larvae (Gifondorwa and Leise, 2006). At the molecular level, similarities exist between the apoptotic networks of mollusks and vertebrates (Kiss, 2010; Heyland et al., 2011; Romero et al., 2011, 2015). Like vertebrates, mollusks use both intrinsic and extrinsic apoptotic mechanisms and the presence of perforin in several bivalves may suggest the existence of a perforin/granzyme apoptotic pathway as well (Romero et al., 2015). These pathways also include caspases that are homologous to vertebrate caspases such as, CASP1, CASP2, CASP3, CASP6, CASP8, and a combination caspase-3/7 (Vogeler et al., 2021). Another similarity between vertebrate and mollusk apoptotic networks is the common convergence of the execution pathway, as initiated by the cleavage of CASP3 (Romero et al., 2015). While apoptosis has not received much attention in invertebrates, there is evidence that it plays an important role in the development of many different species of marine invertebrates. In the hydrozoan ***Hydractinia echinate*** PCD activity in the larval nervous system has been described (Seipp et al., 2010). In

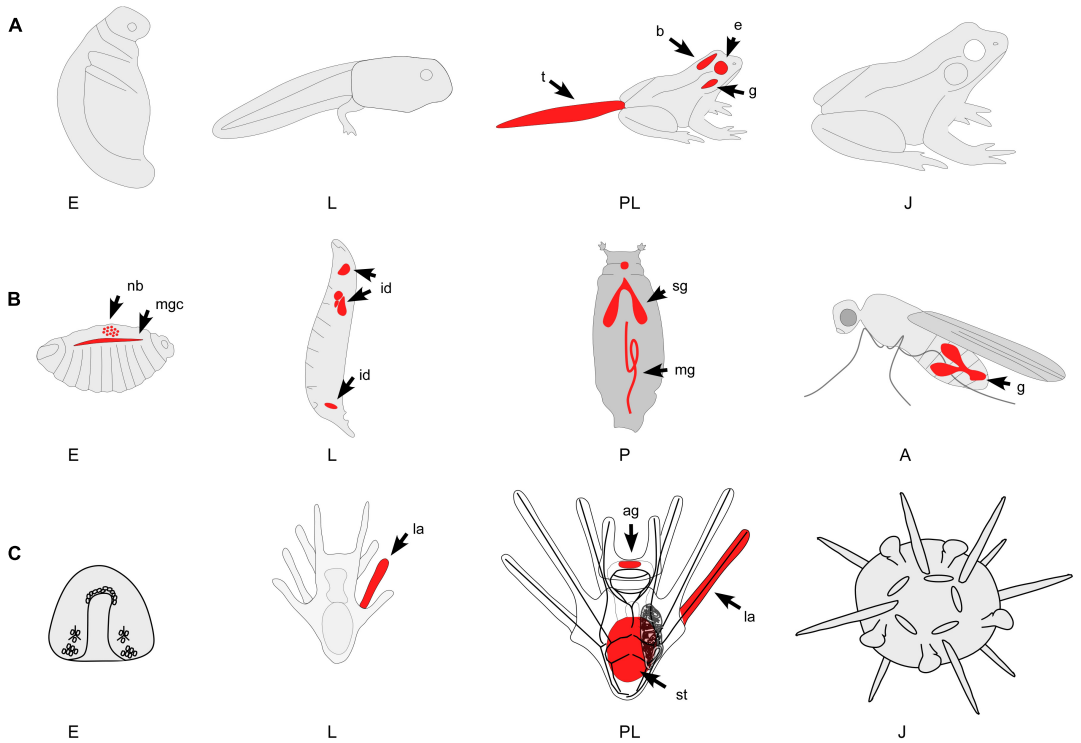


FIGURE 2 | Three representative examples of animals with indirect life histories and metamorphic transitions. Red color indicates major areas of PCD during development. Note that only major areas of PCD are depicted in these diagrams and many more have been identified. **(A)** Indirectly developing frog. The metamorphic transition of frogs impacts most physiological and morphological systems. While PCD occurs during embryogenesis (E – gastrulation and early cleavage stages) most work has identified the mechanisms of PCD during tail (t) regression, gill (g) and brain (b) remodeling, and the restructuring of many other organ systems. **(B)** Holometabolous insect. PCD occurs throughout holometabolous development, including the larval epidermis, neuroblasts (nb), imaginal disks (id), salivary gland (sg), midgut (mg), mushroom bodies, and gonads (g). The pupal stage (P) is a time of active tissue remodeling and patterning that is strongly associated with PCD. **(C)** Indirectly developing sea urchin. PCD has been identified throughout sea urchin development, including the embryo (E) and the larval (L) tissues. During settlement, many larval structures such as the arms (la), the ciliated band and parts of the stomach (st) degenerate, a process that has been shown to involve PCD. A, adult; E, embryo; J, juvenile; L, larva; P, pupa; PL, pre-metamorphic larva; ag, apical ganglion; b, brain; e, eyes; g, gills; g, gonads; id, imaginal disks; la, larval arms; mg, midgut; mgc, midline glial cells; nb, neurblasts; sg, salivary gland; st, stomach; t, tail.

ascidians, a marine basal chordate, apoptosis has been identified during the transition of the larval (tadpole) body to the juvenile (Karaiskou et al., 2015). Apoptosis acts as a primary driver of tissue remodeling in the larvae of the cnidarian ***Clytia hemisphaerica*** (Krasovec et al., 2021). Furthermore, an expansion of the caspase complement has been described in the ascidian ***Ciona intestinalis*** in comparison to closely related species and this expansion may be in part driven by tail regression in the tadpole (Weill et al., 2005).

PCD IN SEA URCHIN DEVELOPMENT AND METAMORPHOSIS

Programmed cell death has also been studied in the development of several sea urchin species. As in other animal groups, PCD functions in oocyte development, embryonic development, and metamorphosis. Understanding the function of PCD in the developmental context of a whole animal will help to understand the relationships between PCD, hormones and metamorphosis. Here we briefly summarize this work to elucidate PCD function in the life cycle of sea urchins and then provide an up-to-date annotation of PCD related genes from the sea urchin *S. purpuratus* with insights into their potential functions in PCD regulation.

Sea urchins possess a functional apoptosis machinery that can act even during the development and maturation of oocytes and early embryos (Voronina and Wessel, 2001). Although PCD in actively dividing cells is widely described because of its role in tissue development, experimental evidence suggests that apoptosis occurs in cells that are not actively dividing but still metabolically active – often called quiescent cells (Voronina and Wessel, 2001). This means that not only is the apoptotic machinery present in germ cells, but it is functional as well (Voronina and Wessel, 2001). Still the function of apoptosis and other forms of programmed cell death in early embryonic development remain largely unclear. Similar to other, more studied organisms such as *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, cleavage stages are mostly characterized by necrotic and pathological cell death rather than regulated apoptosis and it is not until gastrulation that a marked increase of apoptosis has been

observed (Thurber and Epel, 2007; Galasso et al., 2019). Abundant experimental evidence shows that cell death can also be induced in early sea urchin development via cytotoxic treatments, pollutants, and UV radiation (Lesser et al., 2003; Chiarelli et al., 2011). During sea urchin gastrulation apoptosis appears to be a necessary component of normal development, as pharmacological induction of apoptosis or the inhibition of caspases results in deformed larvae and reduced survival in *Stronglyocentrotus purpuratus* (Thurber and Epel, 2007; Heyland pers. obs.). Galasso et al. (2019) found that many death genes are differentially expressed during this important developmental process and that apoptosis and autophagy during gastrulation are likely involved in cavity formation and the removal of inner ectodermal cells. Intriguingly, induction of apoptosis in gastrulation can result in significant acceleration of larval development of the surviving embryos compared to controls (Thurber and Epel, 2007). In larval development, PCD, specifically apoptosis, play a prominent role in sculpting tissues in the arms and the ciliary band and a function of apoptosis has been proposed in the adaptive phenotypic plastic response of larval arm and ciliary band length to microalgae concentration in the environment (Thurber and Epel, 2007; Agnello and Roccheri, 2010).

Data from a broad range of animals suggests a prominent role of PCD in the process of metamorphosis as summarized above and links between PCD and sea urchin metamorphosis and settlement have been studied in several species [reviewed in Heyland and Moroz (2006)]. As in other marine invertebrate species, sea urchins with an indirect life history undergo a drastic ecological, developmental, and physiological transition between the larval and juvenile stage. Settlement, the transition from the planktonic to the benthic environment can be preceded by major morphological or developmental changes but does not have to. In fact, some species, such as cnidarian, ascidian, and echinoderm species complete a large portion of morphological changes during or after settlement (Rodriguez et al., 1993; Heyland and Hodin, 2014; Krasovec et al., 2021). In sea urchins, a hallmark of settlement is the larval arm retraction and subsequent transition from a bilateral to a pentaradial organism. During this process cell death, proliferation, migration, and differentiation occur in a relatively short period of time (Cameron et al., 1989; Heyland and Moroz, 2006; Hodin, 2006; Lutek et al., 2018). Other changes, such as the formation of the juvenile mouth and nervous system develop on a much longer time scale, taking typically days to week to fully form (Thet et al., 2004; Emlet, 2010; Fadl et al., 2017, 2019). Apoptotic cells have been identified in the arms and ciliary bands of metamorphically competent larvae (the developmental stage preceding settlement) as well as in juveniles (Roccheri et al., 2002; Lutek et al., 2018). Moreover, specific signaling molecules have been implicated in the induction of PCD in sea urchin metamorphosis and settlement. Glutamine and some of its derivatives, compounds that have been shown to accelerate metamorphic development, can induce apoptosis in the larval epithelium in the sea urchin *Hemicentrotus pulcherrimus* (Sato et al., 2006). Furthermore, recent research shows that histamine has a dual function in larval development of the sea urchin *S. purpuratus*. While it aids in the attainment of metamorphic competence, it also keeps larvae in that state by inhibiting the settlement process (Lutek et al., 2018). The latter process is, at least in part, the result of the inhibition of apoptosis in the larval arms by histamine signaling via the sea urchin histamine receptor (Lutek et al., 2018). Histamine may interact with TH signaling, a hormone that can be endogenously synthesized (Heyland et al., 2006b,a; Miller and Heyland, 2009, 2013) or derived from unicellular algae that echinoid larvae feed on (van Bergeijk et al., 2013) and that has been shown to regulate development, including metamorphic development in sea urchins and sand dollars (Heyland and Hodin, 2004; Heyland et al., 2004). Recent evidence from the sea urchin *S. purpuratus* also suggests that THs may signal via integrins in addition to their nuclear hormone receptor pathway (Taylor and Heyland, 2018). A distinct characteristic of the effect TH signaling in sea urchin and sand dollar larvae is the acceleration of juvenile structures while reducing the length of larval arms (Heyland and Hodin, 2004). It is the ladder effect of THs that is in part the result of PCD (Wynen and Heyland, 2021).

APOPTOTIC GENES IN THE SEA URCHIN (*S. purpuratus*)

Due to the important role apoptosis plays in development and homeostasis, the apoptotic toolkit may become complex and contain many redundancies within specific lineages. As far as metazoans are concerned, there have been many documented cases of gene losses and expansion (Zmasek and Godzik, 2013; Romero et al., 2015; Green and Fitzgerald, 2016). This makes it difficult to provide a comprehensive overview of apoptosis in specific groups for which little functional data is available. Keeping this in mind, we used an earlier PCD annotation of the sea urchin genome as a starting point (Robertson et al., 2006) and further annotated the sea urchin apoptotic tool kit (sea urchin genome assembly and annotation v. 5.0).

As outlined above, the intrinsic pathway responds to intracellular signals produced by non-receptor mediated stimuli that leads to the activation of Bcl-2 family members. One type of non-receptor mediated stimuli that leads to intracellular signaling is exposure to ultraviolet radiation. As discussed earlier, DNA damage caused by radiation can lead to the activation of the gene p53 (Lesser et al., 2003). The protein p53 leads to a downregulation of anti-apoptotic Bcl-2 proteins which can lead to apoptosis (Bourgarel-Rey et al., 2009). The Bcl-2 family may also be responsible for the absence of early developmental apoptosis in sea urchins (Thurber and Epel, 2007). Bcl-2 members that were identified in the sea urchin genome included Bcl-2, BAX, BAK1, BAG1, BLK, and BCL10 (**Table 1**). In sea urchins, orthologs for both BAX and BAK1 have been identified (**Table 1**) and similarly to vertebrates, these proteins share a Bcl-2 homology 3 (BH3) motif at the N-terminus of the Bcl-2 domain (Robertson et al., 2006). While the function of a protein or domain can only be conclusively determined by experimental work, examining the presence or absence of specific domains or comparing their structure to known proteins can still provide a good foundation to generate hypotheses and

predictions about their role. The Bcl-2 family of proteins has a few characteristic domains that, while difficult to presume their function, at least allow for possible identification. These include the BH1, BH2, BH3, and BH4 domains (Moroy et al., 2009). The BH3 domain is a particularly well-known part of the Bcl-2 protein family and has been identified as a mediator of cell death (Moroy et al., 2009). The BH3 domain appears in pro-apoptotic proteins such as BAX, BAD and BIK (Moroy et al., 2009). Another class of pro-apoptotic Bcl-2 family members called the BH3-only proteins bind with anti-apoptotic proteins like Bcl-2 and BCL2L1 through the BH3 domain in order to suppress their anti-apoptotic activity (Moroy et al., 2009). The BH domains are important for facilitating interactions between the Bcl-2 protein family members (Gurudutta et al., 2005; Aouacheria et al., 2013).

The proteins involved in the formation of the apoptosome, cycs, APAF1, and CASP9, are well-represented in sea urchins. While four predictions for APAF1-like proteins exist in the sea urchin genome, two are nearly identical and generally regarded as paralogs (Robertson et al., 2006) and one APAF1 -like protein lacks the N-terminal caspase recruitment domain (CARD) found in humans, flies and nematodes, and instead possessing a death domain (Robertson et al., 2006). The final APAF1 -like protein is very similar in architecture to human APAF1 (**Table 1**; Robertson et al., 2006). The CARD domain of APAF1 is important due to its interaction with the CARD domain of CASP9 (Wang et al., 2017). As mentioned previously, formation of the apoptosome can be blocked by AVEN which uses the BH1 domain of BCL2L1 to bind it and APAF1 to prevent activation of CASP9 by the apoptosome; all of which we found orthologs for (**Table 1**; Chau et al., 2000; Elmore, 2007). Should the apoptosome form, then CASP9 is activated (Elmore, 2007). The CASP9 family underwent an echinoderm-specific expansion which resulted in several CASP9-like proteins (Robertson et al., 2006; Agnello and Roccheri, 2010). When analyzed by Robertson et al. (2006) four CASP9-like proteins were reported and only one featured a recognizable CARD domain (LOC594735). In the updated genome, we were able to annotate three proteins that are similar to CASP9 that have a complete CARD domain (LOC586121, LOC115924698, and LOC584219) and one protein that is very similar in sequence to CASP9 and has an incomplete CARD domain (LOC586121). Furthermore, the protein that Robertson et al. (2006) originally identified, does not appear to have a CARD domain or show as much similarity to human CASP9 as the proteins reported above. The CARD domain is important for binding of procaspase-9 to APAF1 and may play a role in stabilizing interactions within the core of CASP9 (Huber et al., 2018). Since this domain is so important, it may help to identify the function of CASP9-like proteins. This function can be blocked however, when the baculoviral IAP repeat 3 (BIR3) domain of XIAP binds with CASP9 in order to prevent the start of the execution pathway (Shiozaki et al., 2003). This action can be blocked by DIABLO which competes with CASP9 for binding of the BIR3 domain (Gao et al., 2007). Additionally, binding of DIABLO with the BIR2 domain of XIAP can antagonize inhibition of CASP3 by XIAP (Gao et al., 2007). HtrA2 is able to inhibit XIAP in a similar way (**Figure 1**; Verhagen et al., 2002; Elmore, 2007; Obexer and Ausserlechner, 2014). The protein AIFM1, which is released from the outer mitochondrial membrane plays a role triggering DNA fragmentation and was also found upon analysis (**Table 1**; Elmore, 2007).

We found fewer orthologous proteins in the extrinsic complement than in the intrinsic pathway. Specifically, we were unable to find a clear ortholog of TNF, even though we found an ortholog for its receptor TNFRSF1A (**Table 1**). There are a few couplings that are well known in this pathway: TNF/TNFRSF1A, FasLG/Fas, TNFSF12/TNFRSF25, TNFSF10/TNFRSF10A, and TNFSF10/TNFRSF10B (Elmore, 2007). We were able to identify possible orthologs of FasLG, Fas, TNFSF12 and TNFSF10 however, we were unable to find clear orthologs for TNFRSF25, TNFRSF10A, and TNFRSF10B (**Table 1**). Interestingly, there seems to be similarity between TNFRSF10A, TNFRSF10B and *S. purpuratus* TNFR16 (LOC586563). While this similarity is relatively low, it may provide valuable insight into the extrinsic pathway of sea urchins and the absence of so many extrinsic receptors. Of the proteins that are involved in recruiting procaspase-8 to the DISC, possible orthologs for FADD and RIP were identified but not TRADD (**Table 1**). Like the CASP9-like protein family, there seems to have been some expansion of the CASP8 family in the sea urchin genome (Robertson et al., 2006; Agnello and Roccheri, 2010). Upon analysis, Robertson et al. (2006) found five caspase-like-proteins, two of which contained clear linkages to DEDs, similar to human CASP8. Of these two proteins, only one has a complete caspase domain and therefore is a strong candidate for a human CASP8 ortholog (**Table 1**; Robertson et al., 2006).

The granzyme/perforin pathway contains fewer proteins than the intrinsic and extrinsic pathway but still seems to be well represented in the sea urchin genome (**Table 1**). In the perforin/granzyme pathway, sea urchin orthologs for GZMA, GZMB, CASP10, and NME1 were identified (**Table 1**).

All three of these pathways converge at the execution pathway which is also well represented in the sea urchin genome, likely due to the important role caspases play in this part of apoptosis (**Table 1**). One of the biggest differences between mammals and sea urchins seems to be the lack of clear distinction between CASP3 and CASP7 (Robertson et al., 2006). Due to this fact, it is difficult to conclusively identify separate orthologs for these caspases and the LOCs represented in the table may indicate paralogs of one gene (**Table 1**). We have been able to identify orthologs for CASP6, PARP1 and SPTAN1 (**Table 1**).

CONCLUSION

As others have pointed out, endocrine mechanisms are intricately linked to the mechanisms underlying metamorphic transitions in animals and PCD is one important output of these mechanisms (Nakajima et al., 2005; Heyland and Moroz, 2006; Mané-Padrós et al., 2010; Heyland et al., 2018). In addition to frogs and flies, for which many of these mechanisms have been studied in detail, our analysis also revealed that PCD is an intricate part of metamorphosis and settlement of many marine invertebrate groups with indirect life histories. Specifically, the target of PCD in these groups is the larval nervous system and feeding and digestive structures such as the ciliated band and larval arms.

Studies into the molecular and cellular mechanisms underlying these processes require further research. While the annotation of PCD related genes in target species can be difficult due to species specific expansions and diversification, it is a valuable exercise and can help shed light on the ecological and environmental factors driving these processes (Lespinet et al., 2002). For example, caspases in sea urchins have undergone such expansions and may play a role in important developmental processes such as metamorphosis and settlement by removing larval tissue. Although necessary to switch between environments, metamorphosis, and settlement can be rapid and resource intense processes and elucidating the putative function of caspases in these processes may help shed light onto the environmental conditions driving the expansion of the caspase family in sea urchins. Finally, the role of endocrine mechanisms in the regulation of metamorphosis and settlement as well as PCD may be significantly more wide-spread and future studies should explore this link in more details in marine invertebrate phyla.

AUTHOR CONTRIBUTIONS

HW and AH contributed to the concept and writing of the article. Both authors contributed to the article and approved the submitted version.

FUNDING

Funding for this work was provided, in part by NSERC Discovery Grant to AH (400230).

ACKNOWLEDGMENTS

We would like to thank members of the Heylandlab for feedback on earlier versions of the manuscript and help with the annotation of apoptosis genes form the sea urchin.

REFERENCES

Agnello, M., and Roccheri, M. C. (2010). Apoptosis: focus on sea urchin development. *Apoptosis* 15, 322–330. doi: 10.1007/s10495-009-0420-0

Amiesen, J. C. (2002). On the origin, evolution and nature of programmed cell death: a timeline of four billion years. *Cell Death Differ.* 9, 367–393. doi: 10.1038/sj.cdd.4400950

Andrade, R., Crisol, L., Prado, R., Boyano, M. D., Arluzea, J., and Aréchaga, J. (2010). Plasma membrane and nuclear envelope integrity during the blebbing stage of apoptosis: a time-lapse study. *Biol. Cell* 102, 25–35. doi: 10.1042/bc20090077

Aouacheria, A., Rech, de Laval, V., Combet, C., and Hardwick, M. J. (2013). Evolution of Bcl-2 homology motifs: homology versus homoplasy. *Trends Cell Biol.* 23, 103–111. doi: 10.1016/j.tcb.2012.10.010

Balon, E. (1999). Alternative ways to become a juvenile or a definitive phenotype (and on some persisting linguistic offenses). *Environ. Biol. Fishes* 56, 17–38. doi: 10.1007/978-94-017-3678-7_2

Bishop, A., and Anderson, J. E. (2005). NO signaling in the CNS: from the physiological to the pathological. *Toxicology* 208, 193–205. doi: 10.1016/j.tox.2004.11.034

Bishop, C. D., and Brandhorst, B. P. (2001). NO/cGMP signaling and HSP90 activity represses metamorphosis in the sea urchin *Lytechinus pictus*. *Biol. Bull.* 201, 394–404. doi: 10.2307/1543617

Bishop, C. D., and Brandhorst, B. P. (2003). On nitric oxide signaling, metamorphosis, and the evolution of biphasic life cycles. *Evol. Dev.* 5, 542–550. doi: 10.1046/j.1525-142x.2003.03059.x

Bishop, C. D., Huggett, M. J., Heyland, A., Hodin, J., and Brandhorst, B. P. (2006). Interspecific variation in metamorphic competence in marine invertebrates: the significance for comparative investigations into the timing of metamorphosis. *Integr. Comp. Biol.* 46, 662–682. doi: 10.1093/icb/icl043

Bourgarel-Rey, V., Savry, A., Hua, G., Carré, M., Bressin, C., Chacon, C., et al. (2009). Transcriptional down-regulation of Bcl-2 by vinorelbine: identification of a novel binding site of p53 on Bcl-2 promoter. *Biochem. Pharmacol.* 78, 1148–1156. doi: 10.1016/j.bcp.2009.06.025

Brown, D. D., Cai, L., Das, B., Marsh-Armstrong, N., Schreiber, A. M., and Juste, R. (2005). Thyroid hormone controls multiple independent programs required for limb development in *Xenopus laevis* metamorphosis. *Proc. Natl. Acad. Sci. USA* 35, 12455–12458. doi: 10.1073/pnas.0505989102

Cameron, A. R., Britten, R. J., and Davidson, E. H. (1989). Expression of two actin genes during larval development in the sea urchin *Strongylocentrotus purpuratus*. *Mol. Reprod. Dev.* 1, 149–155. doi: 10.1002/mrd.1080010302

Chau, B. N., Cheng, E. H., Kerr, D. A., and Hardwick, J. M. (2000). Aven, a novel inhibitor of caspase activation, binds Bcl-xL and Apaf-1. *Mol. Cell* 6, 31–40. doi: 10.1016/s1097-2765(05)00021-3

Chiarelli, R., Agnello, M., and Roccheri, M. C. (2011). Sea urchin embryos as a model system for studying autophagy induced by cadmium stress. *Autophagy* 7, 1028–1034. doi: 10.4161/auto.7.9.16450

Coleman, M. L., Sahai, E. A., Yeo, M., Bosch, M., Dewar, A., and Olson, M. F. (2001). Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. *Nat. Cell Biol.* 3, 339–345. doi: 10.1038/35070009

Daniel, P. T., Schulze-Osthoff, K., Belka, C., and Güner, D. (2003). Guardians of cell death: the Bcl-2 family proteins. *Essays Biochem.* 39, 73–88. doi: 10.1042/bse0390073

Das, B., Cai, L., Carter, M. G., Piao, Y. L., Sharov, A. A., Ko, M. S. H., et al. (2006). Expression changes at metamorphosis induced by thyroid hormone in *Xenopus laevis* tadpoles. *Dev. Biol.* 291, 342–355. doi: 10.1016/j.ydbio.2005.12.032

Das, B., Schreiber, A. M., Huang, H., and Brown, D. D. (2002). Multiple thyroid hormone-induced muscle growth and death programs during metamorphosis in *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA*. 99, 12230–12235. doi: 10.1073/pnas.182430599

Davidson, B., and Swalla, B. J. (2002). A molecular analysis of ascidian metamorphosis reveals activation of an innate immune response. *Development* 129, 4739–4751. doi: 10.1242/dev.129.20.4739

Degnan, B. M. (2001). "Settlement and metamorphosis of the tropical ascidian Herdmania curvata," in *The Biology of Ascidians*, eds H. Sawada, H. Yokosawa, and C. C. Lambert (Tokyo: Springer), 258–263. doi: 10.1007/978-4-431-66982-1_41

Degterev, A., and Yuan, J. (2008). Expansion and evolution of cell death programs. *Nat. Rev. Mol. Cell Biol.* 9, 378–290. doi: 10.1038/nrm2393

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149, 1060–1072. doi: 10.1016/j.cell.2012.03.042

Doherty, J., and Baehrecke, E. H. (2018). Life, death and autophagy. *Nat. Cell Biol.* 20, 1110–1117.

Dondelinger, Y., Hulpiau, P., Saeys, Y., Bertrand, M. J. M., and Vandenabeele, P. (2016). An evolutionary perspective on the necroptotic pathway. *Trends Cell Biol.* 26, 721–732. doi: 10.1016/j.tcb.2016.06.004

Du, C., Fang, M., Li, Y., Li, L., and Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* 102, 33–42. doi: 10.1016/s0092-8674(00)00008-8

Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35, 495–516.

Emlet, R. B. (2010). Morphological evolution of newly metamorphosed sea urchins—a phylogenetic and functional analysis. *Integr. Comp. Biol.* 50, 571–588. doi: 10.1093/icb/icq073

Eroglu, M., and Derry, W. B. (2016). Your neighbours matter – non-autonomous control of apoptosis in development and disease. *Cell Death Differ.* 23, 1110–1118. doi: 10.1038/cdd.2016.41

Fadl, A. E. A., Mahfouz, M. E., El-Gamal, M. M. T., and Heyland, A. (2017). New biomarkers of post-settlement growth in the sea urchin *Strongylocentrotus purpuratus*. *Heliyon* 3:e00412. doi: 10.1016/j.heliyon.2017.e00412

Fadl, A. E. A., Mahfouz, M. E., El-Gamal, M. M. T., and Heyland, A. (2019). Onset of feeding in juvenile sea urchins and its relation to nutrient signalling. *Invertebr. Reprod. Dev.* 63, 11–22. doi: 10.1080/07924259.2018.1513873

Faunes, F., and Larraín, J. (2016). Conservation in the involvement of heterochronic genes and hormones during developmental transitions. *Dev. Biol.* 416, 3–17. doi: 10.1016/j.ydbio.2016.06.013

Fuchs, Y., and Steller, H. (2011). Programmed cell death in animal development and disease. *Cell* 147, 742–758. doi: 10.1016/j.cell.2011.10.033

Fulda, S., and Debatin, K. M. (2006). Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene* 25, 4798–4811. doi: 10.1038/sj.onc.1209608

Galasso, C., D'Aniello, S., Sansone, A., and Romano, G. (2019). Identification of cell death genes in sea urchin *Paracentrotus lividus* and their expression patterns during embryonic development. *Genome Biol. Evol.* 11, 586–596. doi: 10.1093/gbe/evz020

Gao, Z., Tian, Y., Wang, J., Yin, Q., Wu, H., Li, Y. M., et al. (2007). A dimeric Smac/diablo peptide directly relieves caspase-3 inhibition by XIAP. dynamic and cooperative regulation of XIAP by Smac/Diablo. *J. Biol. Chem.* 282, 30718–30727. doi: 10.1074/jbc.m705258200

Gifondorwa, D. J., and Leise, E. M. (2006). Programmed cell death in the apical ganglion during larval metamorphosis of the marine mollusc *Ilyanassa obsolete*. *Biol. Bull.* 210, 109–120. doi: 10.2307/4134600

Green, D. R., and Fitzgerald, P. (2016). Just so stories about the evolution of apoptosis. *Curr. Biol.* 26, R620–R627.

Gurudutta, G. U., Verma, Y. K., Singh, V. K., Gupta, P., Raj, H. G., Sharma, R. K., et al. (2005). Structural conservation of residues in BH1 and BH2 domains of Bcl-2 family proteins. *FEBS. Lett.* 579, 3503–3507. doi: 10.1016/j.febslet.2005.05.015

Götz, R., Wiese, S., Takayama, S., Camarero, G., Rossol, W., Schweizer, U., et al. (2005). Bag1 is essential for differentiation and survival of hematopoietic and neuronal cells. *Nat. Neurosci.* 8, 1169–1178. doi: 10.1038/nn1524

Hadfield, M. G. (2000). Why and how marine-invertebrate larvae metamorphose so fast. *Semin. Cell Dev. Biol.* 11, 437–443. doi: 10.1006/scdb.2000.0197

Hadfield, M. G. (2011). Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annu. Rev. Mar. Sci.* 3, 453–470. doi: 10.1146/annurev-marine-120709-142753

Hadfield, M. G., Freckelton, M. L., and Nedved, B. T. (2021). The natural sequence of events in larval settlement and metamorphosis of *Hydroides elegans* (Polychaeta; Serpulidae). *PLoS One* 16:e0249692. doi: 10.1371/journal.pone.0249692

Hanson, B. (2016). Necroptosis: a new way of dying? *Cancer Biol. Ther.* 17, 899–910. doi: 10.1080/15384047.2016.1210732

Hegde, R., Srinivasula, S. M., Ahmad, M., Fernandes-Alnemri, T., and Alnemri, E. S. (1998). Blk, a BH3-containing mouse protein that interacts with Bcl-2 and Bcl-xL, is a potent death agonist. *J. Biol. Chem.* 273, 7783–7786. doi: 10.1074/jbc.273.14.7783

Heyland, A., and Hodin, J. (2004). Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of nonfeeding development. *Evolution* 58, 524–538. doi: 10.1554/03-243

Heyland, A., and Hodin, J. (2014). A detailed staging scheme for late larval development in *Stronglyocentrotus purpuratus* focused on readily-visible juvenile structures within the rudiment. *BMC Dev. Biol.* 14:22. doi: 10.1186/1471-213x-14-22

Heyland, A., and Moroz, L. L. (2006). Signaling mechanisms underlying metamorphic transitions in animals. *Integr. Comp. Biol.* 46, 743–759. doi: 10.1093/icb/icl023

Heyland, A., Price, D., Bodnarova-Buganova, M., and Moroz, L. (2006a). Thyroid hormone metabolism and peroxidase function in two non-chordate animals. *J. Exp. Zool.* 306B, 551–566. doi: 10.1002/jez.b.21113

Heyland, A., Reitzel, A. M., and Hodin, J. (2004). Thyroid hormones determine developmental mode in sand dollars (Echinodermata: Echinoidea). *Evol. Dev.* 6, 382–392. doi: 10.1111/j.1525-142x.2004.04047.x

Heyland, A., Reitzel, A. M., Price, D. A., and Moroz, L. L. (2006b). Endogenous thyroid hormone synthesis in facultative planktotrophic larvae of the sand dollar *Clypeaster rosaceus*: implications for the evolutionary loss of larval feeding. *Evol. Dev.* 8, 568–579. doi: 10.1111/j.1525-142x.2006.00128.x

Heyland, A., Schuh, N., and Rast, J. (2018). Sea urchin larvae as a model for postembryonic development. *Mar. Organ. Model Syst. Biol. Med.* 65, 137–161. doi: 10.1007/978-3-319-92486-1_8

Heyland, A., Vue, Z., Voolstra, C. R., Medina, M., and Moroz, L. L. (2011). Developmental transcriptome of *Aplysia californica*. *J. Exp. Zool. B Mol. Dev. Evol.* 316B, 113–134. doi: 10.1002/jez.b.21383

Hodin, J. (2006). Expanding networks: signaling components in and a hypothesis for the evolution of metamorphosis. *Integr. Comp. Biol.* 46, 719–742. doi: 10.1093/icb/icl038

Hodin, J., Lutek, K., and Heyland, A. (2016). A newly identified left-right asymmetry in larval sea urchins. *R. Soc. Open. Sci.* 3:160139. doi: 10.1098/rsos.160139

Holze, C., Michaudel, C., Mackowiak, C., Haas, D. A., Benda, C., Hubel, P., et al. (2018). Oxeiptosis, a ROS-induced caspase-independent apoptosis-like cell-death pathway. *Nat. Immunol.* 19, 130–140. doi: 10.1038/s41590-017-0013-y

Huber, K. L., Serrano, B. P., and Hardy, J. A. (2018). Caspase-9 CARD : core domain interactions require a properly formed active site. *Biochem. J.* 475, 1177–1196. doi: 10.1042/bcj20170913

Ishizuya-Oka, A., Hasebe, T., and Shi, Y. B. (2010). Apoptosis in amphibian organs during metamorphosis. *Apoptosis* 15, 350–364. doi: 10.1007/s10495-009-0422-y

Kalkavan, H., and Green, D. R. (2018). MOMP, cell suicide as a BCL-2 family business. *Cell Death Differ.* 25, 46–55. doi: 10.1038/cdd.2017.179

Karaiskou, A., Swalla, B. J., Sasakura, Y., and Chambon, J. P. (2015). Metamorphosis in solitary ascidians. *Genesis* 53, 34–47. doi: 10.1002/dvg.22824

Kawamoto, Y., Nakajima, Y. I., and Kuranaga, E. (2016). Apoptosis in cellular society: communication between apoptotic cells and their neighbors. *Int. J. Mol. Sci.* 17:2144. doi: 10.3390/ijms17122144

Kerr, J. F., Wyllie, A. H., and Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26, 239–257. doi: 10.1038/bjc.1972.33

Kiss, T. (2010). Apoptosis and its functional significance in molluscs. *Apoptosis* 15, 313–321. doi: 10.1007/s10495-009-0446-3

Krasovec, G., Pottin, K., Rosello, M., Quéinnec, É, and Chambon, J. P. (2021). Apoptosis and cell proliferation during metamorphosis of the planula larva of *Clytia hemisphaerica* (Hydrozoa, Cnidaria). *Dev. Dyn.* doi: 10.1002/dvdy.376 Online ahead of print.

Leise, E. M., Kempf, S. C., Durham, N. R., and Gifondorwa, D. J. (2004). Induction of metamorphosis in the marine gastropod Ilyanassa Obsoleta: 5HT, NO and programmed cell death. *Acta Biol. Hung.* 55, 293–300. doi: 10.1556/abiol.55.2004.1-4.35

Lespinet, O., Wolf, Y. I., Koonin, E. V., and Aravind, L. (2002). The role of lineage-specific gene family expansion in the evolution of eukaryotes. *Genome Res.* 12, 1048–1059. doi: 10.1101/gr.174302

Lesser, M. P., Kruse, V. A., and Barry, T. M. (2003). Exposure to ultraviolet radiation causes apoptosis in developing sea urchin embryos. *J. Exp. Biol.* 206, 4097–4103. doi: 10.1242/jeb.00621

Lutek, K., Dhaliwal, R. S., Van Raay, T. J., and Heyland, A. (2018). Sea urchin histamine 1 regulates programmed cell death in larval *Stronglyocentrotus purpuratus*. *Sci. Rep.* 8, 4002–4014.

Mané-Padrós, D., Cruz, J., Vilaplana, L., Nieva, C., Josefa Cruz, Ureña, E., et al. (2010). The hormonal pathway controlling cell death during metamorphosis in a hemimetabolous insect. *Dev. Biol.* 346, 150–160. doi: 10.1016/j.ydbio.2010.07.012

Miller, A. E. M., and Heyland, A. (2009). Endocrine interactions between plants and animals: implications of exogenous hormone sources for the evolution of hormone signaling. *Gen. Comp. Endocrinol.* 166, 455–461. doi: 10.1016/j.ygcen.2009.09.016

Miller, A. E. M., and Heyland, A. (2013). Iodine accumulation in sea urchin larvae is dependent on peroxide. *J. Exp. Biol.* 216, 915–926.

Moroy, G., Martin, E., Dejaegere, A., and Stote, R. H. (2009). Molecular basis for Bcl-2 homology 3 domain recognition in the Bcl-2 protein family: identification of conserved hot spot interactions. *J. Biol. Chem.* 284, 17499–17511. doi: 10.1074/jbc.m805542200

Nakajima, K., Fujimoto, K., and Yaoita, Y. (2005). Programmed cell death during amphibian metamorphosis. *Semin. Cell Dev. Biol.* 16, 271–280. doi: 10.1016/j.semcdb.2004.12.006

Nakayama-Ishimura, A., Chambon, J. P., Horie, T., Satoh, N., and Sasakura, Y. (2009). Delineating metamorphic pathways in the ascidian *Ciona intestinalis*. *Dev. Biol.* 326, 357–367. doi: 10.1016/j.ydbio.2008.11.026

Ninov, N., and Martín-Blanco, E. (2009). Changing gears in the cell cycle: histoblasts and beyond. *Fly* 3, 286–289. doi: 10.4161/fly.10443

Obexer, P., and Ausserlechner, M. J. (2014). X-linked inhibitor of apoptosis protein – a critical death resistance regulator and therapeutic target for personalized cancer therapy. *Front. Oncol.* 4:197.

Oliver, F. J., de la Rubia, G., Rolli, V., Ruiz-Ruiz, M. C., de Murcia, G., and Me'nissier-de Murcia, J. (1998). Importance of Poly(ADP-ribose) Polymerase and its cleavage in apoptosis: lesson from an uncleavable mutant. *J. Biol. Chem.* 50, 33533–33539.

Parasathy, R., and Palli, S. R. (2008). Proliferation and differentiation of intestinal stem cells during metamorphosis of the Red Flour Beetle, *Tribolium castaneum*. *Dev. Dyn.* 237, 893–908. doi: 10.1002/dvdy.21475

Paris, M., Escriva, H., Schubert, M., Brunet, F., Brtko, J., Ciesielski, F., et al. (2008). Amphioxus postembryonic development reveals the homology of chordate metamorphosis. *Curr. Biol.* 18, 825–830. doi: 10.1016/j.cub.2008.04.078

Paris, M., Hillenweck, A., Bertrand, S., Delous, G., Escriva, H., Zalko, D., et al. (2010). Active metabolism of thyroid hormone during metamorphosis of amphioxus. *Integr. Comp. Biol.* 50, 63–74. doi: 10.1093/icb/icq052

Robertson, A. J., Croce, J., Carbonneau, S., Voronina, E., Miranda, E., McClay, D. R., et al. (2006). The genomic underpinnings of apoptosis in *Strongylocentrotus purpuratus*. *Dev. Biol.* 300, 321–334. doi: 10.1016/j.ydbio.2006.08.053

Roccheri, M. C., Tipa, C. T., Bonaventura, R., and Matranga, V. (2002). Physiological and induced apoptosis in sea urchin larvae undergoing metamorphosis. *Int. J. Dev. Biol.* 46, 801–806.

Rodriguez, S. R., Ojeda, F. P., and Inestrosa, N. C. (1993). Settlement of benthic marine invertebrates. *Mar. Ecol. Prog. Ser.* 97, 193–207. doi: 10.3354/meps097193

Romero, A., Estevez-Calvar, N., Dios, S., Figueras, A., Novoa, B., and Bergmann, A. (2011). New insights into the apoptotic process in mollusks: characterization of caspase genes in *Mytilus galloprovincialis*. *PLoS. One* 6:e17003. doi: 10.1371/journal.pone.0017003

Romero, A., Novoa, B., and Figueras, A. (2015). The complexity of apoptotic cell death in mollusks: an update. *Fish Shellfish Immunol.* 46, 79–87. doi: 10.1016/j.fsi.2015.03.038

Sachs, L. M., and Buccholz, D. R. (2019). Insufficiency of Thyroid Hormone in frog metamorphosis and the role of glucocorticoids. *Front. Endocrinol.* 10:287.

Sato, Y., Kaneko, H., Negishi, S., and Yazaki, I. (2006). Larval arm resorption proceeds concomitantly with programmed cell death during metamorphosis of the sea urchin *Hemicentrotus pulcherrimus*. *Cell Tissue Res.* 326, 851–860. doi: 10.1007/s00441-006-0212-6

Seipp, S., Schmich, J., Will, B., Schetter, E., Plickert, G., and Leitz, T. (2010). Neuronal cell death during metamorphosis of *Hydractina echinata* (Cnidaria, Hydrozoa). *Invert. Neurosci.* 10, 77–91. doi: 10.1007/s10158-010-0109-7

Shiozaki, E. N., Jijie, C., Rigotti, D. J., Riedl, S. J., Li, P., Srinivasula, S. M., et al. (2003). Mechanism of XIAP-mediated inhibition of caspase-9. *Mol. Cell* 11, 519–517. doi: 10.1016/s1097-2765(03)00054-6

Sun, G., Fu, L., and Shi, Y. B. (2014). Epigenetic regulation of thyroid hormone-induced adult intestinal stem cell development during anuran metamorphosis. *Cell Biosci.* 4:73. doi: 10.1186/2045-3701-4-73

Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K., and Takahashi, R. (2001). A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol. Cell* 8, 613–621. doi: 10.1016/s1097-2765(01)00341-0

Tata, J. R. (1993). Gene expression during metamorphosis: an ideal model for post-embryonic development. *Bioessays* 15, 239–248. doi: 10.1002/bies.950150404

Taylor, E., and Heyland, A. (2018). Thyroid hormones accelerate initiation of skeletogenesis via MAPK (ERK1/2) in larval sea urchins (*Stronglyocentrotus purpuratus*). *Front. Endocrinol.* 9, 439–439. doi: 10.3389/fendo.2018.00439

Tettamanti, G., and Casartelli, M. (2019). Cell death during complete metamorphosis. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 374:1783.

Thet, M. M., Noguchi, M., and Yazaki, I. (2004). Larval and juvenile development of the echinometrid sea urchin Colobocentrotus mertensii: emergence of the peculiar form of spines. *Zool. Sci.* 21, 265–274. doi: 10.2108/zsj.21.265

Thurber, R. V., and Epel, D. (2007). Apoptosis in early development of the sea urchin, *Strongylocentrotus purpuratus*. *Dev. Biol.* 303, 336–346. doi: 10.1016/j.ydbio.2006.11.018

van Bergeijk, S. A., Hernández Javier, L., Heyland, A., Manchado, N., and Pedro Cañavate, J. (2013). Uptake of iodide in the marine haptophyte Isochrysis sp. (T.ISO) driven by iodide oxidation. *J. Phycol.* 49, 640–647. doi: 10.1111/jpy.12073

Verhagen, A. M., Silke, J., Ekert, P. G., Moritz, R. L., Simpson, R. J., and Vaux, D. (2002). HtrA2 promotes cell death through Its Serine Protease activity and its ability to antagonize inhibitor of apoptosis proteins. *J. Biol. Chem.* 277, 445–454. doi: 10.1074/jbc.m109891200

Vogeler, S., Carboni, A., Li, X., and Joyce, A. (2021). Phylogenetic analysis of the caspase family in bivalves: implications for programmed cell death, immune response and development. *BMC Genom.* 22:80.

Voronina, E., and Wessel, G. M. (2001). Apoptosis in sea urchin oocytes, eggs, and early embryos. *Mol. Reprod. Dev.* 60, 553–561. doi: 10.1002/mrd.1120

Wang, L., Qiao, Q., and Wu, H. (2017). Understanding CARD tricks in apoptosomes. *Structure* 25, 575–577. doi: 10.1016/j.str.2017.03.013

Weill, M., Philips, A., Chourrout, D., and Fort, P. (2005). The caspase family in urochordates: distinct evolutionary fates in ascidians and larvaceans. *Biol. Cell* 97, 857–866. doi: 10.1042/bc20050018

Williams, S. T., Smith, A. N., Cianci, C. D., Morrow, J. S., and Brown, T. L. (2003). Identification of the primary caspase 3 cleavage site in alpha II-spectrin during apoptosis. *Apoptosis* 8, 353–361.

Wong, E., Mölter, J., Anggono, V., Degnan, S. M., and Degnan, B. M. (2019). Co-expression of synaptic genes in the sponge *Amphimedon queenslandica* uncovers ancient neural submodules. *Sci. Rep.* 10:15781.

Wray, G. A., and Raff, R. A. (1990). Origins of lineage founder cells in the direct-developing sea urchin *Heliocidaris erythrogamma*. *Dev. Biol.* 141, 41–54. doi: 10.1016/0012-1606(90)90100-w

Wynen, H., and Heyland, A. (2021). *Thyroid hormones induce programmed cell death in sea urchin (Stronglyocentrotus purpuratus) larval arms prior to metamorphosis.* (Manuscript in preparation).

Yui, D., Yoneda, T., Oono, K., Katayama, T., Imaizumi, K., and Tohyama, M. (2001). Interchangeable binding of Bcl10 to TRAF2 and cIAPs regulates apoptosis signaling. *Oncogene* 20, 4317–4323. doi: 10.1038/sj.onc.1204576

Zmasek, C. M., and Godzik, A. (2013). Evolution of the animal apoptosis network. *Cold Spring Harb. Perspect. Biol.* 5:a008649. doi: 10.1101/cshperspect.a008649

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

REVIEW
published: 23 September 2021
doi: 10.3389/fevo.2021.715029

Neuroendocrine Regulation of Reproductive Dormancy in the Fruit Fly *Drosophila melanogaster*: A Review of Juvenile Hormone-Dependent Regulation

Yoshitomo Kurogi[1], Yosuke Mizuno[1], Eisuke Imura[2†] and Ryusuke Niwa[2]*

[1] Degree Programs in Life and Earth Sciences, Graduate School of Science and Technology, University of Tsukuba, Tsukuba, Japan, [2] Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance, University of Tsukuba, Tsukuba, Japan

OPEN ACCESS

Edited by:
Takashi Koyama, University of Copenhagen, Denmark

Reviewed by:
Gabriele Andreatta, Max F. Perutz Laboratories GmbH, Austria
Manolis Lyrakis, University of Veterinary Medicine Vienna, Austria

***Correspondence:**
Ryusuke Niwa
ryusuke-niwa@tara.tsukuba.ac.jp

†Present address:
Eisuke Imura, Department of Entomology, Institute for Integrative Genome Biology, University of California, Riverside, Riverside, CA, United States

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *26 May 2021*
Accepted: *30 August 2021*
Published: *23 September 2021*

Citation:
Kurogi Y, Mizuno Y, Imura E and Niwa R (2021) Neuroendocrine Regulation of Reproductive Dormancy in the Fruit Fly Drosophila melanogaster: A Review of Juvenile Hormone-Dependent Regulation. Front. Ecol. Evol. 9:715029. doi: 10.3389/fevo.2021.715029

Animals can adjust their physiology, helping them survive and reproduce under a wide range of environmental conditions. One of the strategies to endure unfavorable environmental conditions such as low temperature and limited food supplies is dormancy. In some insect species, this may manifest as reproductive dormancy, which causes their reproductive organs to be severely depleted under conditions unsuitable for reproduction. Reproductive dormancy in insects is induced by a reduction in juvenile hormones synthesized in the *corpus allatum* (pl. *corpora allata*; CA) in response to winter-specific environmental cues, such as low temperatures and short-day length. In recent years, significant progress has been made in the study of dormancy-inducing conditions dependent on CA control mechanisms in *Drosophila melanogaster*. This review summarizes dormancy control mechanisms in *D. melanogaster* and discusses the implications for future studies of insect dormancy, particularly focusing on juvenile hormone-dependent regulation.

Keywords: reproductive dormancy, *corpus allatum (corpora allata)*, juvenile hormone (JH), monoamine, insulin-like peptides (ILPs), ecdysone, ecdysis-triggering hormone, circadian clock

INTRODUCTION

In animals, neuroendocrine systems play a crucial role in facilitating adaptation to a wide variety of environments (Schmidt-Nielsen, 1997). In the temperate zone, winter is a challenging season for many animals because of the freezing temperatures and food shortages, which are sustained over several months. Hence, in winter, animals often suspend or slow down their normal physical functions, a process known as dormancy (Hand et al., 2016). To date, dormancy is recognized as an adaptive phenomenon in a wide variety of animal species (Hand et al., 2016).

Insects are one of the main animal groups that have been intensively used in dormancy studies. This is because control of insect dormancy could benefit many aspects of industry and agriculture. For example, since the silkworm *Bombyx mori* possesses an egg dormancy stage, a technology to flexibly regulate the induction and end of egg dormancy is useful to enable sericulture throughout the year (Yamashita and Yaginuma, 1991). In addition, pest dormancy studies are thought to

be important because understanding the seasonal distribution of pests is critical for generating predictive models to accurately determine when pests are destructive (Denlinger, 2008). Moreover, there have been attempts to select specific dormancy phenotypes in biocontrol agents, which cannot become dormant in a season when crop pest populations continue to grow (Lirakis and Magalhães, 2019). In this regard, it is noteworthy that a branch of the United Nations promoted a coordinated research project on dormancy management to enable mass-rearing and increase the efficacy of sterile insects and natural enemies from 2014 to 2019 (Food and Agriculture Organization of the United Nations, 2013).

Previous studies have revealed that the developmental stages of dormancy differ between insect species (Tauber et al., 1986; Danks, 1987, 2006; Schiesari and O'Connor, 2013). For example, as described above, *B. mori* enters dormancy only in the egg stage (Yamashita and Yaginuma, 1991). However, in other species, dormancy is not restricted to the egg but can occur at other stages, including the adult life. Adult dormancy results in many metabolic and behavioral changes, such as the slow-down of reproduction, decreased food ingestion, suppressed metabolism, increased stress resistance, and extended adult lifespan (Danks, 1987; Tatar and Yin, 2001; Hahn and Denlinger, 2011; Kubrak et al., 2014). Among the multiple changes, reproductive slow-down is thought to be a key event of adult dormancy, known as reproductive dormancy. Since adult insects allocate a considerable amount of energy to gametogenesis, reproductive dormancy allows insects to reduce their energy consumption and produce offspring again in the spring when winter is over. Reproductive dormancy is found in both male and female adults; In particular, females exhibit a drastic suppression of gametogenesis (oogenesis) by inhibiting vitellogenesis, accessory gland activity, and mating behavior (Saunders et al., 1989; Pener, 1992; Kubrak et al., 2016).

Previous studies have shown that reproductive dormancy and other types of dormancy are regulated by a complex interplay of multiple hormones and neurotransmitters in insects (Denlinger, 2002; Emerson et al., 2009a; Denlinger et al., 2012). In particular, several major hormones influence reproductive dormancy, including juvenile hormones (JHs), insulin-like peptides, and ecdysteroids (Denlinger, 2002; Emerson et al., 2009a; Denlinger et al., 2012). All of three classes of hormones are known to have a stimulatory effect on ovarian development, and a decrease in these signals in dormant individuals suppresses ovarian development (Denlinger, 2002; Emerson et al., 2009a; Denlinger et al., 2012; Uryu et al., 2015; Lenaerts et al., 2019; Santos et al., 2019; Swevers, 2019; Semaniuk et al., 2021). Among these three, JHs, the arthropod-specific sesquiterpenoid hormones, have been the most intensively studied in the long history of entomology to unravel their vital role in regulating reproductive dormancy in female adults. Since JHs are essential for promoting vitellogenesis in normal (non-dormancy-inducing) conditions, the reduction in JH levels is conversely required for suppressing vitellogenesis, leading to reproductive dormancy in females (Denlinger et al., 2012; Santos et al., 2019). This mechanism was well demonstrated in a study using the Colorado potato beetle *Leptinotarsa decemlineata* (de Wilde and de Boer, 1961, 1969; Schooneveld et al., 1977; Kort, 1990), which has frequently been used for studies on reproductive dormancy. After hatching under short-day condition, the adults burrow into the soil and enter dormancy. When the endocrine organ known as the *corpus allatum* (*pl. corpora allata*; CA), responsible for biosynthesizing JHs, is dissected out from a non-dormant beetle and then transplanted to a dormant beetle, the dormant status is released from the beetle receiving the transplant. Such release of dormancy is also observed when JH is applied to a dormant beetle. In contrast, when the CA of a non-dormant beetle is surgically ablated, the ovarian development is inhibited even under long-day (non-dormancy-inducing) conditions. These results indicate that the reduced concentration of JH, which is biosynthesized in the CA, causes reproductive dormancy in *L. decemlineata* females. After this discovery, the importance of JH reduction to induce female reproductive dormancy has been confirmed in many insect species (Denlinger et al., 2012; Hand et al., 2016). Therefore, there must be essential, common mechanisms by which information about dormancy-inducing environmental cues, such as low temperature and short-day condition, are transmitted to the CA to control JH biosynthesis or release. However, the mechanisms at the molecular, cellular, and neuroendocrine levels have not yet been fully elucidated.

Among several insects used in laboratories, the fruit fly *Drosophila melanogaster* has contributed substantially to the discovery of various biological phenomena based on powerful genetic tools such as the GAL4-UAS system (Brand and Perrimon, 1993; Neckameyer and Argue, 2013). In the last decade, significant progress has been made in the study of reproductive dormancy in females of *D. melanogaster*. Specifically, there have been several recent studies to address how dormancy-inducing stimuli are transmitted to the CA to regulate reproductive dormancy through the reduction of JH titer. Importantly, a part of *D. melanogaster* research is beginning to contribute to understanding the molecular mechanisms of reproductive dormancy in other insects, as we describe below. This review summarizes current knowledge about the mechanisms of reproductive dormancy in *D. melanogaster* females and discusses the remaining questions of the *D. melanogaster* dormancy, particularly focusing on juvenile hormone-dependent regulation.

AN OVERVIEW OF *DROSOPHILA MELANOGASTER* DORMANCY

First, we would like to define our usage of the term "dormancy." In general, dormancy can be broadly classified into two types, diapause and quiescence. Diapause is induced by seasonal cues that do not directly prevent development but foreshow the arrival of unfavorable conditions. On the other hand, quiescence is characterized by slowed metabolism and directly results from unfavorable environmental conditions such as low temperature (Koštál, 2006; Schiesari et al., 2011; Schiesari and O'Connor, 2013). There is still a debate about whether reproductive dormancy in *D. melanogaster* should be classified as diapause

or quiescence (Tatar et al., 2001; Koštál, 2006; Lirakis et al., 2018). However, it seems obvious that reproductive dormancy in *D. melanogaster* is influenced by both short-day conditions and low temperature (Schiesari et al., 2016; Zonato et al., 2017; **Figure 1**). This fact implies that reproductive dormancy in *D. melanogaster* has both quiescence and diapause properties. Therefore, most papers published in recent years simply use "dormancy" to describe the short-day and low temperature-induced changes in *D. melanogaster* adults (Kubrak et al., 2016; Liu et al., 2016; Andreatta et al., 2018; Lirakis et al., 2018; Ojima et al., 2018; Nagy et al., 2019; Abrieux et al., 2020). In this paper, as is customary, we will refer to the *D. melanogaster* phenotypes in terms of dormancy.

In laboratories, *D. melanogaster* dormancy is commonly induced by transferring virgin females into dormancy-inducing conditions shortly after (usually 2–6 h) eclosion under non-dormancy-inducing conditions. Such newly eclosed female flies are supposed to have previtellogenic or even few early vitellogenic egg chambers but not mature eggs. When the newly eclosed females are maintained in dormancy-inducing conditions, the flies continuously keep the immature ovaries even 2 or 3 weeks after eclosion (Zonato et al., 2017; Lirakis et al., 2018; **Figure 1**). However, since reproductive dormancy is a "slow-down" and not a complete arrest, in ovarian development, the percentage of non-dormant flies increases about 4 weeks after hatching (Schiesari et al., 2016; Zonato et al., 2017; Lirakis et al., 2018). Meanwhile, rearing flies under conditions of temperature and day length that mimic winter can prolong the slow-down of oogenesis and maintain a higher reproductive dormancy rate than simple dormancy-inducing conditions even 3 months after eclosion (Zonato et al., 2017). It is still unclear why such environmental fluctuation is important to extend reproductive dormancy.

Under dormancy-inducing conditions in laboratories, *D. melanogaster* adults display some physiological changes, such as reduced metabolism (Saunders et al., 1989; Saunders and Gilbert, 1990; Tatar and Yin, 2001; Tatar et al., 2001; Kubrak et al., 2014; Anduaga et al., 2018). Previous studies have shown that absolute amounts of trehalose and glucose positively correlate with the dormancy rate (Kubrak et al., 2014; Anduaga et al., 2018). In addition, trehalose/glycogen and trehalose/glucose ratios also positively correlate with the dormancy rate (Watanabe et al., 2002; Kubrak et al., 2014; Anduaga et al., 2018). These observations raise a hypothesis that the biosynthesis of trehalose, an energy source and an anti-freezing material, is activated in dormant flies (Crowe, 2007; MacRae, 2010; Hahn and Denlinger,

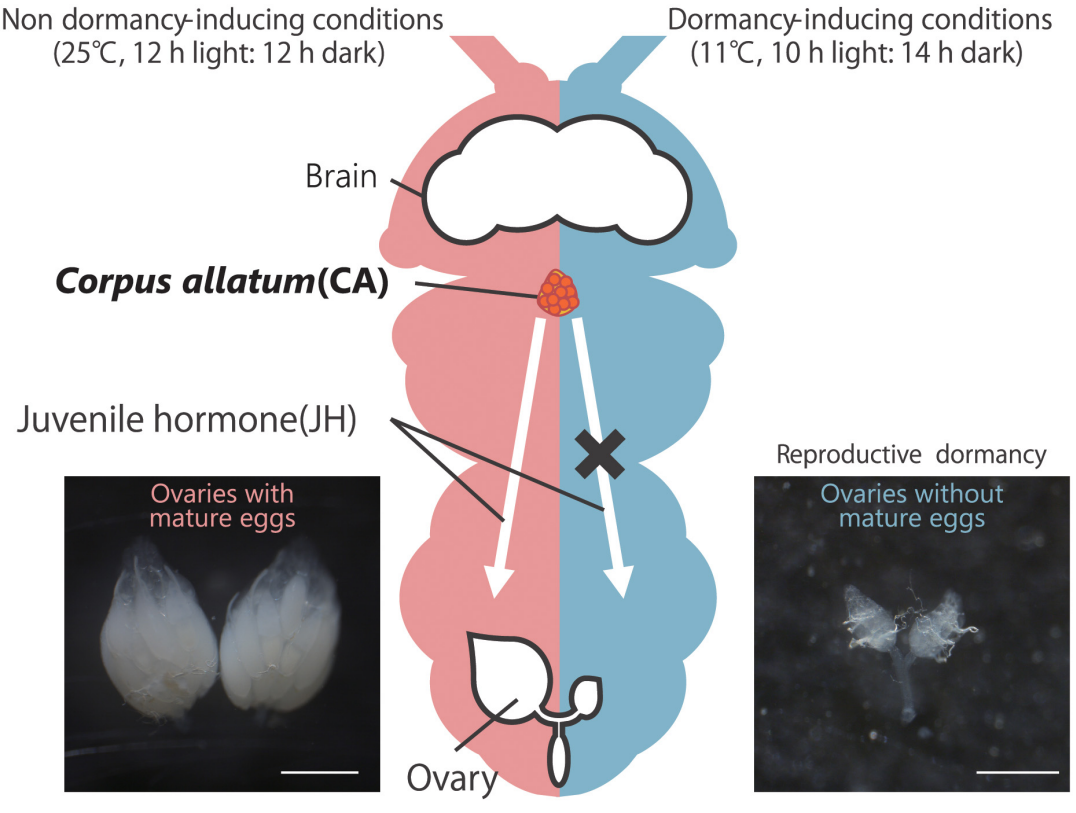


FIGURE 1 | Reproductive dormancy in *Drosophila melanogaster* adult females. Under non-dormancy-inducing conditions (25°C, 12 h light:12 h dark; left), approximately 20–60 mature eggs, in which yolk proteins are well accumulated, are observed in the female abdomen. On the other hand, under dormancy-inducing conditions (11°C, 10 h light:14 h dark; right), oogenesis is severely suppressed, and mature eggs with yolk protein are rarely observed. In non-dormant flies, yolk protein accumulation is positively controlled by juvenile hormone (JH) produced in and secreted from the *corpus allatum* (CA, orange). Conversely, in dormant flies, JH biosynthesis in the CA is suppressed, leading to the slow-down of oogenesis. Scale bars, 0.5 mm.

2011). On the other hand, protein amount does not correlates with dormancy rate (Kubrak et al., 2014; Anduaga et al., 2018). Dormancy-associated physiological changes also include an increase in stress tolerance, an extended lifespan, and the activation of the innate immune system in both male and female fly adults (Saunders et al., 1989; Saunders and Gilbert, 1990; Tatar et al., 2001; Tatar and Yin, 2001; Kubrak et al., 2014; Kuèerová et al., 2016; Anduaga et al., 2018). In addition, in *D. melanogaster* dormant females, the intestine is markedly shortened (Kubrak et al., 2014). Some of these physiological changes are also observed in other insects when they enter dormancy (Tatar and Yin, 2001; Denlinger, 2002; Hahn and Denlinger, 2007, 2011; Sim and Denlinger, 2013).

Along with the changes in metabolic state, suppression of vitellogenesis in oogenesis is another major aspect of dormancy (**Figure 2**); hence this process is called reproductive dormancy. Among the 14 distinct stages of *D. melanogaster* oogenesis (King, 1970; Bastock and St Johnston, 2008), vitellogenesis occurs from stage 8 (Bownes, 1994; Soller et al., 1997). Therefore, oocytes before and at stage 7 are defined as previtellogenic oocytes, while those after and at stage 8 are defined as vitellogenic oocytes. Most studies classify flies as dormant if their ovaries have only previtellogenic egg chambers, while flies with ovaries containing vitellogenic egg chambers are characterized as non-dormant (Saunders et al., 1989, 1990; Saunders, 1990; Saunders and Gilbert, 1990; Williams, 1993; Richard et al., 1998; Tatar et al., 2001; Schmidt and Conde, 2006; Williams et al., 2006; Schmidt and Paaby, 2008; Schmidt et al., 2008; Emerson et al., 2009b; Lee et al., 2011; Fabian et al., 2015; Schiesari et al., 2016; Zhao et al., 2016; Zonato et al., 2017; Andreatta et al., 2018; Ojima et al., 2018; Nagy et al., 2019; **Figure 1**). However, there is still an ongoing debate about whether this commonly used classification is truly valid, as discussed in several papers (Tatar et al., 2001; Lee et al., 2011; Lirakis et al., 2018; Erickson et al., 2020). These papers claim that oogenesis in dormancy-inducing conditions is not arrested at provitellogenic stages but rather at stage 9 corresponding to early vitellogenesis. In fact, stage 9 oocytes are largely different from stage 10 and later oocytes from the following perspectives. First, stage 10 oocytes massively grow and are drastically enlarged compared to stage 9 oocytes (He et al., 2011). Second, stage 9 oocytes possess a very small amount of yolk, but stage 10 oocytes accumulate it well (He et al., 2011). Third, stage 8 oocytes self-synthesize yolk proteins, while stage 10 oocytes incorporate extracellular yolk proteins synthesized in the fat body cells and released into the hemolymph (Brennan et al., 1982). Importantly, several studies have shown that dormant oocytes incorporate hemolymph yolk proteins in lesser amounts (Saunders, 1990; Saunders et al., 1990; Richard et al., 2001b). This issue is still being debated.

Previous laboratory studies have reported that, like other insects, low temperature and short-day condition influence the induction of dormancy in *D. melanogaster* adults (Saunders et al., 1989; Saunders and Gilbert, 1990; Allen, 2007; **Figure 1**). However, in contrast to the importance of short-day condition to induce dormancy in many other insect species, it is known that low temperature, but not short-day condition, is the crucial dormancy stimulus in *D. melanogaster* (Saunders and Gilbert, 1990; Zonato et al., 2017; Anduaga et al., 2018). In other words, *D. melanogaster* adults enter dormancy at temperatures less than 13°C even in long-day condition, but they never enter dormancy in short-day condition if the temperature is higher than 13°C. In *D. melanogaster*, the photoperiod appears to modulate reproductive dormancy within a 10–13°C range of permissiveness temperature (Saunders et al., 1989; Saunders and Gilbert, 1990; Schiesari et al., 2011). Curiously, a recent study has suggested that a very subtle difference in temperature, such as a 0.3°C difference, possibly has a significant impact on the induction of dormancy (Anduaga et al., 2018).

A concern about most laboratory experiments is that these previous data were obtained under artificial light on/off conditions, which is the unnatural constant light-constant dark photoperiodic cycle. However, a recent study has claimed that such artificial light on/off conditions does not reflect natural light/dark change and, thus, underestimate the effect of photoperiod on *D. melanogaster* reproductive dormancy (Nagy et al., 2018). This study revealed that a photoperiod-dependent reproductive dormancy is more clearly observed in *D. melanogaster* European strains when the animals are reared under gradual elevation and reduction of light that mimic the natural light/dark change (Nagy et al., 2018). In addition to low temperature and short-day condition, starvation is an additional factor that enhances dormancy rate (Lirakis et al., 2018; Ojima et al., 2018).

In the wild, it has been well described that there are geographic variations in reproductive dormancy in *D. melanogaster* (Williams et al., 2006; Tauber et al., 2007; Sandrelli et al., 2007; Schmidt and Paaby, 2008; Schmidt et al., 2008; Emerson et al., 2009b; Kolaczkowski et al., 2011; Lee et al., 2011; Fabian et al., 2012; Bergland et al., 2014; Kapun et al., 2016; Zhao et al., 2016; Zonato et al., 2017; Lirakis et al., 2018; Betancourt et al., 2021; Machado et al., 2021). *D. melanogaster* originated in sub-Saharan Africa and subsequently expanded its habitat, possibly in association with humans and agriculture (Flatt, 2020). The flies have colonized temperate habitats in Eurasia and, more recently, North America and Australia. Genotypes from some populations enter reproductive dormancy readily in response to low temperature and short-day conditions, whereas others have low or zero dormancy propensity (Williams, 1993; Schmidt and Paaby, 2008; Emerson et al., 2009b; Fabian et al., 2015). Seasonal high-latitude populations show much greater dormancy inducibility than flies from subtropical/tropical low-latitude populations. In addition, geographic variations in reproductive dormancy in wild *D. melanogaster* are associated with single nucleotide polymorphisms in several loci, as we discuss later, suggesting that the traits of reproductive dormancy are genetically constrained.

THE ROLE OF JUVENILE HORMONES IN *DROSOPHILA MELANOGASTER* REPRODUCTIVE DORMANCY

As in other insect species, the induction of *D. melanogaster* reproductive dormancy is associated with reduced JH levels

(**Figure 1**). A previous study using an *in vitro* assay system has shown that the dissected CA from animals under reproductive dormancy-inducing conditions exhibits reduced JH biosynthesis (Saunders et al., 1990). In contrast, administration of the JH analog methoprene can break reproductive dormancy (Tatar and Yin, 2001). Phenotypes of reproductive dormancy are also observed in female flies in which JH biosynthetic enzymes are enhanced or suppressed. For example, overexpression of a gene encoding juvenile hormone acid *O*-methyltransferase (*Jhamt*) significantly reduces the percentage of dormant flies (Shinoda and Itoyama, 2003; Niwa et al., 2008; Noriega, 2014; Ojima et al., 2018). Conversely, the CA-specific transgenic double-strand RNA interference (RNAi) of *Jhamt* increases the percentage of dormant flies (Ojima et al., 2018). Taken together, these observations are consistent with the fact that JH accelerates oogenesis in most insects, including *D. melanogaster*, under non-dormancy-inducing conditions.

The role of JH in the regulation of *D. melanogaster* reproductive dormancy has also been investigated at the level of oogenesis. It is well known that JH is essential for facilitating vitellogenesis from the oocyte stage 8 (Bownes, 1994; Soller et al., 1997). More precisely, JH promotes yolk protein production, which occurs mainly in the fat body, and also acts on the ovarian follicle cells to promote vitellogenesis by enhancing yolk protein uptake to oocytes (Postlethwait and Weiser, 1973; Bownes, 1989; Soller et al., 1997, 1999; Richard et al., 2001a; Santos et al., 2019). The suppression of yolk protein production in the dormancy-inducing condition correlates well with reproductive dormancy (Saunders et al., 1990; **Figure 2**). Experimentally, application of the JH analog to dormant females increased yolk protein production and the number of vitellogenic oocytes as compared to an experimental control group (Saunders et al., 1990; Tatar et al., 2001). These data indicate that, in *D. melanogaster* and many other insects, reproductive dormancy is due to the suppression of vitellogenesis, at least in part, by JH titer reduction.

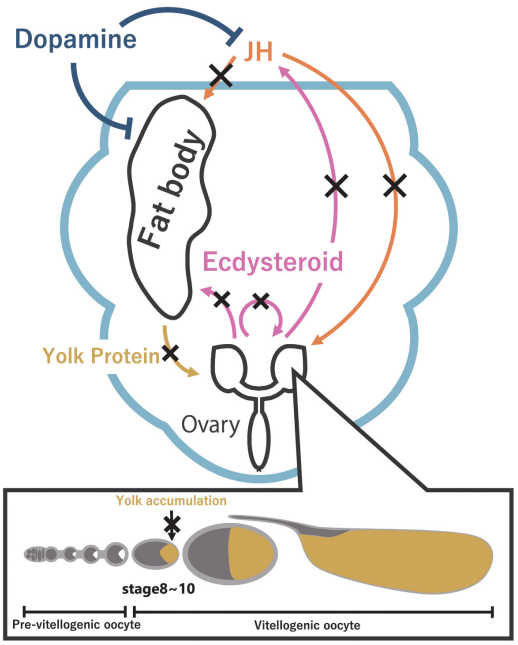


FIGURE 2 | Reproductive dormancy is regulated by JH through the production of yolk protein in the fat body. JH (orange) promotes the production of yolk protein in the fat body and the uptake of yolk protein in the ovary. On the other hand, ecdysteroids (pink) promote yolk protein production in the fat body, affect JH biosynthesis in the CA, and regulate oogenesis. Under dormancy-inducing conditions, a decrease in JH level and ecdysteroid level suppresses the yolk protein accumulation (dark yellow) in oocytes, leading to reproductive dormancy. Furthermore, dopamine (dark blue) might be involved in inducing reproductive dormancy via yolk protein production in the fat body.

NEUROENDOCRINE MECHANISMS THAT DIRECTLY OR INDIRECTLY CONTROL JUVENILE HORMONE BIOSYNTHESIS IN THE *CORPUS ALLATUM* IN *DROSOPHILA MELANOGASTER*

To understand how JH biosynthesis in the CA is suppressed when the animals are placed in dormancy-inducing conditions, it is important to unravel the mechanisms transmitting environmental cues to the CA to influence the activity of JH biosynthesis. Although the mechanisms have not yet been fully elucidated, recent studies on *D. melanogaster* have collectively argued that several neuroendocrine factors are crucial for inducing JH-mediated reproductive dormancy. Hereafter we are describing the recent knowledge of neuroendocrine regulation of reproductive dormancy in *D. melanogaster*. As we described earlier, insect reproductive dormancy is regulated not only by JHs but also many other hormones, including insulin-like peptides and ecdysteroids. Although it might be plausible to consider that insulin-like peptides and ecdysteroids have roles in reproductive dormancy independent of JHs, recent *D. melanogaster* studies have indicated that insulin-like peptides and ecdysteroids influence JH biosynthesis in the CA in multiple way. Therefore, in this review, instead of deliberately focusing on the whole picture of hormone interactions, we dare to focus on JHs and discuss the involvement of other hormones and neurotransmitters.

Mechanisms of Insulin Signaling-Mediated Reproductive Dormancy

Insulin signaling controls development, growth, and physiology by regulating nutrient metabolism and energy expenditure in animals. In *D. melanogaster*, insulin signaling has versatile functions, including reproductive dormancy (Sim and Denlinger, 2013; Kubrak et al., 2014; Liu et al., 2016; Schiesari et al., 2016; Ojima et al., 2018; Semaniuk et al., 2021). *Drosophila* insulin-like peptides (Dilps), orthologs of vertebrate insulin and insulin growth factors, are the endocrine factors activating insulin signaling in *D. melanogaster* (Semaniuk et al., 2021; **Figures 3A,B**). Their secretion is regulated by the activity of

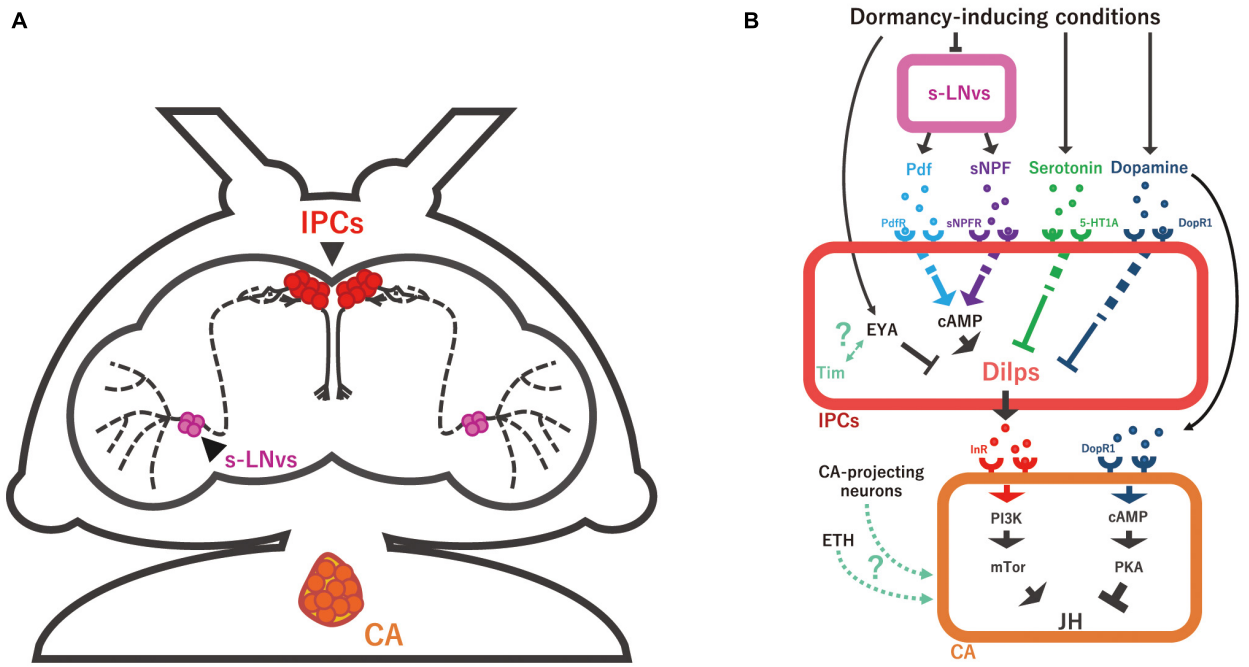


FIGURE 3 | Schematic representation of the neuroendocrine mechanisms regulating JH biosynthesis. **(A)** Anatomy of small ventral lateral neurons (s-LNv; magenta), insulin-producing cells (IPCs; red), and the *corpus allatum* (CA) in *D. melanogaster* adult female. s-LNvs are the circadian clock pacemaker neurons whose axons seem to have direct synaptic connections with IPCs. **(B)** The s-LNv-IPC-CA axis and multiple humoral factors to regulate JH biosynthesis in the CA. Under dormant conditions, the secretion of pigment-dispersing factor (Pdf) and short neuropeptide F (sNPF) from s-LNv is reduced, followed by the suppression of IPC activity and consequently that of Dilp production. Serotonin and dopamine are also involved in inducing reproductive dormancy by repressing *Dilp* expression in IPCs. It is noteworthy that, whereas it is generally considered that serotonin receptor (5-HT1A) and dopamine receptor (DopR1) positively evoke intracellular cAMP level, it is unclear how serotonin-5-HT1A and dopamine-DopR1 signaling negatively regulate *Dilp* expression, differently from Pdf-Pdf receptor (PdfR) and sNPF-sNPF receptor (sNPFR) signaling. In addition to these hormones and neurotransmitters, *Dilp* expression is intracellularly regulated by the transcriptional regulator Eye absent (Eya) and possibly the core circadian clock gene *timeless* (Tim). Dilps and Ecdysis-triggering hormone (ETH) have a function to promote JH biosynthesis in the CA. On the other hand, dopamine has a function to suppress JH production in the CA under dormancy-inducing conditions. In many insects, direct projection nerves of the CA are essential for the regulation of reproductive dormancy, while it is still unclear whether this is also the case in *D. melanogaster.*

insulin-producing cells (IPCs), which are neurons located in the pars intercerebralis (PI) of the brain. Dilps are then released from IPCs into the hemolymph (Semaniuk et al., 2021; **Figure 3A**). A previous study has demonstrated that, in flies reared at 11°C, short-day condition results in lower neuronal activity of IPCs than long-day condition (Ojima et al., 2018). Furthermore, when IPCs are artificially activated by overexpression of a transient receptor potential cation channel under dormancy-inducing conditions, the percentage of dormant flies decreases. Conversely, an artificial inhibition of IPCs by overexpressing an inward rectifier potassium channel increases the percentage of dormant flies (Venken et al., 2011; Schiesari et al., 2016; Ojima et al., 2018). These results suggest that IPCs negatively regulate reproductive dormancy. Phenotypes of reproductive dormancy are also observed in loss- and gain-of-function of *Dilps* and insulin receptor (*InR*). For example, overexpression of *Dilp2* and *Dilp5* in the IPCs suppresses the induction of dormancy (Schiesari et al., 2016). On the other hand, IPC-specific RNAi of *Dilp2*, *Dilp5* upregulates the induction of dormancy (Schiesari et al., 2016). Moreover, molecules downstream of Dilps and InR are also involved in reproductive dormancy. For example, Phosphatidylinositol-3 kinase (PI3K) influences of the rate of reproductive dormancy in the North American population of *D. melanogaster* (Williams et al., 2006). In addition, FoxO, a well-known transcription factor downstream of Dilp signaling, also plays a crucial role in inducing reproductive dormancy, as revealed by an experiment using a FoxO-mediated gene expression reporter (Schiesari et al., 2016). Taken together, the production and secretion of Dilps are greatly suppressed in dormancy-inducing conditions, resulting in reduced insulin signaling, followed by the suppression of oogenesis (**Figure 3B**).

How does the reduction of Dilp signaling suppress oogenesis? One mechanism is that reduced Dilp signaling in the CA downregulates JH biosynthesis (**Figure 3B**). For example, overexpression of constitutively active *InR* or *PI3K* in the CA significantly decreases in flies with ovarian arrest in dormancy-inducing conditions (Ojima et al., 2018). Conversely, *InR* RNAi or dominant-negative PI3K or mTOR, another downstream element of insulin signaling, increases the proportion of flies with ovarian arrest in both normal and dormancy-inducing conditions (Ojima et al., 2018). On the other hand, besides the JH-mediated action of Dilps, there are other mechanisms by which Dilps directly act on ovarian cells to regulate oogenesis or indirectly influence oogenesis through the regulation of

ecdysteroid biosynthesis (Tu et al., 2002, 2005; LaFever and Drummond-Barbosa, 2005).

The role of insulin signaling in controlling developmental arrest has also been suggested in species other than insects, such as the nematode *Caenorhabditis elegans* and the annual killifish *Austrofundulus limnaeus*. In *C. elegans*, low food levels or other unfavorable environmental conditions lead to larval developmental arrest, known as dauer (Hu, 2007). Previous studies have identified and characterized genes mutated in various mutants exhibiting dysregulation of dauer arrest, called *daf* mutants. Some of the identified genes encode insulin-like peptides, insulin receptor, and signal transduction molecules downstream of insulin receptor (Golden and Riddle, 1982, 1984; Cornils et al., 2011; Sim and Denlinger, 2013). In the annual killifish, downregulation of insulin signaling appears essential for regulating embryonic diapause (Woll and Podrabsky, 2017). Considering that JHs are arthropod-specific and neither nematodes nor vertebrates biosynthesize JHs, it would be intriguing to examine whether and how insulin signaling interacts with other hormones and other bioactive molecules to regulate developmental arrest in these animals, such as dafachronic acids in *C. elegans* (Hu, 2007; Niwa and Niwa, 2014) and vitamin D3 in the annual killifish (Romney et al., 2018).

Mechanisms of Aminergic Signaling-Mediated Reproductive Dormancy

Previous studies on pupal dormancy in butterflies and moths have shown that hemolymph levels of aminergic neurotransmitters, such as dopamine and serotonin, are elevated during dormancy (Noguchi and Hayakawa, 1997; Isabel et al., 2001; Hsu et al., 2020; **Figure 3B**). Recent studies have revealed that serotonin and dopamine are also involved in regulating reproductive dormancy in *D. melanogaster* (Andreatta et al., 2018).

In *D. melanogaster*, the dopamine level in the whole adult body significantly increases under dormancy-inducing conditions (Andreatta et al., 2018). Furthermore, the percentage of flies with reproductive dormancy is reduced in mutants of DOPA decarboxylase (Ddc), which is involved in the biosynthesis of dopamine and serotonin (Sherald and Wright, 1974; Wright et al., 1976). On the other hand, activation of the dopaminergic neurons results in an increased proportion of flies with reproductive dormancy. These results indicate that dopamine, in contrast to Dilps, positively regulates reproductive dormancy. However, among the approximately 130 *bona fide* dopaminergic neurons in the *D. melanogaster* brain (Kasture et al., 2018; Karam et al., 2020), the dopaminergic neuron(s) important for inducing reproductive dormancy have not yet been identified.

A previous study has revealed that *Dop1R1*, the gene encoding one of the dopamine receptors, is expressed at the CA and the fat body (Gruntenko et al., 2012; **Figure 3B**). Knockdown of *Dop1R1* either in the CA or the fat body significantly reduces the percentage of dormant flies (Andreatta et al., 2018), suggesting that dopamine induces reproductive dormancy via both the CA and the fat body. It should be remembered that the fat body is the main organ producing yolk protein, which is essential for vitellogenesis (Bownes, 1994). Therefore, these data raise the possibility that dopamine might inhibit yolk protein production in the fat body, which indirectly suppresses vitellogenesis in dormant flies (**Figure 2**). Besides the CA and the fat body, dopamine seems to act on IPCs as well. While it is unclear whether any of the dopamine receptors are expressed in IPCs, *Dop1R1* knockdown in IPCs also reduces the percentage of dormant flies (Andreatta et al., 2018). Therefore, Dilps production may be reduced when the IPCs receive dopamine (**Figure 3B**). Taken together, these data indicate that dopamine simultaneously acts on multiple tissues to regulate reproductive dormancy.

It is known that Dop1R1 is coupled with the stimulatory trimeric G-protein, known as Gs, and utilizes intracellular cyclic AMP (cAMP) as a second messenger (Gotzes et al., 1994; Sugamori et al., 1995). A previous study has shown that the CA-specific forced expression of a dominant-negative form of protein kinase A (PKA), a cAMP-activating kinase (Francis and Corbin, 1994), represses reproductive dormancy in the dormancy-inducing condition (Andreatta et al., 2018). Moreover, the animals in this study exhibited expression changes in JH-responsive genes, which seemed to reflect the increased JH titer. These results are also consistent with the involvement of Dop1R1 signaling in the CA in regulating reproductive dormancy (**Figure 3B**).

In addition to dopamine, serotonin also regulates reproductive dormancy in *D. melanogaster*. Artificial neuronal activation of serotonergic neurons by overexpressing a voltage-gated ion channel causes a significant increase in the percentage of dormant flies (Venken et al., 2011; Andreatta et al., 2018). Furthermore, one of the serotonin receptors, 5-HT1A, is expressed in IPCs and negatively regulates IPCs (Luo et al., 2012, 2014). Knockdown of *5-HT1A* in IPCs greatly reduces the percentage of dormant flies. Consistent with this observation, the IPC-specific knockdown of *5-HT1A* enhances the expression levels of *Dilp2* and *Dilp5*. These results indicate that serotonin acts on IPCs to promote dormancy via regulation of insulin signaling (**Figure 3B**).

Other bioactive monoamines include octopamine and γ-aminobutyric acid (GABA) (Nässel, 2018). It has been demonstrated that the activation of octopaminergic neurons suppresses reproductive dormancy, while GABAergic signaling does not consistently affect dormancy (Andreatta et al., 2018). Since the octopaminergic signal is known to influence the production and release of Dilps in non-dormancy-inducing conditions (Enell et al., 2010; Luo et al., 2014), a previous study has examined whether octopamine regulates reproductive dormancy by acting on IPCs. However, the silencing of the octopamine receptor in mushroom bodies (OAMB) in the IPCs does not affect the percentage of dormant flies (Andreatta et al., 2018). On the other hand, a previous study has suggested that octopamine suppresses JH degradation, leading to a rise in JH titer under heat stress (Gruntenko et al., 2000; Rauschenbach et al., 2001). Although this study does not focus on reproductive dormancy, this observation implies that activation of octopaminergic neurons may facilitate reproductive dormancy. However, it is still unclear which tissues receive

octopamine and whether octopamine-dependent control of reproductive dormancy requires JH signaling.

Mechanisms of Ecdysteroid Signaling-Mediated Reproductive Dormancy

The classical scheme of insect endocrinology describes how insect developmental transitions are regulated by three primary circulating factors: prothoracicotropic hormone, JHs, and ecdysteroids (Jindra et al., 2013; Truman, 2019). In *D. melanogaster*, besides JHs, ecdysteroids are also known to play an important role in regulating reproductive dormancy (Richard et al., 1998, 2001b). Ovaries of reproductively dormant flies exhibit lower ecdysteroid biosynthesis activity *in vitro* compared to those of non-dormant flies (Richard et al., 1998). On the other hand, the ovarian ecdysteroid level is elevated during the dormancy-breaking process (Richard et al., 1998). The elevation of the ovarian ecdysteroids occurs before mature eggs start to be produced in the dormancy-broken ovaries. Moreover, microinjection of 20-hydroxyecdysone (20E), the most active form of ecdysteroid, to reproductively dormant females evokes vitellogenesis (Richard et al., 1998, 2001b). Notably, vitellogenesis is severely suppressed in the ecdysone receptor (EcR) loss-of-function ovaries in non-dormant flies (Carney and Bender, 2000). Taken together, these results indicate that ecdysteroid biosynthesis in the ovary needs to be suppressed in the dormancy-inducing condition, while the elevation of the ovarian ecdysteroid level and the activation of 20E signaling are required for breaking reproductive dormancy (**Figure 2**).

Currently, however, the role of ecdysteroids in the regulation of reproductive dormancy in *D. melanogaster* has not been as well documented as that of the JHs. It is also unclear how the ovarian ecdysteroid levels are suppressed in the dormancy-inducing condition. Nevertheless, it has been hypothesized that three major pathways might be involved in the 20E-mediated regulation of reproductive dormancy. First, the downregulation of 20E signaling in the fat body seems to suppress yolk protein production in the dormancy-inducing condition, as 20E stimulates the fat body in non-dormant females, leading to the promotion of yolk protein production (Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980; Bownes, 1982; Handler and Maroy, 1989; Bownes et al., 1996; Richard et al., 2001a). Second, the downregulation of 20E signaling in the ovary might suppress many aspects of oogenesis in the dormancy-inducing condition. Previous studies have indicated that 20E has versatile roles in *D. melanogaster* oogenesis, including niche formation, germline stem cell proliferation and maintenance, cystocyte differentiation, and the developmental checkpoint at the stage 8–10 egg chambers (Belles and Piulachs, 2015; Uryu et al., 2015; Ameku and Niwa, 2016; Ameku et al., 2017; Swevers, 2019). Third, the downregulation of 20E signaling might suppress JH biosynthesis in the CA in the dormancy-inducing condition. In fact, very recent studies have just revealed the relationship between 20E signaling and JH biosynthesis, which is at least in part mediated by the peptide hormone ecdysis-triggering hormone (ETH). We provide more details in the next section.

Mechanisms of Ecdysis-Triggering Hormone-Mediated Reproductive Dormancy

ETH has been identified and characterized as the peptide hormone playing an essential role in regulating innate ecdysis behavior in the tobacco horn moth *Manduca sexta* and *D. melanogaster* (Žitòan et al., 1996; Kingan et al., 1997; Park et al., 1999). ETH is produced by Inka cells located close to tracheal pits in the thorax and abdomen and then secreted into the hemolymph. While early studies have shown that the released ETH is received in the brain, after which there is activation of a neuroendocrine mechanism to control ecdysis behavior, the recent studies using non-dormant *D. melanogaster* have also revealed that ETH has a function to promote JH biosynthesis in the CA, leading to ovarian development in females and mating behavior in males (Lee et al., 2017; Meiselman et al., 2017). In females, knockdown of *ETH* results in the reduction of JH levels and slow-down of ovarian development (Lee et al., 2017; Meiselman et al., 2017). In addition, live imaging experiments have shown that ETH is directly received by the ETH receptor on the CA to elevate intracellular Ca^{2+} level (Lee et al., 2017; Meiselman et al., 2017). Curiously, it is known that ETH production is promoted by 20E (Park et al., 1999). Therefore, it is plausible to expect that, in dormancy-inducing conditions, the downregulations of ecdysteroid biosynthesis and 20E signaling indirectly suppress JH biosynthesis via ETH, yet this fascinating possibility has not been experimentally tested in *D. melanogaster* so far (**Figure 3B**).

Interestingly, a recent study has shown that ecdysteroids regulate dormancy via ETH in the cabbage beetle *Colaphellus bowringi* (Guo et al., 2021). In this insect, reduction of ecdysteroid biosynthesis is required for suppressed oogenesis and accumulated lipid storage in the fat body in dormancy-inducing conditions. The reduction of ecdysteroid biosynthesis causes a decrease in ETH expression level, leading to a decrease in JH biosynthesis, which is essential for entering reproductive dormancy. The study on *C. bowringi* beetle nicely demonstrated that the ecdysteroid-ETH-JH relay is important to regulate reproductive dormancy. This study is an excellent example of how findings on the regulation of JH biosynthesis from analyses with *D. melanogaster* led to an understanding of the mechanisms of reproductive dormancy in other insects. Conversely, it would be intriguing to examine whether the ecdysteroid-ETH-JH-mediated mechanism found in *C. bowringi* also exists in *D. melanogaster*.

Is There a Direct Neuronal Regulation of Juvenile Hormone Biosynthesis in the *Corpus Allatum* in *Drosophila melanogaster*?

In some insects other than *D. melanogaster*, JH-mediated reproductive dormancy is regulated by not only humoral factors remotely acting on the CA but also by direct inputs from neurons projecting to the CA (de Wilde and de Boer, 1969; Shiga and Numata, 2000; Shimokawa et al., 2008). For example, in the

northern blowfly *P. terraenovae* and the bean bug *R. pedestris*, the neurons projecting to the CA (hereafter referred to as the CA-projecting neurons) are essential for inducing reproductive dormancy in dormancy-inducing conditions (Shiga et al., 2003; Shimokawa et al., 2008). In adults of the brown-winged green bug *Plautia stali*, the CA-projecting neurons co-produce several neuropeptides, such as Diuretic hormone 44, Insulin-like peptide 1, and *Plautia stali* myoinhibitory protein (Plast-MIP). In particular, Plast-MIP is considered to suppress JH biosynthesis in the CA directly (Matsumoto et al., 2017; Hasegawa et al., 2020; Hasebe and Shiga, 2021). In these insects, reproductive dormancy can be broken by surgically removing the CA-projecting neurons, confirming the importance of the CA-projecting neurons in the regulation of reproductive dormancy. However, in almost all cases, except *P. stali*, the neurotransmitter and/or neurohormone produced in the CA-projecting neurons has not been clarified. For a better understanding of the neuroendocrine mechanisms of JH biosynthesis in the CA, clarification and characterization of such neurotransmitters and/or neurohormones are crucial.

In *D. melanogaster* larvae, two types of neurons, designated CA-LP1 and CA-LP2 neurons, have been reported to directly innervate the CA (Siegmund and Korge, 2001). These neurons are involved in the epithelial movement of male genitalia by inhibiting JH biosynthesis during pupal development in non-dormancy-inducing condition (Ádám et al., 2003). It is possible that these neurons are also present in the adult stage and positively regulate reproductive dormancy via inhibiting JH biosynthesis. In addition, our recent study has reported new CA-projection neurons that produce the neuropeptide hugin in *D. melanogaster* adults, which is unexpected given that the CA-projecting hugin neurons may not have a significant impact on JH biosynthesis in the CA (Mizuno et al., 2021). Further studies would be needed to clarify the role of the CA-projecting neurons in reproductive dormancy in *D. melanogaster* (**Figure 3B**).

MECHANISMS OF DORMANCY CONTROL BY CIRCADIAN CLOCKS

In general, dormancy is significantly influenced by photoperiodism (Denlinger, 2002; Saunders, 2014). In *D. melanogaster*, although a low temperature is the main environmental cue to induce reproductive dormancy, photoperiodism additionally influences the dormancy entry; the short-day but not the long-day condition accelerates the entry of reproductive dormancy in *D. melanogaster* (Saunders et al., 1989; Saunders and Gilbert, 1990; Nagy et al., 2018). In a wide range of insects, photoperiodism and photoperiodism-associated dormancy are under the control of the circadian clock system (Bloch et al., 2013; Meuti and Denlinger, 2013; Meuti et al., 2015; Saunders, 2020). Indeed, photoperiodism significantly influences JH titers in *D. melanogaster* (Saunders, 1990) and other insects (Kramer, 1978; Khan et al., 1982; Meuti and Denlinger, 2013; Matsumoto et al., 2017), although molecular and cellular mechanisms of this phenomenon remain largely unclear. On the other hand, recent studies have shown that circadian clock neurons and circadian clock genes play an important role in modulating the neuroendocrine system to control reproductive dormancy in *D. melanogaster*. Below, we mainly summarize the role of clock neurons and circadian clock genes in reproductive dormancy, and briefly mention a possible contribution of the clock machinery to JH biosynthesis in the CA.

The molecular machinery of the circadian clock has been extensively studied in *D. melanogaster*, where the circadian master clock comprises about 150 neurons located in the central brain (Helfrich-Förster, 2004). Among the identified circadian clock neurons, the small ventral lateral neurons (s-LNvs) act as central circadian pacemakers to regulate various daily changes in *D. melanogaster* physiology. Indeed, s-LNvs are involved in the dormancy-dependent regulation of neuronal activity of IPCs (Nagy et al., 2019). The axons of *D. melanogaster* s-LNvs project to the dorsal protocerebrum, where the cell bodies and dendrites of IPCs are located, implying that s-LNvs and IPCs might have direct synaptic contact with each other (**Figure 3A**).

The s-LNvs are known to produce and release two neuropeptides required for the normal functioning of the behavioral circadian rhythm, namely pigment-dispersing factor (Pdf) (Park et al., 2000) and short neuropeptide F (sNPF) (Johard et al., 2009). Overexpression of either Pdf or sNPF, specifically in s-LNvs, decreases the percentage of reproductively dormant flies in dormancy-inducing condition. Furthermore, inhibition of the sNPF receptor only in IPCs also increases the percentage of dormant flies in dormancy-inducing condition. Consistent with these results, administration of synthetic Pdf or sNPF ligand to cultured brains evokes an increase in intracellular cAMP, which acts as a second messenger for the Pdf receptor and sNPF receptor in IPCs (Nagy et al., 2019). According to these data, it is hypothesized that the change in day length during winter suppresses the production and secretion of Pdf and sNPF in s-LNvs, which might provide less stimulation for IPCs and consequently induce reproductive dormancy (**Figure 3B**). Therefore, the suppression of IPC neuronal activity might be due to reduced activation of the receptors for Pdf and sNPF (Ojima et al., 2018). In addition, it is very likely that Dilp-mediated regulation of JH biosynthesis in the CA would be under the control of Pdf- and sNPF-dependent regulation of the circadian rhythm; however, this hypothesis has not been closely examined.

It has been reported that Pdf-dependent regulation of reproductive dormancy has been observed in the blow fly *Protophormia terraenovae* (Shiga and Numata, 2009), the mosquito *Culex pipiens* (Meuti et al., 2015), and the bean bug *Riptortus pedestris* (Ikeno et al., 2014). These data imply that Pdf-producing clock neurons are involved in reproductive dormancy beyond Drosophilidae species.

Besides the regulation mediated by the central clock pacemaker neurons, the activity of IPCs is also regulated by transcription regulators expressed in IPCs. This mechanism has been revealed in a study on a gene called *eyes absent* (*eya*) (Abrieux et al., 2020). *Eya* encodes a highly conserved transcriptional coactivator and protein phosphatase that plays vital roles in multiple developmental processes in organisms ranging from *Drosophila* to humans. While the best-known function of Eya is to control eye formation, it is also known that *eya* is expressed in IPCs. Knockdown of *eya* in IPCs causes an

increase in the size of the ovary, implying that Eya negatively regulates oogenesis through IPCs. Interestingly, the expression of *eya* has a circadian rhythm (Abrieux et al., 2020). More precisely, the peak of Eya protein levels is delayed in the short-day condition in comparison to the long-day condition. In addition, the protein levels of Eya in IPCs are higher under the short-day condition than the long-day condition. In parallel, Eya protein level is also upregulated under low-temperature conditions. These observations raise the possibility that the upregulation of Eya protein in dormancy-inducing conditions is necessary for oogenesis suppression (**Figure 3B**).

In the same study (Abrieux et al., 2020), the authors have demonstrated that the *timeless* (*tim*) gene also functions in IPCs to regulate reproductive dormancy. The *tim* gene is notable for its role in the transcriptional-translational autoregulatory feedback loops of the circadian core clock system in *D. melanogaster* (Dunlap, 1999; Hardin, 2005). In the *tim* mutant, a higher rate of reproductive dormancy is observed in any daylight length condition compared to wild type, and the impact of the short-day condition is less effective to enhance reproductive dormancy (Tauber et al., 2007; Abrieux et al., 2020). Interestingly, Tim is involved in the regulation of the Eya protein level. For example, the circadian rhythm of Eya protein is abolished in the *tim* mutant under non-dormancy-inducing conditions (Abrieux et al., 2020). Furthermore, the increased level of Eya protein at low temperatures is thought to be mediated by Tim. The Tim-mediated upregulation of Eya seems to be regulated by a unique splice isoform generated only at low temperature (Anduaga et al., 2019; Foley et al., 2019), which physically interacts with EYA protein (Abrieux et al., 2020). Based on these findings, it is hypothesized that the low temperature-specific Tim splice isoform binds Eya to suppress IPC activity, while the nature of the cell types in which this physical interaction takes place has not been precisely elucidated. Taken together, IPCs activity seems to be regulated by the external and internal regulatory system of the circadian clock in *D. melanogaster*, both of which might be required for regulating reproductive dormancy by inhibiting JH biosynthesis in the CA (**Figure 3B**).

Lastly, we would like to mention that a part of the circadian clock-mediated mechanisms of photoperiodism in *D. melanogaster* might be conserved even in mammals. For example, *EYA3*, one of the mammalian orthologs of *eya*, is required for the photoperiodism-dependent expression change of the *thyroid-stimulating hormone* gene in the mouse and the sheep (Dardente et al., 2010; Masumoto et al., 2010; Hut, 2011; Wood and Loudon, 2014; Wood et al., 2015).

FUTURE PERSPECTIVES

Although the studies on *D. melanogaster* in the last decades have improved our understanding of JH-mediated regulation of reproductive dormancy, several issues remain to be clarified. Here, we list the five main possible research lines, in our opinion.

(1) Do hemolymph JH titers change during reproductive dormancy induction in *D. melanogaster* and how do they change? In fact, no previous study using *D. melanogaster* has made a conclusive report about the temporal fluctuation of hemolymph JH titers. This information must be a solid foothold for *D. melanogaster* reproductive dormancy studies and, therefore, should be obtained by the *D. melanogaster* research community in the near future. Liquid chromatography-mass spectrometry-based methods to measure hemolymph JH titers have already been reported by several groups (Reiff et al., 2015; Lee et al., 2017; Meiselman et al., 2017; Ramirez et al., 2020; Zhang et al., 2021).

(2) How does the information regarding low temperature eventually get transmitted to the CA? It has been revealed that the antenna is a vital organ for detecting cold sensations (Gallio et al., 2011; Li and Gong, 2017; Alpert et al., 2020). Therefore, there must be a neuronal circuit from the antennal cold-sensitive sensilla to the CA; however, the nature of this circuit is largely unclear. At least, it is likely that the neuronal circuit may contain some dopaminergic neurons, based on the observations that dopamine suppresses JH biosynthesis and is elevated in dormancy-inducing conditions (Andreatta et al., 2018). On the other hand, it should be noted that low temperature suppresses overall biological activity, including feeding. Since a lower level of feeding results in the suppression of energy expenditure, the dormancy-inducing low temperature might negatively affect IPC activity, possibly leading to reproductive dormancy. Meanwhile, curiously and paradoxically, some studies have reported that IPCs are activated by cold-sensing neurons at 18°C rather than 25°C (Li and Gong, 2015; Zhang et al., 2019), whereas the function of cold-sensing neurons in dormancy-inducing conditions has not yet been investigated. Further studies will be needed to clarify how low temperatures influence IPC activity to induce reproductive dormancy.

(3) How does the reproductive dormancy status influence the physiology and remodeling in each tissue? It has been reported that reproductive dormancy suppresses oogenesis and is also associated with several changes in various organs of the body. For example, the intestine is markedly shortened, and the innate immune system is activated during reproductive dormancy in *D. melanogaster* (Kubrak et al., 2014); however, the mechanisms involved are still unknown.

(4) How is male reproductive dormancy regulated? It has been reported that low temperatures and short-day condition also induce male reproductive dormancy, such as the slow-down of spermatogenesis and degeneration of male accessory glands (Kubrak et al., 2016). However, the molecular and neuroendocrine mechanisms of male reproductive dormancy have not been investigated. It has not even been elucidated whether this process depends on JHs. In the future, it would be intriguing to examine whether and how the neuroendocrine regulatory system of female reproductive dormancy resembles that of male reproductive dormancy.

(5) How does the JH-mediated regulation of reproductive dormancy contribute to the evolutionary and ecological adaptation of wild *D. melanogaster*? To date, most studies on *D. melanogaster* reproductive dormancy have been conducted in laboratories. However, since reproductive dormancy is the adaptive response of organisms to the severe winter season, it must be important to investigate the reproductive dormancy of wild animals. As described earlier, geological differences of

genetic bias influence reproductive dormancy in *D. melanogaster*. Indeed, previous studies have shown that geographic variations in reproductive dormancy in wild *D. melanogaster* is associated with single nucleotide polymorphisms in the loci of *tim*, *InR*, *FoxO*, and *couch potato* (Williams et al., 2006; Sandrelli et al., 2007; Tauber et al., 2007; Schmidt et al., 2008; Paaby et al., 2010, 2014; Kolaczkowski et al., 2011; Lee et al., 2011; Fabian et al., 2012; Bergland et al., 2014; Kapun et al., 2016; Zhao et al., 2016; Betancourt et al., 2021; Machado et al., 2021). In the case of *tim*, it is hypothesized that the allelic frequencies of two distinct isoforms, *ls-tim* and *s-tim* (Rosato et al., 1997), may have evolved to cope with winter when *D. melanogaster* expanded into Europe (Tauber et al., 2007). This hypothesis is based on the observation that flies expressing *ls-tim* have a higher dormancy rate than those with *s-tim* (Sandrelli et al., 2007; Tauber et al., 2007). It would be interesting to investigate whether such genetic variations and allelic frequencies among these geographically distinct *D. melanogaster* populations affect neuroendocrine mechanisms for reproductive dormancy.

(6) How does the JH-mediated regulation of reproductive dormancy contribute to the evolutionary and ecological adaptation of other Drosophilidae species? Other Drosophilidae species also enter adult dormancy in the winter, and many biological events during dormancy are essentially the same as we found in *D. melanogaster*, although the ease of dormancy varies among species (Carson and Stalker, 1948; Lumme et al., 1974; Schmidt and Conde, 2006; Yamada and Yamamoto, 2011; Salminen and Hoikkala, 2013; Zhai et al., 2016; Lirakis et al., 2018). Therefore, wild non-*melanogaster* Drosophilidae species are interesting research subjects for understanding the evolutionary significance of genetic and ecological variations in reproductive dormancy, which may influence flies' fitness in nature.

Well-developed and sophisticated genetic tools for *D. melanogaster* will shed light on the fundamental mechanisms of JH-mediated reproductive dormancy, not only in *D. melanogaster*, but also in other insect species. As mentioned in the Introduction, studies on reproductive dormancy would increase our understanding of the seasonal distribution of pests (Denlinger, 2008). Notably, in recent years, several researchers have used the spotted wing Drosophilidae fly *D. suzukii* for studying reproductive dormancy (Toxopeus et al., 2016; Wallingford et al., 2016; Zhai et al., 2016, 2019). Since *D. suzukii* is a well-known agricultural pest, the motivation for these studies is to explore new pest control strategies to disrupt the mechanisms of reproductive dormancy. Therefore, future studies on reproductive dormancy in Drosophilidae species, including *D. melanogaster*, have the potential to contribute to the fields of basic animal physiology and applied agricultural biology in the future.

AUTHOR CONTRIBUTIONS

YK and RN wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by grant from the JSPS KAKENHI 21J20365 to YK.

ACKNOWLEDGMENTS

YK and EI were recipients of a fellowship from the Japan Society for the Promotion of Science. We would like to thank Louis John Irving and Editage (www.editage.com) for English language editing.

REFERENCES

Abrieux, A., Xue, Y., Cai, Y., Lewald, K. M., Nguyen, H. N., Zhang, Y., et al. (2020). EYES ABSENT and TIMELESS integrate photoperiodic and temperature cues to regulate seasonal physiology in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 117, 15293–15304. doi: 10.1073/pnas.2004262117

Ádám, G., Perrimon, N., and Noselli, S. (2003). The retinoic-like juvenile hormone controls the looping of left-right asymmetric organs in *Drosophila*. *Development* 130, 2397–2406. doi: 10.1242/dev.00460

Allen, M. J. (2007). What makes a fly enter diapause? *Fly* 1, 307–310. doi: 10.4161/fly.5532

Alpert, M. H., Frank, D. D., Kaspi, E., Flourakis, M., Zaharieva, E. E., Allada, R., et al. (2020). A circuit encoding absolute cold temperature in *Drosophila*. *Curr. Biol.* 30, 2275–2288.e5. doi: 10.1016/j.cub.2020.04.038

Ameku, T., and Niwa, R. (2016). Mating-induced increase in germline stem cells via the neuroendocrine system in female *Drosophila*. *PLoS Genet.* 12:e1006123. doi: 10.1371/journal.pgen.1006123

Ameku, T., Yoshinari, Y., Fukuda, R., and Niwa, R. (2017). Ovarian ecdysteroid biosynthesis and female germline stem cells. *Fly* 11, 185–193. doi: 10.1080/19336934.2017.1291472

Andreatta, G., Kyriacou, C. P., Flatt, T., and Costa, R. (2018). Aminergic signaling controls ovarian dormancy in *Drosophila*. *Sci. Rep.* 8:2030. doi: 10.1038/s41598-018-20407-z

Anduaga, A. M., Evanta, N., Patop, I. L., Bartok, O., Weiss, R., and Kadener, S. (2019). Thermosensitive alternative splicing senses and mediates 2 temperature adaptation in *Drosophila*. *eLife* 8:e44642. doi: 10.7554/eLife.44642

Anduaga, A. M., Nagy, D., Costa, R., and Kyriacou, C. P. (2018). Diapause in *Drosophila melanogaster* – Photoperiodicity, cold tolerance and metabolites. *J. Insect Physiol.* 105, 46–53. doi: 10.1016/j.jinsphys.2018.01.003

Bastock, R., and St Johnston, D. (2008). *Drosophila* oogenesis. *Curr. Biol.* 18, R1082–R1087. doi: 10.1016/j.cub.2008.09.011

Belles, X., and Piulachs, M. D. (2015). Ecdysone signalling and ovarian development in insects: from stem cells to ovarian follicle formation. *Biochim. Biophys. Acta Gene Regul. Mech.* 1849, 181–186. doi: 10.1016/j.bbagrm.2014.05.025

Bergland, A. O., Behrman, E. L., O'Brien, K. R., Schmidt, P. S., and Petrov, D. A. (2014). Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. *PLoS Genet.* 10:e1004775. doi: 10.1371/JOURNAL.PGEN.1004775

Betancourt, N. J., Rajpurohit, S., Durmaz, E., Fabian, D. K., Kapun, M., Flatt, T., et al. (2021). Allelic polymorphism at foxo contributes to local adaptation in *Drosophila melanogaster*. *Mol. Ecol.* 30, 2817–2830. doi: 10.1111/MEC.15939

Bloch, G., Hazan, E., and Rafaeli, A. (2013). Circadian rhythms and endocrine functions in adult insects. *J. Insect Physiol.* 59, 56–69. doi: 10.1016/j.jinsphys.2012.10.012

Bownes, M. (1982). The role of 20-hydroxy-ecdysone in yolk-polypeptide synthesis by male and female fat bodies of *Drosophila melanogaster*. *J. Insect Physiol.* 28, 317–328. doi: 10.1016/0022-1910(82)90043-9

Bownes, M. (1989). The roles of juvenile hormone, ecdysone and the ovary in the control of *Drosophila* vitellogenesis. *J. Insect Physiol.* 35, 409–413. doi: 10.1016/0022-1910(89)90115-7

Bownes, M. (1994). The regulation of the yolk protein genes, a family of sex differentiation genes in *Drosophila melanogaster*. *Bioessays* 16, 745–752. doi: 10.1002/bies.950161009

Bownes, M., Ronaldson, E., and Mauchline, D. (1996). 20-Hydroxyecdysone, but not juvenile hormone, regulation of yolk protein gene expression can be mapped to cis-acting DNA sequences. *Dev. Biol.* 173, 475–489. doi: 10.1006/dbio.1996.0041

Brand, A. H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401–415.

Brennan, M. D., Weiner, A. J., Goralski, T. J., and Mahowald, A. P. (1982). The follicle cells are a major site of vitellogenin synthesis in *Drosophila melanogaster*. *Dev. Biol.* 89, 225–236. doi: 10.1016/0012-1606(82)90309-8

Carney, G. E., and Bender, M. (2000). The *Drosophila* ecdysone receptor (EcR) gene is required maternally for normal oogenesis. *Genetics* 154, 1203–1211. doi: 10.1093/genetics/154.3.1203

Carson, H. L., and Stalker, H. D. (1948). Reproductive diapause in *Drosophila robusta*. *Proc. Natl. Acad. Sci. U.S.A.* 34, 124–129. doi: 10.1073/pnas.34.3.124

Cornils, A., Gloeck, M., Chen, Z., Zhang, Y., and Alcedo, J. (2011). Specific insulin-like peptides encode sensory information to regulate distinct developmental processes. *Development* 138, 1183–1193. doi: 10.1242/DEV.060905

Crowe, J. H. (2007). Trehalose As a "Chemical Chaperone". *Adv. Exp. Med. Biol.* 594, 143–158. doi: 10.1007/978-0-387-39975-1_13

Danks, H. (1987). *Insect Dormancy: An Ecological Perspective*. Ottawa: Entomological Society of Canada.

Danks, H. V. (2006). Key themes in the study of seasonal adaptations in insects II. Life-cycle patterns. *Appl. Entomol. Zool.* 41, 1–13. doi: 10.1303/aez.2006.1

Dardente, H., Wyse, C. A., Birnie, M. J., Dupré, S. M., Loudon, A. S. I., Lincoln, G. A., et al. (2010). A molecular switch for photoperiod responsiveness in mammals. *Curr. Biol.* 20, 2193–2198. doi: 10.1016/j.cub.2010.10.048

de Wilde, J., and de Boer, J. A. (1961). Physiology of diapause in the adult Colorado beetle-II. Diapause as a case of pseudo-allatectomy. *J. Insect Physiol.* 6, 152–161. doi: 10.1016/0022-1910(61)90037-3

de Wilde, J., and de Boer, J. A. (1969). Humoral and nervous pathways in photoperiodic induction of diapause in *Leptinotarsa decemlineata*. *J. Insect Physiol.* 15, 661–675. doi: 10.1016/0022-1910(69)90263-7

Denlinger, D. L. (2002). Regulation of diapause. *Annu. Rev. Entomol.* 47, 93–122. doi: 10.1146/annurev.ento.47.091201.145137

Denlinger, D. L. (2008). Why study diapause? *Entomol. Res.* 38, 1–9. doi: 10.1111/j.1748-5967.2008.00139.x

Denlinger, D. L., Yocum, G. D., and Rinehart, J. P. (2012). Hormonal Control of Diapause. *Insect Endocrinol.* 3, 430–463. doi: 10.1016/B978-0-12-384749-2.10010-X

Dunlap, J. C. (1999). Molecular bases for circadian clocks. *Cell* 96, 271–290. doi: 10.1016/S0092-8674(00)80566-8

Emerson, K. J., Bradshaw, W. E., and Holzapfel, C. M. (2009a). Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. *Trends Genet.* 25, 217–225. doi: 10.1016/j.tig.2009.03.009

Emerson, K. J., Uyemura, A. M., McDaniel, K. L., Schmidt, P. S., Bradshaw, W. E., and Holzapfel, C. M. (2009b). Environmental control of ovarian dormancy in natural populations of *Drosophila melanogaster*. *J. Comp. Physiol. A Neuroethol. Sensory Neural Behav. Physiol.* 195, 825–829. doi: 10.1007/s00359-009-0460-5

Enell, L. E., Kapan, N., Söderberg, J. A. E., Kahsai, L., and Nässe, D. R. (2010). Insulin signaling, lifespan and stress resistance are modulated by metabotropic GABA receptors on insulin producing cells in the brain of *Drosophila*. *PLoS One* 5:15780. doi: 10.1371/journal.pone.0015780

Erickson, P. A., Weller, C. A., Song, D. Y., Bangerter, A. S., Schmidt, P., and Bergland, A. O. (2020). Unique genetic signatures of local adaptation over space and time for diapause, an ecologically relevant complex trait, in *Drosophila melanogaster*. *PLoS Genet.* 16:e1009110. doi: 10.1371/JOURNAL.PGEN.1009110

Fabian, D. K., Kapun, M., Nolte, V., Kofler, R., Schmidt, P. S., Schlötterer, C., et al. (2012). Genome-wide patterns of latitudinal differentiation among populations of *Drosophila melanogaster* from North America. *Mol. Ecol.* 21, 4748–4769. doi: 10.1111/J.1365-294X.2012.05731.X

Fabian, D. K., Lack, J. B., Mathur, V., Schlötterer, C., Schmidt, P. S., Pool, J. E., et al. (2015). Spatially varying selection shapes life history clines among populations of *Drosophila melanogaster* from sub-Saharan Africa. *J. Evol. Biol.* 28, 826–840. doi: 10.1111/jeb.12607

Flatt, T. (2020). Life-history evolution and the genetics of fitness components in *Drosophila melanogaster*. *Genetics* 214, 3–48. doi: 10.1534/genetics.119.300160

Foley, L. E., Ling, J., Joshi, R., Evantal, N., Kadener, S., and Emery, P. (2019). *Drosophila* PSI controls circadian period and the phase of circadian behavior under temperature cycle via tim splicing. *eLife* 8:e50063. doi: 10.7554/eLife.50063

Food and Agriculture Organization of the United Nations (2013). *New Coordinated Research Projects, Insect Pest Control - NAFA*. Available online at: http://www-naweb.iaea.org/nafa/ipc/crp/new-crps-ipc-1.html (accessed May 1, 2021).

Francis, S. H., and Corbin, J. D. (1994). Structure and function of cyclic nucleotide-dependent protein kinases. *Annu. Rev. Physiol.* 56, 237–272. doi: 10.1146/annurev.ph.56.030194.001321

Gallio, M., Ofstad, T. A., Macpherson, L. J., Wang, J. W., and Zuker, C. S. (2011). The coding of temperature in the *Drosophila* brain. *Cell* 144, 614–624. doi: 10.1016/j.cell.2011.01.028

Golden, J., and Riddle, D. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. *Science* 218, 578–580. doi: 10.1126/SCIENCE.6896933

Golden, J. W., and Riddle, D. L. (1984). The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev. Biol.* 102, 368–378. doi: 10.1016/0012-1606(84)90201-X

Gotzes, F., Balfanz, S., and Baumann, A. (1994). Primary structure and functional characterization of a *Drosophila* dopamine receptor with high homology to human D(1/5) receptors. *Recept. Channels* 2, 131–141.

Gruntenko, N., Wilson, T., Monastirioti, M., and Rauschenbach, I. (2000). Stress-reactivity and juvenile hormone degradation in *Drosophila melanogaster* strains having stress-related mutations. *Insect Biochem. Mol. Biol.* 30, 775–783. doi: 10.1016/S0965-1748(00)00049-7

Gruntenko, N. Y., Laukhina, O. V., and Rauschenbach, I. Y. (2012). Role of D1- and D2-like receptors in age-specific regulation of juvenile hormone and 20-hydroxyecdysone levels by dopamine in *Drosophila*. *J. Insect Physiol.* 58, 1534–1540. doi: 10.1016/j.jinsphys.2012.09.005

Guo, S., Tian, Z., Wu, Q. W., King-Jones, K., Liu, W., Zhu, F., et al. (2021). Steroid hormone ecdysone deficiency stimulates preparation for photoperiodic reproductive diapause. *PLoS Genet.* 17:e1009352. doi: 10.1371/JOURNAL.PGEN.1009352

Hahn, D. A., and Denlinger, D. L. (2007). Meeting the energetic demands of insect diapause: nutrient storage and utilization. *J. Insect Physiol.* 53, 760–773. doi: 10.1016/J.JINSPHYS.2007.03.018

Hahn, D. A., and Denlinger, D. L. (2011). Energetics of insect diapause. *Annu. Rev. Entomol.* 56, 103–121. doi: 10.1146/annurev-ento-112408-085436

Hand, S. C., Denlinger, D. L., Podrabsky, J. E., and Roy, R. (2016). Mechanisms of animal diapause: recent developments from nematodes, crustaceans, insects, and fish. *Am. J. Physiol.Regul. Integr. Comp. Physiol.* 310, R1193–R1211. doi: 10.1152/ajpregu.00250.2015

Handler, A. M., and Maroy, P. (1989). Ecdysteroid receptors in *Drosophila melanogaster* adult females. *Mol. Cell. Endocrinol.* 63, 103–109. doi: 10.1016/0303-7207(89)90086-5

Hardin, P. E. (2005). The circadian timekeeping system of *Drosophila*. *Curr. Biol.* 15, R714–R722. doi: 10.1016/j.cub.2005.08.019

Hasebe, M., and Shiga, S. (2021). Photoperiodic response in the pars intercerebralis neurons, including Plast-MIP neurons, in the brown-winged green bug, *Plautia stali*. *Zoolog. Sci.* 38, 317–325. doi: 10.2108/zs210005

Hasegawa, T., Hasebe, M., and Shiga, S. (2020). Immunohistochemical and direct mass spectral analyses of *Plautia stali* myoinhibitory peptides in the cephalic ganglia of the brown-winged green bug *Plautia stali*. *Zoolog. Sci.* 37, 42–49. doi: 10.2108/zs190092

He, L., Wang, X., and Montell, D. J. (2011). Shining light on *Drosophila* oogenesis: live imaging of egg development. *Curr. Opin. Genet. Dev.* 21, 612–619. doi: 10.1016/J.GDE.2011.08.011

Helfrich-Förster, C. (2004). The circadian clock in the brain: a structural and functional comparison between mammals and insects. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 190, 601–613. doi: 10.1007/s00359-004-0527-2

Hsu, S. K., Jakšiæ, A. M., Nolte, V., Lirakis, M., Kofler, R., Barghi, N., et al. (2020). Rapid sex-specific adaptation to high temperature in *Drosophila*. *eLife* 9:e53237. doi: 10.7554/ELIFE.53237

Hu, P. (2007). *"Dauer," in WormBook, ed. W. The C. elegans Research Community*. Available online at: http://www.wormbook.org (accessed August 08, 2007).

Hut, R. A. (2011). Photoperiodism: Shall EYA compare thee to a summers day? *Curr. Biol.* 21, R22–R25. doi: 10.1016/j.cub.2010.11.060

Ikeno, T., Numata, H., Goto, S. G., and Shiga, S. (2014). Involvement of the brain region containing pigment-dispersing factor-immunoreactive neurons in the photoperiodic response of the bean bug, *Riptortus pedestris*. *J. Exp. Biol.* 217, 453–462. doi: 10.1242/jeb.091801

Isabel, G., Gourdoux, L., and Moreau, R. (2001). Changes of biogenic amine levels in haemolymph during diapausing and non-diapausing status in *Pieris brassicae* L. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 128, 117–127. doi: 10.1016/S1095-6433(00)00284-1

Jindra, M., Palli, S. R., and Riddiford, L. M. (2013). The juvenile hormone signaling pathway in insect development. *Annu. Rev. Entomol.* 58, 181–204. doi: 10.1146/annurev-ento-120811-153700

Johard, H. A. D., Yoishii, T., Dircksen, H., Cusumano, P., Rouyer, F., Helfrich-Förster, C., et al. (2009). Peptidergic clock neurons in *Drosophila*: ion transport peptide and short neuropeptide F in subsets of dorsal and ventral lateral neurons. *J. Comp. Neurol.* 516, 59–73. doi: 10.1002/cne.22099

Jowett, T., and Postlethwait, J. H. (1980). The regulation of yolk polypeptide synthesis in *Drosophila* ovaries and fat body by 20-hydroxyecdysone and a juvenile hormone analog. *Dev. Biol.* 80, 225–234. doi: 10.1016/0012-1606(80)90510-2

Kapun, M., Fabian, D. K., Goudet, J., and Flatt, T. (2016). Genomic evidence for adaptive inversion clines in *Drosophila melanogaster*. *Mol. Biol. Evol.* 33, 1317–1336. doi: 10.1093/MOLBEV/MSW016

Karam, C. S., Jones, S. K., and Javitch, J. A. (2020). Come Fly with Me: an overview of dopamine receptors in *Drosophila melanogaster*. *Basic Clin. Pharmacol. Toxicol.* 126, 56–65. doi: 10.1111/bcpt.13277

Kasture, A. S., Hummel, T., Sucic, S., and Freissmuth, M. (2018). Big lessons from tiny flies: *Drosophila melanogaster* as a model to explore dysfunction of dopaminergic and serotonergic neurotransmitter systems. *Int. J. Mol. Sci.* 19:1788. doi: 10.3390/ijms19061788

Khan, M. A., Doderer, A., Koopmanschap, A. B., and de Kort, C. A. D. (1982). Improved assay conditions for measurement of corpus allatum activity *in vitro* in the adult Colorado potato beetle, *Leptinotarsa decemlineata*. *J. Insect Physiol.* 28, 279–284. doi: 10.1016/0022-1910(82)90088-9

King, R. C. (1970). The meiotic behavior of the *Drosophila* oocyte. *Int. Rev. Cytol.* 28, 125–168. doi: 10.1016/S0074-7696(08)62542-5

Kingan, T. G., Gray, W., Zitnan, D., and Adams, M. E. (1997). Regulation of ecdysis-triggering hormone release by eclosion hormone. *J. Exp. Biol.* 200, 3245–3256. doi: 10.1242/jeb.200.24.3245

Kolaczkowski, B., Kern, A. D., Holloway, A. K., and Begun, D. J. (2011). Genomic differentiation between temperate and tropical Australian populations of *Drosophila melanogaster*. *Genetics* 187, 245–260. doi: 10.1534/GENETICS.110.123059

Kort, C. A. D. (1990). Thirty-five years of diapause research with the Colorado potato beetle. *Entomol. Exp. Appl.* 56, 1–13. doi: 10.1111/j.1570-7458.1990.tb01376.x

Koštál, V. (2006). Eco-physiological phases of insect diapause. *J. Insect Physiol.* 52, 113–127. doi: 10.1016/j.jinsphys.2005.09.008

Kramer, S. J. (1978). Age-dependent changes in corpus allatum activity *in vitro* in the adult Colorado potato beetle, *Leptinotarsa decemlineata*. *J. Insect Physiol.* 24, 461–464. doi: 10.1016/0022-1910(78)90046-X

Kubrak, O. I., Kuèerová, L., Theopold, U., and Nässel, D. R. (2014). The sleeping beauty: how reproductive diapause affects hormone signaling, metabolism, immune response and somatic maintenance in *Drosophila melanogaster*. *PLoS One* 9:e113051. doi: 10.1371/journal.pone.0113051

Kubrak, O. I., Kucerová, L., Theopold, U., Nylin, S., and Nässel, D. R. (2016). Characterization of reproductive dormancy in male *Drosophila melanogaster*. *Front. Physiol.* 7:572. doi: 10.3389/fphys.2016.00572

Kuèerová, L., Kubrak, O. I., Bengtsson, J. M., Strnad, H., Nylin, S., Theopold, U., et al. (2016). Slowed aging during reproductive dormancy is reflected in genome-wide transcriptome changes in *Drosophila melanogaster*. *BMC Genomics* 17:50. doi: 10.1186/s12864-016-2383-1

LaFever, L., and Drummond-Barbosa, D. (2005). Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*. *Science* 309, 1071–1073. doi: 10.1126/SCIENCE.1111410

Lee, S. F., Sgro, C. M., Shirriffs, J., Wee, C. W., Rako, L., van Heerwaarden, B., et al. (2011). Polymorphism in the couch potato gene clines in eastern Australia but is not associated with ovarian dormancy in *Drosophila melanogaster*. *Mol. Ecol.* 20, 2973–2984. doi: 10.1111/J.1365-294X.2011.05155.X

Lee, S. S., Ding, Y., Karapetians, N., Rivera-Perez, C., Noriega, F. G., and Adams, M. E. (2017). Hormonal signaling cascade during an early-adult critical period required for courtship memory retention in *Drosophila*. *Curr. Biol.* 27, 2798–2809.e3. doi: 10.1016/j.cub.2017.08.017

Lenaerts, C., Monjon, E., Van Lommel, J., Verbakel, L., and Vanden Broeck, J. (2019). Peptides in insect oogenesis. *Curr. Opin. Insect Sci.* 31, 58–64. doi: 10.1016/J.COIS.2018.08.007

Li, K., and Gong, Z. (2017). Feeling hot and cold: thermal sensation in *Drosophila*. *Neurosci. Bull.* 33, 317–322. doi: 10.1007/s12264-016-0087-9

Li, Q., and Gong, Z. (2015). Cold-sensing regulates *Drosophila* growth through insulin-producing cells. *Nat. Commun.* 6:10083. doi: 10.1038/ncomms10083

Lirakis, M., Dolezal, M., and Schlötterer, C. (2018). Redefining reproductive dormancy in *Drosophila* as a general stress response to cold temperatures. *J. Insect Physiol.* 107, 175–185. doi: 10.1016/j.jinsphys.2018.04.006

Lirakis, M., and Magalhães, S. (2019). Does experimental evolution produce better biological control agents? A critical review of the evidence. *Entomol. Exp. Appl.* 167, 584–597. doi: 10.1111/EEA.12815

Liu, Y., Liao, S., Veenstra, J. A., and Nässel, D. R. (2016). *Drosophila* insulin-like peptide 1 (DILP1) is transiently expressed during non-feeding stages and reproductive dormancy. *Sci. Rep.* 6:26620. doi: 10.1038/srep26620

Lumme, J., Oikarinen, A., Lakovaara, S., and Alatalo, R. (1974). The environmental regulation of adult diapause in *Drosophila* littoralis. *J. Insect Physiol.* 20, 2023–2033. doi: 10.1016/0022-1910(74)90109-7

Luo, J., Becnel, J., Nichols, C. D., and Nässel, D. R. (2012). Insulin-producing cells in the brain of adult *Drosophila* are regulated by the serotonin 5-HT 1A receptor. *Cell. Mol. Life Sci.* 69, 471–484. doi: 10.1007/s00018-011-0789-0

Luo, J., Lushchak, O. V., Goergen, P., Williams, M. J., and Nässel, D. R. (2014). *Drosophila* insulin-producing cells are differentially modulated by serotonin and octopamine receptors and affect social behavior. *PLoS One* 9:e99732. doi: 10.1371/journal.pone.0099732

Machado, H. E., Bergland, A., Taylor, R. W., Tilk, S., Behrman, E., Dyer, K., et al. (2021). Broad geographic sampling reveals the shared basis and environmental correlates of seasonal adaptation in *Drosophila*. *eLife* 10:e67577. doi: 10.7554/ELIFE.67577

MacRae, T. H. (2010). Gene expression, metabolic regulation and stress tolerance during diapause. *Cell. Mol. Life Sci.* 67, 2405–2424. doi: 10.1007/S00018-010-0311-0

Masumoto, K. H., Ukai-Tadenuma, M., Kasukawa, T., Nagano, M., Uno, K. D., Tsujino, K., et al. (2010). Acute induction of Eya3 by late-night light stimulation triggers TSHβ expression in photoperiodism. *Curr. Biol.* 20, 2199–2206. doi: 10.1016/j.cub.2010.11.038

Matsumoto, K., Suetsugu, Y., Tanaka, Y., Kotaki, T., Goto, S. G., Shinoda, T., et al. (2017). Identification of allatostatins in the brown-winged green bug *Plautia stali*. *J. Insect Physiol.* 96, 21–28. doi: 10.1016/j.jinsphys.2016.10.005

Meiselman, M., Lee, S. S., Tran, R.-T., Dai, H., Ding, Y., Rivera-Perez, C., et al. (2017). Endocrine network essential for reproductive success in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 114, E3849–E3858. doi: 10.1073/pnas.1620760114

Meuti, M. E., and Denlinger, D. L. (2013). Evolutionary links between circadian clocks and photoperiodic diapause in insects. *Integr. Comp. Biol.* 53, 131–143. doi: 10.1093/icb/ict023

Meuti, M. E., Stone, M., Ikeno, T., and Denlinger, D. L. (2015). Functional circadian clock genes are essential for the overwintering diapause of the Northern house mosquito, *Culex pipiens*. *J. Exp. Biol.* 218, 412–422. doi: 10.1242/jeb.113233

Mizuno, Y., Imura, E., Kurogi, Y., Shimada-Niwa, Y., Kondo, S., Tanimoto, H., et al. (2021). A population of neurons that produce hugin and express the diuretic hormone 44 receptor gene projects to the corpora allata in *Drosophila melanogaster*. *Dev. Growth Differ.* 63, 249–261. doi: 10.1111/dgd.12733

Nagy, D., Andreatta, G., Bastianello, S., Anduaga, A. M., Mazzotta, G., Kyriacou, C. P., et al. (2018). A semi-natural approach for studying seasonal diapause in *Drosophila melanogaster* reveals robust photoperiodicity. *J. Biol. Rhythms* 33, 117–125. doi: 10.1177/0748730417754116

Nagy, D., Cusumano, P., Andreatta, G., Anduaga, A. M., Hermann-Luibl, C., Reinhard, N., et al. (2019). Peptidergic signaling from clock neurons regulates reproductive dormancy in *Drosophila melanogaster*. *PLoS Genet.* 15:e1008158. doi: 10.1371/journal.pgen.1008158

Nässel, D. R. (2018). Substrates for neuronal cotransmission with neuropeptides and small molecule neurotransmitters in *Drosophila*. *Front. Cell. Neurosci.* 12:83. doi: 10.3389/fncel.2018.00083

Neckameyer, W. S., and Argue, K. J. (2013). Comparative approaches to the study of physiology: *Drosophila* as a physiological tool. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R177–R188. doi: 10.1152/ajpregu.00084.2012

Niwa, R., Niimi, T., Honda, N., Yoshiyama, M., Itoyama, K., Kataoka, H., et al. (2008). Juvenile hormone acid O-methyltransferase in *Drosophila melanogaster*. *Insect Biochem. Mol. Biol.* 38, 714–720. doi: 10.1016/j.ibmb.2008.04.003

Niwa, R., and Niwa, Y. (2014). Enzymes for ecdysteroid biosynthesis: their biological functions in insects and beyond. *Biosci. Biotechnol. Biochem.* 78, 1283–1292. doi: 10.1080/09168451.2014.942250

Noguchi, H., and Hayakawa, Y. (1997). Role of dopamine at the onset of pupal diapause in the cabbage armyworm *Mamestra brassicae*. *FEBS Lett.* 413, 157–161. doi: 10.1016/S0014-5793(97)00848-X

Noriega, F. G. (2014). Juvenile hormone biosynthesis in insects: What is new, what do we know, and what questions remain? *Int. Sch. Res. Not.* 2014:967361. doi: 10.1155/2014/967361

Ojima, N., Hara, Y., Ito, H., and Yamamoto, D. (2018). Genetic dissection of stress-induced reproductive arrest in *Drosophila melanogaster* females. *PLoS Genet.* 14:e1007434. doi: 10.1371/journal.pgen.1007434

Paaby, A. B., Bergland, A. O., Behrman, E. L., and Schmidt, P. S. (2014). A highly pleiotropic amino acid polymorphism in the *Drosophila* insulin receptor contributes to life-history adaptation. *Evolution* 68, 3395–3409. doi: 10.1111/EVO.12546

Paaby, A. B., Blacket, M. J., Hoffman, A. A., and Schmidt, P. S. (2010). Identification of a candidate adaptive polymorphism for *Drosophila* life history by parallel independent clines on two continents. *Mol. Ecol.* 19, 760–774. doi: 10.1111/J.1365-294X.2009.04508.X

Park, J. H., Helfrich-Förster, C., Lee, G., Liu, L., Rosbash, M., and Hall, J. C. (2000). Differential regulation of circadian pacemaker output by separate clock genes in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3608–3613. doi: 10.1073/pnas.070036197

Park, Y., Zitnan, D., Gill, S. S., and Adams, M. E. (1999). Molecular cloning and biological activity of ecdysis-triggering hormones in *Drosophila melanogaster*. *FEBS Lett.* 463, 133–138. doi: 10.1016/S0014-5793(99)01622-1

Pener, M. P. (1992). Environmental cues, endocrine factors, and reproductive diapause in Male insects. *Chronobiol. Int.* 9, 102–113. doi: 10.3109/07420529209064521

Postlethwait, J. H., and Handler, A. M. (1979). The roles of juvenile hormone and 20-hydroxy-ecdysone during vitellogenesis in isolated abdomens of *Drosophila melanogaster*. *J. Insect Physiol.* 25, 455–460. doi: 10.1016/0022-1910(79)90014-3

Postlethwait, J. H., and Weiser, K. (1973). Vitellogenesis induced by juvenile hormone in the female sterile mutant apterous-four in *Drosophila melanogaster*. *Nat. New Biol.* 244, 284–285. doi: 10.1038/newbio244284a0

Ramirez, C. E., Nouzova, M., Michalkova, V., Fernandez-Lima, F., and Noriega, F. G. (2020). Common structural features facilitate the simultaneous identification and quantification of the five most common juvenile hormones by liquid chromatography-tandem mass spectrometry. *Insect Biochem. Mol. Biol.* 116, 103287. doi: 10.1016/J.IBMB.2019.103287

Rauschenbach, I. Y., Gruntenko, N. E., Chentsova, N. S., Hirashima, A., Sukhanova, M. Z., Andreenkova, E. V., et al. (2001). Regulation of reproductive function in *Drosophila* females due to hormonal interaction under stress is genetically determined. *Russ. J. Genet.* 37, 1041–1048. doi: 10.1023/A:1011961514711

Reiff, T., Jacobson, J., Cognigni, P., Antonello, Z., Ballesta, E., Tan, K. J., et al. (2015). Endocrine remodelling of the adult intestine sustains reproduction in *Drosophila*. *eLife* 4:e06930. doi: 10.7554/eLife.06930

Richard, D. S., Gilbert, M., Crum, B., Hollinshead, D. M., Schelble, S., and Scheswohl, D. (2001a). Yolk protein endocytosis by oocytes in *Drosophila melanogaster*: immunofluorescent localization of clathrin, adaptin and the yolk protein receptor. *J. Insect Physiol.* 47, 715–723. doi: 10.1016/S0022-1910(00)00165-7

Richard, D. S., Jones, J. M., Barbarito, M. R., Cerula, S., Detweiler, J. P., Fisher, S. J., et al. (2001b). Vitellogenesis in diapausing and mutant *Drosophila melanogaster*: further evidence for the relative roles of ecdysteroids and juvenile hormones. *J. Insect Physiol.* 47, 905–913. doi: 10.1016/S0022-1910(01)00063-4

Richard, D. S., Watkins, N. L., Serafin, R. B., and Gilbert, L. I. (1998). Ecdysteroids regulate yolk protein uptake by *Drosophila melanogaster* oocytes. *J. Insect Physiol.* 44, 637–644. doi: 10.1016/S0022-1910(98)00020-1

Romney, A. L. T., Davis, E. M., Corona, M. M., Wagner, J. T., and Podrabsky, J. E. (2018). Temperature-dependent vitamin D signaling regulates developmental trajectory associated with diapause in an annual killifish. *Proc. Natl. Acad. Sci. U.S.A.* 115, 12763–12768. doi: 10.1073/PNAS.1804590115

Rosato, E., Trevisan, A., Sandrelli, F., Zordan, M., Kyriacou, C. P., and Costa, R. (1997). Conceptual translation of *timeless* reveals alternative initiating methionines in *Drosophila*. *Nucleic Acids Res.* 25, 455–457. doi: 10.1093/nar/25.3.455

Salminen, T. S., and Hoikkala, A. (2013). Effect of temperature on the duration of sensitive period and on the number of photoperiodic cycles required for the induction of reproductive diapause in *Drosophila montana*. *J. Insect Physiol.* 59, 450–457. doi: 10.1016/j.jinsphys.2013.02.005

Sandrelli, F., Tauber, E., Pegoraro, M., Mazzotta, G., Cisotto, P., Landskron, J., et al. (2007). A molecular basis for natural selection at the timeless locus in *Drosophila melanogaster*. *Science* 316, 1898–1900. doi: 10.1126/science.1138426

Santos, C. G., Humann, F. C., and Hartfelder, K. (2019). Juvenile hormone signaling in insect oogenesis. *Curr. Opin. Insect Sci.* 31, 43–48. doi: 10.1016/j.cois.2018.07.010

Saunders, D. S. (1990). The circadian basis of ovarian diapause regulation in *Drosophila melanogaster*: Is the period gene causally involved in photoperiodic time measurement?: *J. Biol. Rhythms* 5, 315–331. doi: 10.1177/074873049000500404

Saunders, D. S. (2014). Insect photoperiodism: effects of temperature on the induction of insect diapause and diverse roles for the circadian system in the photoperiodic response. *Entomol. Sci.* 17, 25–40. doi: 10.1111/ens.12059

Saunders, D. S. (2020). Dormancy, diapause, and the role of the circadian system in insect photoperiodism. *Annu. Rev. Entomol.* 65, 373–389. doi: 10.1146/annurev-ento-011019-025116

Saunders, D. S., and Gilbert, L. I. (1990). Regulation of ovarian diapause in *Drosophila melanogaster* by photoperiod and moderately low temperature. *J. Insect Physiol.* 36, 195–200. doi: 10.1016/0022-1910(90)90122-V

Saunders, D. S., Henrich, V. C., and Gilbert, L. I. (1989). Induction of diapause in *Drosophila melanogaster*: photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. *Proc. Natl. Acad. Sci. U.S.A.* 86, 3748–3752. doi: 10.1073/pnas.86.10.3748

Saunders, D. S., Richard, D. S., Applebaum, S. W., Ma, M., and Gilbert, L. I. (1990). Photoperiodic diapause in *Drosophila melanogaster* involves a block to the juvenile hormone regulation of ovarian maturation. *Gen. Comp. Endocrinol.* 79, 174–184. doi: 10.1016/0016-6480(90)90102-R

Schiesari, L., Andreatta, G., Kyriacou, C. P., O'Connor, M. B., and Costa, R. (2016). The insulin-like proteins dILPs-2/5 determine diapause inducibility in *Drosophila*. *PLoS One* 11:e0163680. doi: 10.1371/journal.pone.0163680

Schiesari, L., Kyriacou, C. P., and Costa, R. (2011). The hormonal and circadian basis for insect photoperiodic timing. *FEBS Lett.* 585, 1450–1460. doi: 10.1016/j.febslet.2011.02.026

Schiesari, L., and O'Connor, M. B. (2013). Diapause. Delaying the developmental clock in response to a changing environment. *Curr. Top. Dev. Biol.* 105, 213–246. doi: 10.1016/B978-0-12-396968-2.00008-7

Schmidt, P. S., and Conde, D. R. (2006). Environmental heterogeneity and the maintenance of genetic variation for reproductive diapause in *Drosophila melanogaster*. *Evolution* 60, 1602–1611. doi: 10.1111/j.0014-3820.2006.tb00505.x

Schmidt, P. S., and Paaby, A. B. (2008). Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. *Evolution* 62, 1204–1215. doi: 10.1111/j.1558-5646.2008.00351.x

Schmidt, P. S., Zhu, C.-T., Das, J., Batavia, M., Yang, L., and Eanes, W. F. (2008). An amino acid polymorphism in the couch potato gene forms the basis for climatic adaptation in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 105, 16207–16211. doi: 10.1073/PNAS.0805485105

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th Edn. Cambridge: Cambridge University Press.

Schooneveld, H., Otazo Sanchez, A., and de Wilde, J. (1977). Juvenile hormone-induced break and termination of diapause in the Colorado potato beetle. *J. Insect Physiol.* 23, 689–696. doi: 10.1016/0022-1910(77)90085-3

Semaniuk, U., Piskovatska, V., Strilbytska, O., Strutynska, T., Burdyliuk, N., Vaiserman, A., et al. (2021). *Drosophila* insulin-like peptides: from expression to functions – a review. *Entomol. Exp. Appl.* 169, 195–208. doi: 10.1111/eea.12981

Sherald, A. F., and Wright, T. R. F. (1974). The analog inhibitor, α-methyl dopa, as a screening agent for mutants elevating levels of dopa decarboxylase activity in *Drosophila melanogaster*. *Mol. Gen. Genet.* 133, 25–36. doi: 10.1007/BF00268674

Shiga, S., Hamanaka, Y., Tatsu, Y., Okuda, T., and Numata, H. (2003). Juvenile hormone biosynthesis in diapause and nondiapause females of the adult blow fly *Protophormia terraenovae*. *Zoolog. Sci.* 20, 1199–1206. doi: 10.2108/zsj.20.1199

Shiga, S., and Numata, H. (2000). The role of neurosecretory neurons in the pars intercerebralis and pars lateralis in reproductive diapause of the blowfly, *Protophormia terraenovae*. *Naturwissenschaften* 87, 125–128. doi: 10.1007/s001140050689

Shiga, S., and Numata, H. (2009). Roles of PER immunoreactive neurons in circadian rhythms and photoperiodism in the blow fly, *Protophormia terraenovae*. *J. Exp. Biol.* 212, 867–877. doi: 10.1242/jeb.027003

Shimokawa, K., Numata, H., and Shiga, S. (2008). Neurons important for the photoperiodic control of diapause in the bean bug, *Riptortus pedestris*. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 194, 751–762. doi: 10.1007/s00359-008-0346-y

Shinoda, T., and Itoyama, K. (2003). Juvenile hormone acid methyltransferase: a key regulatory enzyme for insect metamorphosis. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11986–11991. doi: 10.1073/pnas.2134232100

Siegmund, T., and Korge, G. (2001). Innervation of the ring gland of *Drosophila melanogaster*. *J. Comp. Neurol.* 431, 481–491.

Sim, C., and Denlinger, D. L. (2013). Insulin signaling and the regulation of insect diapause. *Front. Physiol.* 4:189. doi: 10.3389/fphys.2013.00189

Soller, M., Bownes, M., and Kubli, E. (1997). Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Droosophila melanogaster*. *Eur. J. Biochem.* 243, 732–738. doi: 10.1111/j.1432-1033.1997.00732.x

Soller, M., Bownes, M., and Kubli, E. (1999). Control of oocyte maturation in sexually mature *Drosophila* females. *Dev. Biol.* 208, 337–351. doi: 10.1006/dbio.1999.9210

Sugamori, K. S., Demchyshyn, L. L., McConkey, F., Forte, M. A., and Niznik, H. B. (1995). A primordial dopamine D1-like adenylyl cyclase-linked receptor from *Drosophila melanogaster* displaying poor affinity for benzazepines. *FEBS Lett.* 362, 131–138. doi: 10.1016/0014-5793(95)00224-W

Swevers, L. (2019). An update on ecdysone signaling during insect oogenesis. *Curr. Opin. Insect Sci.* 31, 8–13. doi: 10.1016/j.cois.2018.07.003

Tatar, M., Chien, S. A., and Priest, N. K. (2001). Negligible senescence during reproductive dormancy in *Drosophila melanogaster*. *Am. Nat.* 158, 248–258. doi: 10.1086/321320

Tatar, M., and Yin, C. M. (2001). Slow aging during insect reproductive diapause: Why butterflies, grasshoppers and flies are like worms. *Exp. Gerontol.* 36, 723–738. doi: 10.1016/S0531-5565(00)00238-2

Tauber, E., Zordan, M., Sandrelli, F., Pegoraro, M., Osterwalder, N., Breda, C., et al. (2007). Natural selection favors a newly derived timeless allele in *Drosophila melanogaster*. *Science* 316, 1895–1898. doi: 10.1126/science.1138412

Tauber, M., Tauber, C., and Masaki, S. (1986). *Seasonal Adaptations of Insects*. Oxford: Oxford University Press.

Toxopeus, J., Jakobs, R., Ferguson, L. V., Gariepy, T. D., and Sinclair, B. J. (2016). Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii*. *J. Insect Physiol.* 89, 37–51. doi: 10.1016/j.jinsphys.2016.03.006

Truman, J. W. (2019). The evolution of insect metamorphosis. *Curr. Biol.* 29, R1252–R1268. doi: 10.1016/j.cub.2019.10.009

Tu, M.-P., Yin, C.-M., and Tatar, M. (2002). Impaired ovarian ecdysone synthesis of *Drosophila melanogaster* insulin receptor mutants. *Aging Cell* 1, 158–160. doi: 10.1046/J.1474-9728.2002.00016.X

Tu, M. P., Yin, C. M., and Tatar, M. (2005). Mutations in insulin signaling pathway alter juvenile hormone synthesis in *Drosophila melanogaster*. *Gen. Comp. Endocrinol.* 142, 347–356. doi: 10.1016/J.YGCEN.2005.02.009

Uryu, O., Ameku, T., and Niwa, R. (2015). Recent progress in understanding the role of ecdysteroids in adult insects: Germline development and circadian clock in the fruit fly *Drosophila melanogaster*. *Zool. Lett.* 1:32. doi: 10.1186/s40851-015-0031-2

Venken, K. J. T., Simpson, J. H., and Bellen, H. J. (2011). Genetic manipulation of genes and cells in the nervous system of the fruit fly. *Neuron* 72, 202–230. doi: 10.1016/j.neuron.2011.09.021

Wallingford, A. K., Lee, J. C., and Loeb, G. M. (2016). The influence of temperature and photoperiod on the reproductive diapause and cold tolerance of spotted-wing *Drosophila*, *Drosophila suzukii*. *Entomol. Exp. Appl.* 159, 327–337. doi: 10.1111/eea.12443

Watanabe, M., Kikawada, T., Minagawa, N., Yukuhiro, F., and Okuda, T. (2002). Mechanism allowing an insect to survive complete dehydration and extreme temperatures. *J. Exp. Biol.* 205, 2799–2802. doi: 10.1242/JEB.205.18.2799

Williams, K. D. (1993). Diapause in *Drosophila melanogaster* females: a genetic analysis. *Heredity* 71, 315–317. doi: 10.1038/hdy.1993.141

Williams, K. D., Busto, M., Suster, M. L., So, A. K.-C., Ben-Shahar, Y., Leevers, S. J., et al. (2006). Natural variation in *Drosophila melanogaster* diapause due to the insulin-regulated PI3-kinase. *Proc. Natl. Acad. Sci. U.SA.* 103, 15911–15915. doi: 10.1073/PNAS.0604592103

Woll, S. C., and Podrabsky, J. E. (2017). Insulin-like growth factor signaling regulates developmental trajectory associated with diapause in embryos of the annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* 220, 2777–2786. doi: 10.1242/JEB.151373

Wood, S., and Loudon, A. (2014). Clocks for all seasons: unwinding the roles and mechanisms of circadian and interval timers in the hypothalamus and pituitary. *J. Endocrinol.* 222, R39–R59.

Wood, S. H., Christian, H. C., Miedzinska, K., Saer, B. R. C., Johnson, M., Paton, B., et al. (2015). Binary wwitching of calendar cells in the pituitary defines the phase of the circannual cycle in mammals. *Curr. Biol.* 25, 2651–2662. doi: 10.1016/j.cub.2015.09.014

Wright, T. R. F., Bewley, G. C., and Sherald, A. F. (1976). The genetics of dopa decarboxylase in *Drosophila melanogaster*. II. Isolation and characterization of dopa decarboxylase deficient mutants and their relationship to the α methyl dopa hypersensitive mutants. *Genetics* 84, 287–310.

Yamada, H., and Yamamoto, M. T. (2011). Association between circadian clock genes and diapause incidence in *Drosophila triauraria*. *PLoS One* 6:e27493. doi: 10.1371/journal.pone.0027493

Yamashita, O., and Yaginuma, T. (1991). "Silkworm eggs at low temperatures: implications for sericulture," in *Insects at Low Temperature*, eds R. E. Lee and D. L. Denlinger (Boston, MA: Springer), 424–445. doi: 10.1007/978-1-4757-0190-6_18

Zhai, Y., Dong, X., Gao, H., Chen, H., Yang, P., Li, P., et al. (2019). Quantitative proteomic and transcriptomic analyses of metabolic regulation of adult reproductive diapause in *Drosophila* suzukii (Diptera: Drosophilidae) females. *Front. Physiol.* 10:344. doi: 10.3389/fphys.2019.00344

Zhai, Y., Lin, Q., Zhang, J., Zhang, F., Zheng, L., and Yu, Y. (2016). Adult reproductive diapause in *Drosophila suzukii* females. *J. Pest Sci.* 89, 679–688. doi: 10.1007/s10340-016-0760-9

Zhang, S. X., Glantz, E. H., Miner, L. E., Rogulja, D., and Crickmore, M. A. (2021). Hormonal control of motivational circuitry orchestrates the transition to sexuality in *Drosophila*. *Sci. Adv.* 7:eabg6926. doi: 10.1126/SCIADV.ABG6926

Zhang, X. O., Cui, X., and Zhu, D. (2019). Insulin producing cells monitor the cold and compensate the cold-induced sleep in *Drosophila*. *bioRxiv* [Preprint]. doi: 10.1101/419200

Zhao, X., Bergland, A. O., Behrman, E. L., Gregory, B. D., Petrov, D. A., and Schmidt, P. S. (2016). Global transcriptional profiling of diapause and climatic

adaptation in *Drosophila melanogaster*. *Mol. Biol. Evol.* 33, 707–720. doi: 10.1093/MOLBEV/MSV263

Žitòan, D., Kingan, T. G., Hermesman, J. L., and Adams, M. E. (1996). Identification of ecdysis-triggering hormone from an epitracheal endocrine system. *Science* 271, 88–91. doi: 10.1126/science.271.5245.88

Zonato, V., Collins, L., Pegoraro, M., Tauber, E., and Kyriacou, C. P. (2017). Is diapause an ancient adaptation in *Drosophila*? *J. Insect Physiol.* 98, 267–274. doi: 10.1016/j.jinsphys.2017.01.017

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

REVIEW
published: 30 September 2021
doi: 10.3389/fevo.2021.735924

Hormonal Regulation of Diapause and Development in Nematodes, Insects, and Fishes

*Xantha Karp**

Department of Biology, Central Michigan University, Mount Pleasant, MI, United States

Diapause is a state of developmental arrest adopted in response to or in anticipation of environmental conditions that are unfavorable for growth. In many cases, diapause is facultative, such that animals may undergo either a diapause or a non-diapause developmental trajectory, depending on environmental cues. Diapause is characterized by enhanced stress resistance, reduced metabolism, and increased longevity. The ability to postpone reproduction until suitable conditions are found is important to the survival of many animals, and both vertebrate and invertebrate species can undergo diapause. The decision to enter diapause occurs at the level of the whole animal, and thus hormonal signaling pathways are common regulators of the diapause decision. Unlike other types of developmental arrest, diapause is programmed, such that the diapause developmental trajectory includes a pre-diapause preparatory phase, diapause itself, recovery from diapause, and post-diapause development. Therefore, developmental pathways are profoundly affected by diapause. Here, I review two conserved hormonal pathways, insulin/IGF signaling (IIS) and nuclear hormone receptor signaling (NHR), and their role in regulating diapause across three animal phyla. Specifically, the species reviewed are *Austrofundulus limnaeus* and *Nothobranchius furzeri* annual killifishes, *Caenorhabditis elegans* nematodes, and insect species including *Drosophila melanogaster*, *Culex pipiens*, and *Bombyx mori*. In addition, the developmental changes that occur as a result of diapause are discussed, with a focus on how IIS and NHR pathways interact with core developmental pathways in *C. elegans* larvae that undergo diapause.

Keywords: diapause, insulin/IGF, nuclear hormone receptor, hormone, developmental trajectory, life histories

OPEN ACCESS

Edited by:
Sarah Kelly McMenamin, Boston College, United States

Reviewed by:
Jason E. Podrabsky, Portland State University, United States
Jr-Kai Yu, Institute of Cellular and Organismic Biology, Academia Sinica, Taiwan
Richard Roy, McGill University, Canada

***Correspondence:**
Xantha Karp
karp1x@cmich.edu

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *03 July 2021*
Accepted: *08 September 2021*
Published: *30 September 2021*

Citation:
Karp X (2021) Hormonal Regulation of Diapause and Development in Nematodes, Insects, and Fishes. Front. Ecol. Evol. 9:735924. doi: 10.3389/fevo.2021.735924

INTRODUCTION

Diapause is a stress-resistant and developmentally arrested stage that can be adopted in order to increase the chance of survival in adverse environmental conditions (Hand et al., 2016). Diapause can occur as an obligate part of development, but in many species, diapause is facultative, occurring in response to environmental cues that signal that an unfavorable environment either exists or is likely to exist in the near future. For example, temperature, nutrient availability, and photoperiod are common cues that influence the decision to enter diapause (Hand et al., 2016). This type of facultative diapause exists in many animal species, both vertebrate and invertebrate. In these

species, there are two separate life histories or developmental trajectories available: one that includes diapause and one that does not.

The diapause developmental trajectory differs in several respects from the non-diapause trajectory. First, there is a pre-diapause stage where the animal prepares to enter diapause. Then there is diapause itself which can last a variable amount of time. Next there is a period of recovery, and finally there is post-diapause development. Therefore, developmental pathways must accommodate all of these aspects of the diapause developmental trajectory to allow development to occur normally. This review describes hormonal regulation of diapause and the effects of diapause on development.

DEVELOPMENTAL SYSTEMS AND CONTEXT FOR DIAPAUSE

Diapause occurs in a wide range of animal species including nematodes, insects and other arthropods, fishes, and mammals (Hand et al., 2016). This review will focus on a few well-studied examples of these systems that undergo facultative diapause. A brief description of how diapause occurs in each system is provided in this section, along with the ecological context which diapause serves in this organism.

Diapause in Nematodes: *Caenorhabditis elegans*

Nematodes are highly abundant and widespread animals. Nematode species generally have two sexes, which can either be male and female or male and self-fertilizing hermaphrodite (Ellis and Lin, 2014). Each female or hermaphrodite lays hundreds of embryos, contributing to a boom-and-bust life cycle (Frézal and Félix, 2015). Many species within the order Rhabditida have a facultative diapause stage called dauer that can be used to survive the bust periods (Ley, 2006). Dauer occurs midway through larval development and is thought to be analogous to the infective stage adopted by parasitic nematodes (Crook, 2013). While diapause affects many rhabditids, the *Caenorhabditis elegans (C. elegans)* model system provides the most detailed dissection of both dauer formation and the developmental pathways that are affected, and this review will focus on *C. elegans* to represent the nematodes.

After embryogenesis, *C. elegans* develops through four larval stages (L1-L4) separated by molts before becoming a reproductively mature adult (Byerly et al., 1976). *C. elegans* can undergo environmentally induced developmental arrest at a number of stages, but dauer is the best example of a diapause stage, with a preparatory phase, long-term developmental arrest, recovery, and post-diapause stages (Baugh and Hu, 2020). Adverse environmental conditions sensed in the L1 stage will trigger entry into the pre-diapause L2d stage (Golden and Riddle, 1984b; Schaedel et al., 2012). During L2d, larvae prepare for diapause, for example by fat storage, and continue to sense their environment. If adverse conditions persist, L2d larvae will molt into the dauer stage (Golden and Riddle, 1984b; Schaedel et al., 2012). Dauer larvae are highly stress-resistant and can survive for months, longer than the lifespan of a worm that developed through the non-dauer trajectory (Cassada and Russell, 1975; Klass and Hirsh, 1976). If favorable conditions are encountered, dauer larvae undergo a recovery process and then proceed with development normally (Cassada and Russell, 1975; Klass and Hirsh, 1976; Liu and Ambros, 1991; Euling and Ambros, 1996; Hall et al., 2010).

In the wild, *C. elegans* can be found across the globe in temperate regions, where it is located in rich soil, compost, and decomposing vegetation (Frézal and Félix, 2015). *C. elegans* eats the microorganisms that are abundant in environments that include rotting fruit and stems. A worm born in the presence of a rich food source will develop quickly to adulthood (Félix and Duveau, 2012). Because *C. elegans* populations exist largely as self-fertilizing hermaphrodites, each worm in these circumstances will produce approximately 300 offspring within about 3–5 days. Thus, in a short time the food will become exhausted.

Young larvae that hatch when food is scarce and the worm population is high will be induced to enter dauer. Dauer larvae can not only survive in the absence of food, but they have dispersal behaviors that increase the chance that they will find another source of food. One dauer-specific dispersal behavior is nictation, where dauer larvae stand on their tails and wave their heads. This behavior increases the chances of associating with a passing invertebrate that can carry the worm to a new location (Félix and Duveau, 2012; Lee et al., 2012). Parasitic larvae in the infective stage also exhibit nictation behavior, reinforcing the similarities between dauer and infective stages (Reed and Wallace, 1965). If dauer larvae successfully move to a new, more favorable location, they will recover, complete development, and produce progeny that will begin a new cycle (Frézal and Félix, 2015). Therefore, the dauer formation decision must be made accurately to allow the population to survive. Failure to enter diapause when food is scarce is likely to lead to death, whereas adoption of diapause when food is plentiful allows time for other animals to consume the available food (Avery, 2014).

The primary sensory cue that induces dauer is a pheromone that is secreted by all non-dauer members of the population (Golden and Riddle, 1982; Kaplan et al., 2011). The pheromone is comprised of a mix of ascarosides (Jeong et al., 2005; Butcher et al., 2007). Ascarosides are derived from the sugar disaccharide ascarylose and then modified with lipid side chains (Ludewig and Schroeder, 2013). The abundance of pheromone is compared to signals from the food source, and it is the pheromone-to-food ratio that dictates dauer formation (Golden and Riddle, 1984a). Finally, these signals are further modulated by temperature, where higher temperatures are more likely to induce dauer formation (Golden and Riddle, 1984a). Nematodes thrive in cooler temperatures; in the lab 15–25°C is the typical range (Stiernagle, 2006).

Diapause in Insects: *Drosophila melanogaster*, *Culex pipiens*, and *Bombyx mori*

Like nematodes, insects are a highly abundant and widespread group of animal species. Unlike nematodes, the need for

diapause in insects is typically seasonal. In temperate climates insects may spend the winter in diapause, whereas in tropical climates seasonal variations in rainfall may create conditions that necessitate diapause. Therefore, photoperiod is the most important cue that induces diapause, with temperature being the second most important. The availability of food, maternal cues, and other factors can also play a role (Denlinger et al., 2012; Hand et al., 2016).

Unlike in nematodes where diapause occurs midway through larval development, each species of insect has a different stage at which it may undergo diapause, including embryonic, larval, pupal, or adult reproductive diapause. Because there is extensive literature on many insect species and existing excellent comprehensive reviews available (Denlinger et al., 2012; Schiesari and O'Connor, 2013), this paper focuses on three species. The three species chosen are the fruit fly *Drosophila melanogaster (D. melanogaster)* for its importance as a model organism, the mosquito *Culex pipiens (C. pipiens),* and the silkmoth *Bombyx mori (B. mori)*. The latter two were chosen for the literature dissecting at least one of the two major hormonal pathways covered in this review.

Drosophila melanogaster is arguably the most intensely studied insect model organism. This species arose in tropical regions in Africa, but has spread widely and is now present in most tropical and temperate regions across the globe (David and Capy, 1988). In response to cold temperatures and reduced photoperiod, young adult females can undergo a reproductive diapause, where ovarian development is arrested at the previtellogenic stage (Saunders et al., 1989; Saunders and Gilbert, 1990). A reproductive dormancy has also been described in males, but less is known about this system (Kubrak et al., 2016).

In contrast to many insects, diapause in *D. melanogaster* is more strongly dependent on temperature than photoperiod (Saunders et al., 1989; Emerson et al., 2009; Anduaga et al., 2018; Nagy et al., 2018). Because the response to temperature can be thought of as an immediate stress-response, rather than a programmed developmental arrest, the term "diapause" is sometimes avoided in favor of quiescence or dormancy. However, there are some features of diapause present in this reproductive arrest in *D. melanogaster,* including stress-resistance, an increase in lipid stores, and lifespan extension (Tatar et al., 2001; Schmidt et al., 2005a,b; Schmidt and Paaby, 2008). The term reproductive diapause will be used for *D. melanogaster* in this review.

The mosquito *C. pipiens* lives in temperate regions and adult females undergo a reproductive diapause in response to short photoperiod and cool temperatures sensed during late larval development and early pupal development (Eldridge, 1966; Sanburg and Larsen, 1973; Spiebnan and Wong, 1973). This diapause is similar to that described for *D. melanogaster,* but photoperiod plays a larger role in inducing diapause, and there is no disagreement that this reproductive arrest is a genuine diapause (Denlinger and Armbruster, 2013). Diapause induces changes in behavior and metabolism, including hypertrophy of the fat body, movement to overwintering sites, and a shift from blood meals to sugar sources such as nectar, with accompanying changes in the production of enzymes involved in digestion (Robich and Denlinger, 2005; Denlinger and Armbruster, 2013; Diniz et al., 2017).

Bombyx mori is a silkmoth that apparently originated from a Chinese strain of *B. mandarina* but has been domesticated for thousands of years. This species is important economically and also serves as a model organism (Arunkumar et al., 2006; Furdui et al., 2014). Diapause in *B. mori* occurs during mid-embryogenesis and is maternally programmed. Environmental conditions sensed by the mother during embryogenesis are translated into diapause-regulating signals for the next generation. Diapause-inducing cues include warmer temperatures of 25°C and longer photoperiods, whereas temperatures of 15°C and below and short photoperiods stimulate non-diapause development (Xu et al., 1995b).

Diapause in Annual Killifishes: *Austrofundulus limnaeus* and *Nothobranchius furzeri*

Annual killifishes live in Africa and South America in small ponds that can be temporary and present only in the rainy season. Diapause is therefore a mechanism to survive when the water evaporates. Diapausing embryos are packed into the pond sediment and can survive until the water returns.

Two species of annual fishes that have emerged as model systems are *Austrofundulus limnaeus* from Venezuela and *Nothobranchius furzeri* from Zimbabwe and Mozambique (Podrabsky et al., 2017; Reichard and Polačik, 2019). Annual killifishes undergo embryonic diapause at three different stages (DI-DIII) (Wourms, 1972). DI occurs early in development, before the embryonic axis is established. DI-promoting environmental cues include anaerobic conditions, low ambient temperatures, and the presence of adult fishes (Wourms, 1972; Levels et al., 1986; Arezo et al., 2017). DII occurs midway through development before the onset of organogenesis. Lower temperatures of approximately 20°C induce DII whereas at 30°C most embryos bypass DII, termed the "escape" developmental trajectory. DIII occurs at the end of embryogenesis, just before hatching, and this diapause seems to occur in nearly every embryo (Wourms, 1972; Podrabsky et al., 2010). DIII may be related to the delayed hatching that can occur in other species without a clear diapause stage (Wourms, 1972; Martin and Podrabsky, 2017).

Under uniform laboratory conditions, embryos in a population are arrested in different diapauses and for different amounts of time, resulting in a mixture of embryos at a range of developmental stages. This range has been thought to represent a bet-hedging strategy to maximize the chance that some embryos will survive despite unpredictable environments. The mechanism behind these differences in growth rate is still unclear (Wourms, 1972; Rowiński et al., 2021). However, a recent study that sampled natural populations of embryos found much tighter developmental synchrony within populations and provided evidence that environmental cues play a much greater role in controlling developmental progression than previously seen in laboratory studies (Polačik et al., 2021).

This review will focus on DII, as this diapause is well-studied and shares the most features with diapause in the invertebrates featured here, including the highest level of stress-resistance and the most facultative (Podrabsky et al., 2016a,b).

HORMONAL REGULATION OF DIAPAUSE ENTRY AND EXIT

The decision to enter diapause occurs in response to sensory cues, and diapause must be adopted across the entire organism. Hormones are thus key to regulating diapause. The details of hormonal regulation of diapause are different across species, and there are many excellent reviews that focus on individual phyla or two-way comparisons (Fielenbach and Antebi, 2008; Denlinger et al., 2012; Schiesari and O'Connor, 2013; Sim and Denlinger, 2013a; Podrabsky et al., 2016b; Baugh and Hu, 2020). An additional important review describes all three phyla, but without the focus on hormonal pathways (Hand et al., 2016). Here, I describe two of the hormonal pathways that play an important role in regulating diapause across nematodes, insects, and fishes: insulin/IGF signaling and nuclear hormone receptor signaling.

Insulin/Insulin-Like Growth Factor Signaling

Insulin and insulin-like growth factor (IGF) signaling (IIS) is an evolutionarily conserved hormonal pathway that regulates growth, metabolism, and stress-resistance (**Figure 1**; Oldham and Hafen, 2003; Tothova and Gilliland, 2007; Murphy and Hu, 2013). Insulin, IGF, or insulin-like peptides (ILPs) are expressed in response to environmental signals. When insulin/IGF agonists are plentiful, they bind to insulin or IGF receptors (IR or IGFR) on the surface of target cells. IR/IGFRs are receptor tyrosine kinases that dimerize upon agonist binding, thereby activating phosphoinositide 3-kinase (PI3K). Activated PI3K catalyzes the addition of a phosphate group to phosphatidylinositol-3,4-bisphosphate (PIP2), forming phosphatidylinositol-3,4,5-trisphosphate (PIP3). The phosphatase and tensin homolog (PTEN) catalyzes the reverse reaction, removing a phosphate group from PIP3. When levels of PIP3 are high, it acts as a second messenger and leads to activation of downstream kinases, including the serine-threonine kinase AKT. AKT can regulate a number of targets, including Forkhead box O (FOXO) transcription factors. When phosphorylated by AKT, FOXO proteins are cytosolic and therefore inactive. FOXO transcription factors regulate expression of genes involved in stress resistance, metabolism, and cell-cycle arrest. Perhaps for these reasons, downregulation of IIS and/or activation of FOXO are common themes in diapausing animals across species (Murphy and Hu, 2013; Schiesari and O'Connor, 2013; Sim and Denlinger, 2013a).

The core components of IIS signaling are conserved in animal species (**Figure 1**). Insulin, IGFs, and/or ILPs are typically expressed from particular cells in response to environmental signals. Secreted insulins/IGFs then travel throughout the body and bind to their cognate receptors. The number of insulins/IGFs and the receptors they bind to differs across species. Vertebrates have seven related ligands: insulin, IGF-I, IGF-II, and four relaxins, though relaxins appear to function via G-protein coupled receptors rather than receptor tyrosine kinases related to IR and IGFR (Hsu et al., 2002). Insulin binds primarily to the insulin receptor. IGFR1 is responsible for the signaling activity of IGFs, while IGFR2 functions as a negative regulator of IIS (Czech, 1989; Lau et al., 1994; Jones and Clemmons, 1995; Vincent and Feldman, 2002). Insects have seven ligands, ILP1-7 and only one receptor (Oldham and Hafen, 2003). In *C. elegans,* the ligands have expanded to include 40 ILPs and a single receptor, DAF-2/IR. The downstream components are also conserved across phyla, including PI3K, PTEN, AKT, and FOXO. Mammals have four FOXO proteins, while several species of fishes that have been examined so far possess seven FOXO proteins, likely due to the teleost-specific genome duplication event (Brunet et al., 1999; Kops et al., 1999; Oldham and Hafen, 2003; Tothova and Gilliland, 2007; Murphy and Hu, 2013; Gao et al., 2019). The IIS components with known connections to diapause are listed in **Table 1** and described in more detail below.

Regulation of Insulin-Like Peptides and Their Receptors During Diapause

All animal species possess multiple insulin-related genes, and these genes appear to work in combination to regulate diapause. In contrast, in the species where IIS signaling has been shown to regulate diapause, these ligands appear to signal through a single receptor. Worms and insects have only a single insulin/IGF receptor, and in fishes IGFR1 is the key receptor that regulates diapause, as described below.

The combinatorial nature of the ligands is most apparent in *C. elegans,* whose genome encodes 40 ILPs. *C. elegans* ILPs have been studied singly and in combination to reveal a complex network of IIS agonists and antagonists that regulate diapause entry, and a slightly different set that regulates diapause exit (Pierce et al., 2001; Cornils et al., 2011; Fernandes-de-Abreu et al., 2014). ILPs that regulate diapause are expressed in sensory neurons, and their expression changes in response to environmental cues (Pierce et al., 2001; Li et al., 2003; Cornils et al., 2011). The best-studied regulators of dauer diapause include *daf-28, ins-6,* and *ins-1. daf-28* and *ins-6* both promote IIS to oppose dauer entry and promote dauer exit; however, *daf-28* plays a more prominent role in dauer entry and *ins-6* plays a more prominent role in dauer exit (Malone et al., 1996; Li et al., 2003; Cornils et al., 2011). By contrast, *ins-1* antagonizes IIS to promote dauer entry and oppose dauer exit (Pierce et al., 2001; Cornils et al., 2011). If the human insulin protein is introduced into *C. elegans* it also antagonizes IIS, similar to *ins-1,* demonstrating the conservation between ILPs across species (Pierce et al., 2001). In addition to these three ILPs that have been studied in detail, systematic studies have shown that many other ILPs also play more subtle roles in regulating dauer entry and/or exit (Pierce et al., 2001; Fernandes-de-Abreu et al., 2014). ILP signaling converges on the sole *C. elegans* IR, DAF-2, which was discovered on the basis of its effect on entry to dauer diapause. Reduction-of-function mutations in *daf-2* cause a dauer formation phenotype whereby larvae enter dauer diapause even in conditions that normally promote non-dauer development

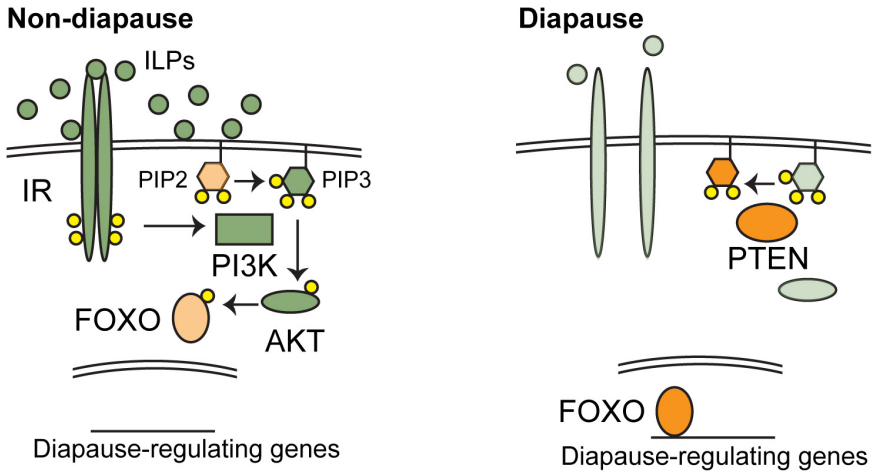


FIGURE 1 | The regulation of diapause by insulin/IGF signaling (IIS). Major, conserved components of IIS signaling are shown in favorable environments (left) and unfavorable environments (right). Orange components promote diapause and green components promote non-diapause development. Favorable environments stimulate the production of IGF-I in annual fishes or insulin-like peptides (ILPs) in *C. elegans* and insects. These ligands activate IIS, leading to the phosphorylation and nuclear exclusion of FOXO transcription factors by AKT. When active FOXO proteins regulate downstream genes involved in diapause. See text for additional details.

(Riddle, 1977). Therefore, active DAF-2/IR signaling promotes non-diapause development.

In insects, the role of IIS in regulating diapause has been studied extensively with respect to the reproductive diapause that occurs in adult mosquitos *(C. pipiens)* and fruit flies *(D. melanogaster)*. In *C. pipiens,* levels of two ILPs, ILP-1, and ILP-5, are reduced during diapause compared with non-diapausing animals. RNAi of ILP-1 but not ILP-5 is sufficient to induce adult diapause as measured by ovarian maturation and follicle length (Sim and Denlinger, 2009). Similarly, RNAi of IR induces adult diapause by the same measures (Sim and Denlinger, 2008). Therefore, IIS signaling opposes diapause in *C. pipiens*.

Similarly, in *D. melanogaster,* loss of *dilps* 1-5 strongly induces reproductive diapause. Single *dilp2* and *dilp5,* but not *dilp1,* mutations are sufficient to induce reproductive diapause at low penetrance, whereas overexpression of *dilp2* and *dilp5* from any tissue strongly suppresses reproductive diapause (Kubrak et al., 2014; Liu et al., 2016; Schiesari et al., 2016). Furthermore, hypomorphic mutations in InR or the insulin receptor substrate protein Chico cause reproductive diapause entry (Schiesari et al., 2016). Surprisingly, although dILPs functionally oppose reproductive diapause, mRNA levels of the five ILPs tested *(dilp1, dilp2, dilp3, dilp5, dilp6)* are all upregulated during reproductive diapause and then return to non-diapause levels after recovery from reproductive diapause (Kubrak et al., 2014; Liu et al., 2016). This upregulation may be a compensatory response to reduced IIS signaling under diapause-inducing conditions (Schiesari et al., 2016).

Another insect that undergoes reproductive diapause is the bumblebee *Bombus terrestris*. In this species, genes within the IIS pathway were upregulated in post-diapause animals compared to diapause. Furthermore, injection of ILPs stimulated metabolic changes consistent with non-diapause development and increased the numbers of offspring produced (Chen et al., 2021). Finally, there are many insect species where reduced expression of ILPs or IR has been reported to correlate with diapause at different stages of development, consistent with the notion that downregulation of IIS is a broadly conserved phenomenon. Some examples include egg diapause in the aphid *Acyrthosiphon pisum* and in *Locusta migratoria* (Barberà et al., 2019; Hao et al., 2019), embryonic diapause in *B. mori* (Gong et al., 2020), and pre-pupal diapause in leafcutting bees, *Megachile rotundata* (Cambron et al., 2021).

In the annual fish *A. limnaeus*, levels of IGF-I are reduced before and during DII compared to escape embryos at the same stages. After recovery, IGF-I levels climb, eventually surpassing those of escape embryos (Woll and Podrabsky, 2017). IGF-II levels are similar in escape and diapause embryos before and during DII, but are higher post-DII than in escape embryos. Higher levels of IGF after diapause may be related to the catch-up growth that is seen in post-diapause embryos. In addition to the correlation of IGF levels, treatment of embryos with an IGF1R inhibitor induces diapause in a dose-dependent manner. Pre-DII and DII embryos induced by this treatment show similarities to naturally induced DII, including slowed heart rate and slowed somitogenesis. However, there were a few differences, including the presence of melanocytes and hemoglobin in circulating blood, indicating that IGF signaling is not the sole regulator of diapause (Woll and Podrabsky, 2017).

Regulation of Insulin/IGF Signaling Components Downstream of IR/IGFR Receptor to Control Diapause

The role of downstream components of IIS in the regulation of diapause has been studied primarily in *C. elegans*, where the dauer diapause-regulating pathways have been worked out in detail (Fielenbach and Antebi, 2008; Murphy and Hu, 2013). Each component functions as expected given its role in insulin/IGF

TABLE 1 | IIS components and diapause.

Component	Organism	Gene	Expression in diapause vs. non-diapause	LOF or RNAi phenotype	References
Insulin/ IGF/ ILP	*C. elegans*	*daf-28*	Reduced	↑ diapause	Li et al., 2003; Cornils et al., 2011
		ins-6	Complex[1]	↑ diapause	Cornils et al., 2011
		ins-1	Unchanged in sensory neurons that regulate dauer	↓ diapause	Cornils et al., 2011
	C. pipiens	ILP-1	Reduced	↑ diapause	Sim and Denlinger, 2008, 2009
		ILP-5	Reduced	No effect on diapause	Sim and Denlinger, 2008, 2009
	D. melanogaster	*dilp2*	Increased	↑ diapause	Kubrak et al., 2014; Liu et al., 2016; Schiesari et al., 2016
		dilp5	Increased	↑ diapause	Kubrak et al., 2014; Liu et al., 2016; Schiesari et al., 2016
	A. limnaeus	IGF-I	Reduced	↑ diapause	Woll and Podrabsky, 2017
		IGF-II	Unchanged		Woll and Podrabsky, 2017
IR/IGFR	*C. elegans*	*daf-2*	Decreased[2]	↑ diapause	Riddle, 1977; Kimura et al., 2011
	C. pipiens	IR		↑ diapause	Sim and Denlinger, 2008
	D. melanogaster	InR	Unchanged	↑ diapause	Kubrak et al., 2014; Schiesari et al., 2016
	A. limnaeus	IGFR1		↑ diapause	Woll and Podrabsky, 2017
PI3K	*C. elegans*	*age-1*		↑ diapause	Gottlieb and Ruvkun, 1994
	D. melanogaster	*Dp110*	Unchanged[3]	↑ diapause	Williams et al., 2006
PTEN	*C. elegans*	*daf-18*		↓ diapause	Riddle et al., 1981
AKT	*C. elegans*	*akt-1*		↑ diapause	Ailion and Thomas, 2003; Oh et al., 2005
	C. elegans	*akt-2*		↑ diapause	Ailion and Thomas, 2003; Oh et al., 2005
FOXO	*C. elegans*	*daf-16*	Nuclear import	↓ diapause ↓ lipid stores, remodeling & dauer cuticle	Riddle et al., 1981; Vowels and Thomas, 1992; Gottlieb and Ruvkun, 1994; Lin et al., 2001
	C. pipiens	*FOXO*	Increased	↓ lipid stores & survival during diapause	Sim and Denlinger, 2008, 2013b

[1] *During dauer, ins-6 expression is reduced in the ASI sensory neuron that inhibits dauer entry and increased in the ASJ sensory neuron that inhibits dauer exit (Cornils et al., 2011).*
[2] *daf-2 expression is reduced in response to starvation, but dauer diapause was not tested (Kimura et al., 2011).*
[3] *Dp110 expression was compared between two wild isolates that differ in propensity to enter diapause (Williams et al., 2006).*

signaling. Specifically, when active, the orthologs of PI3K and AKT oppose dauer formation, whereas the ortholog of PTEN promotes dauer formation. The single PI3K in *C. elegans* is encoded by *age-1*, also called *daf-23* (Morris et al., 1996). *age-1* was first identified based on the long-lived phenotype conferred by mutants (Klass, 1983). Separately, this gene was identified in a screen for mutants that enter dauer in conditions that normally promote non-dauer development (Gottlieb and Ruvkun, 1994). Similarly, the single PTEN ortholog, *daf-18* was identified on the basis of the inability of a *daf-18(-)* mutant to enter dauer under dauer-inducing conditions (Riddle et al., 1981). There are two AKT homologs in *C. elegans, akt-1* and *akt-2* that function partially redundantly to oppose dauer diapause. Loss of *akt-1* produces a weak dauer-constitutive phenotype, whereas loss of both *akt-1* and *akt-2* produces a very strong constitutive dauer diapause phenotype (Ailion and Thomas, 2003; Oh et al., 2005).

In insects, the PI3K ortholog, *Dp110* was identified based on the variation in reproductive diapause rates between two natural strains of *D. melanogaster,* one that enters reproductive diapause more frequently than the other. *Dp110* appears to be one locus responsible for this difference (Williams et al., 2006). Although the molecular changes in *Dp110* that lead to this reproductive diapause phenotype were not clear, reducing the dosage of *Dp110* by half increases the percentage of flies adopting reproductive diapause, whereas misexpressing *Dp110* in neurons decreases the percentage of flies adopting reproductive diapause (Williams et al., 2006).

Regulation of Forkhead Box O Activity to Control Diapause

One common output of IIS is regulation of the FOXO transcription factors. IIS negatively regulates FOXO, such that when IIS is active, FOXO proteins are phosphorylated by AKT, which triggers nuclear exit and therefore inactivity (Manning and Cantley, 2007). Therefore, low levels of IIS are required for FOXO nuclear localization and activity. Across species, FOXO transcription factors promote diapause by directly and indirectly controlling the expression of numerous genes that are involved in diapause. Furthermore, if situations are contrived whereby animals within diapause have reduced FOXO activity, those animals lose key characteristics of diapause, suggesting that FOXO promotes both the decision to enter diapause and the

physiological and structural changes that accompany diapause, as explained below.

The specific role of FOXO proteins in diapause has not been studied in fishes, but there is a great deal of information from invertebrate species, which typically have a single FOXO ortholog. In *C. elegans*, the FOXO protein is encoded by *daf-16*. Like most of the genes in the IIS pathway, *daf-16* was also discovered on the basis of its dauer formation phenotype. Whereas loss-of-function mutations in *daf-2* or other IIS components cause dauer formation even in environments favorable for growth, loss-of-function mutations in *daf-16* cause the opposite phenotype: inability to enter dauer diapause even in dauer-inducing conditions. Furthermore, when double mutants are created, the *daf-16* dauer-defective phenotype is observed (Murphy and Hu, 2013). The complete suppression of the *daf-2(-)* dauer-constitutive phenotype by loss of *daf-16* indicates that the major output of IIS with respect to regulation of diapause is *daf-16*/FOXO.

While *daf-16(-)* larvae do not readily enter dauer diapause, it is possible to obtain *daf-16(-)* dauer larvae by making use of dauer-constitutive mutations outside of the IIS pathway, or by the use of very potent dauer-inducing cues. However, these *daf-16(0)* dauer larvae display defects in some aspects of the remodeling that occurs as part of dauer morphogenesis, demonstrating that *daf-16*/FOXO is not only responsible for the decision to enter dauer but for some of the aspects of diapause itself. For this reason, *daf-16(-)* dauer larvae have been referred to as "partial dauers" (Vowels and Thomas, 1992; Larsen et al., 1995; Ogg et al., 1997). For example, *daf-16* mutants lack the particular collagens in their cuticle that are normally enriched in dauer larvae, and perhaps for this reason they are more sensitive to environmental assaults (Gottlieb and Ruvkun, 1994; Nika et al., 2016; Wirick et al., 2021).

The specific gene expression changes that occur in the absence of *daf-16*/FOXO have been studied primarily in non-diapause contexts: adult worms with activated DAF-16 due to a mutation in IR are compared to IR mutants that lack *daf-16*. As in other species, *daf-16* promotes expression of genes involved in stress resistance and metabolism (Tepper et al., 2013). A recent study examined the changes in gene expression of dauer larvae with and without *daf-16*. In this context, genes related to collagens and signaling were differentially expressed (Wirick et al., 2021).

In mosquitos, levels of FOXO are increased in the fat body of diapausing females, compared to non-diapause animals (Sim and Denlinger, 2013b). Fat storage is an important component of diapause and appears to be dependent on FOXO because knockdown of FOXO with dsRNA in diapausing females causes a reduction in lipid stores (Sim and Denlinger, 2008). These females also showed reduced survival over time in diapause. Reduced survival may be due to insufficient fat storage, but because addition of an oxidoreductase (Mn(III)TBAP) partially rescues survival, oxidative stress is also implicated (Sim and Denlinger, 2008). FOXO promotes resistance to oxidative stress across species. Genes bound by FOXO during diapause were identified by ChIP-seq. These target genes are involved in stress-resistance, metabolism, longevity, cell-cycle, development, and circadian rhythm (Sim et al., 2015). RNAi knockdown of three of these targets reduced glycogen and lipid levels during diapause, consistent with the idea that FOXO regulation of target genes is important for diapause characteristics (Olademehin et al., 2020). In *Drosophila*, a FOXO-responsive luciferase reporter is upregulated during reproductive diapause. Abdomens isolated from cold-reared flies in reproductive diapause showed six-fold more activity of this reporter than non-diapausing flies reared under standard lab conditions (Schiesari et al., 2016).

In *Locusta migratoria* egg diapause, if FOXO is knocked down in the mother by RNAi under diapause-inducing conditions (short photoperiod), the percentage of eggs that enter diapause is reduced, indicating that FOXO promotes entry into diapause (Hao et al., 2019). Consistent with the activity of FOXO being important for diapause, the known FOXO target, *MnSod/sod2*, was found to be upregulated under diapause-inducing conditions (Hao et al., 2019).

In the cotton bollworm *Helicoverpa armigera* pupal diapause, levels of total FOXO are high but phospho-FOXO levels are low, indicating that FOXO is in its active state. Interestingly, this regulation occurs by a different mechanism than the typical IIS pathway (Zhang X.-S. et al., 2017). Although ILP levels are low, these ILP levels do not appear to regulate FOXO activity via Akt, because phospho-AKT levels are high in diapause. Instead, high levels of ROS lead to phosphorylation of Akt as well as high levels of protein arginine methyltransferase 1 (PRMT1). PRMT1 methylates FOXO, thus abrogating phosphorylation by Akt. In this way, FOXO remains active to promote diapause at the same time as active Akt can phosphorylate other targets (Zhang X.-S. et al., 2017).

Nuclear Hormone Receptor Signaling

Nuclear hormone receptors (NHRs) are ligand-gated transcription factors comprised of a DNA-binding domain and a ligand-binding domain. The DNA-binding domain directs binding of the NHR to target sequences called hormone response elements (HREs), while ligand binding modulates the regulation of target gene expression. Class I receptors are activated by the binding of steroid ligands but inactive in the absence of ligand (Mangelsdorf et al., 1995). Class II receptors are also activated by ligand binding. However, in the absence of ligand, Class II receptors act with corepressors to repress transcription of target genes. Upon ligand binding, corepressors are released, and subsequent binding of coactivators switches the NHR from a transcriptional repressor to a transcriptional activator (**Figure 2**). Class II receptors typically function as heterodimers with the retinoid X receptor (RXR) (Mangelsdorf and Evans, 1995; Mangelsdorf et al., 1995).

As described above, NHR activity is controlled primarily by the presence or absence of ligand. NHR ligands are small, lipophilic molecules that can pass through membranes without the aid of a transport protein (**Figure 2**). Therefore, signaling is regulated primarily by controlling the production and release of ligand. These ligands are derived from cholesterol or related molecules by a series of biosynthetic steps, as elaborated on in the following subsections. The expression of enzymes that catalyze the biosynthesis of the mature, active form of the ligand

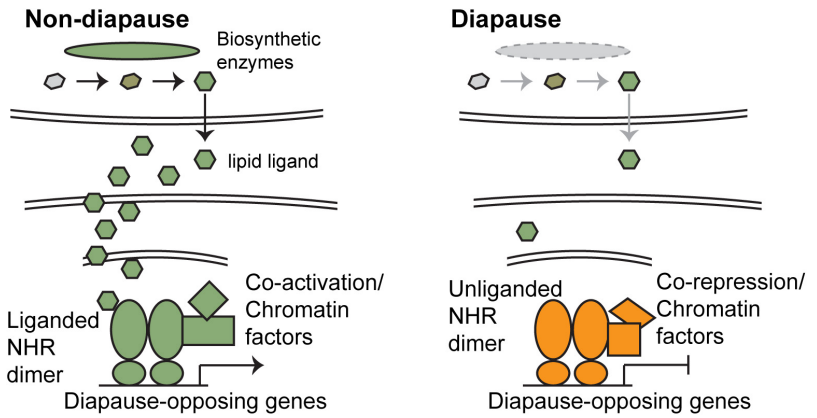


FIGURE 2 | The regulation of diapause by nuclear hormone receptor signaling (NHR). In favorable environments (left), enzymes that catalyze the production of the lipid ligands are present, and these ligands are produced. Because the ligands can freely pass through the plasma and nuclear membrane, they are able to bind to their target NHRs. Ligand-bound NHRs function as transcriptional activators and turn on expression of genes that promote non-dauer development. In unfavorable environments (right), expression of the required enzymes is reduced and little ligand is present. Unliganded NHRs bind to corepressors and repress transcription of target genes, thus promoting diapause. Orange components promote diapause and green components promote non-diapause development. Note that in some insect species, NHR signaling functions opposite to what is described here, in that NHR signaling promotes diapause. See text for additional details.

is regulated to control NHR activity. The components of NHR signaling known to be associated with diapause within the species focused on in this review are listed in **Table 2** and explained in more detail below.

Nuclear Hormone Receptors and the Regulation of Diapause

Class II NHRs play important roles in the regulation of diapause across phyla. The NHRs implicated in diapause are phylogenetically related and include the vitamin D receptor in annual fishes, the ecdysone receptor (EcR) in insects, and DAF-12 in *C. elegans*. In most of these cases, low levels of ligand are associated with diapause whereas ligand-bound NHR leads to non-diapause development (**Figure 2** and **Table 3**).

In *C. elegans, daf-12* was discovered on the basis of the dauer formation phenotypes observed in *daf-12* mutants. Notably, both dauer-defective and dauer-constitutive alleles of *daf-12* were isolated (Antebi et al., 1998). Dauer defective alleles are mutants that failed to enter diapause even in diapause-inducing conditions. Dauer-constitutive alleles enter diapause even in conditions favorable for growth. This complexity stems in part from the two activities of NHRs as activators and repressors. Null alleles remove both activator and repressor functions, whereas other alleles can affect one function more than the other. Null alleles do not enter diapause under any known condition, indicating that *daf-12* activity is required for diapause. By contrast, all of the dauer-constitutive alleles disrupt the ligand binding domain, suggesting that it is the unliganded version of DAF-12 that is required for dauer formation (Antebi et al., 2000). Consistent with this idea, the DIN1S co-repressor was found to bind unliganded DAF-12 and to be required for dauer formation (Ludewig et al., 2004).

As mentioned above, most Class II NHRs function as heterodimers with RXR proteins. However, *C. elegans* lacks a clear RXR ortholog despite the expansion of NHR-encoding genes in *C. elegans*: 284, compared to 48 NHRs in humans (Antebi, 2015). NHRs without clear orthology to RXR have been suggested to play a similar role in some cases, however, whether DAF-12 functions as a homo- or heterodimer is still unknown (Antebi, 2015). DAF-12 does possess a conserved dimerization domain, and the DNA response element to which DAF-12 binds is consistent with either homo- or heterodimerization (Antebi et al., 2000; Shostak et al., 2004; Hochbaum et al., 2011).

In insects, EcR signaling has been studied in depth in *D. melanogaster* and other insects, primarily for its role in molting. Indeed, ecdysteroids are often referred to as molting hormones. In insects, the EcR functions as a heterodimer with the RXR ortholog, Ultraspiracle (USP) (Oro et al., 1990; Koelle et al., 1991; Yao et al., 1992; Thomas et al., 1993). There are three isoforms of EcR: A, B1, and B2, that share DNA-binding and ligand-binding domains but differ at the N-terminus. These isoforms differ in expression pattern and biochemical activity; however, they all function to activate transcription of target genes when bound to ligand, and to repress transcription when ligand-free (Talbot et al., 1993). In some contexts, the unliganded form of EcR is specifically required (Mansilla et al., 2016).

With respect to diapause, EcR signaling plays a regulatory role in many insects, a few of which will be highlighted here. These include reproductive diapause in *D. melanogaster* and embryonic diapause in *B. mori*. For a more comprehensive view of EcR signaling regulating diapause across insect species (see Denlinger et al., 2012). Unlike in *C. elegans*, functional consequences of reduced EcR signaling have not been described in the context of insect diapause, and instead more work has been done manipulating hormone levels, as described in section "Nuclear Hormone Receptor Signaling Ligands and Biosynthetic Enzymes and the Regulation of Diapause."

In the annual fish *A. limnaeus*, a role for the vitamin D receptor (VDR) in the regulation of diapause II (DII) was recently described (Romney et al., 2018). There are two VDR-encoding

TABLE 2 | NHR components and diapause.

Component	Organism	Gene	Expression in diapause vs. non-diapause	References
NHR	*C. elegans*	*daf-12*	Reduced	Antebi et al., 2000
	Insects	*EcR-A*	Reduced[1]	Gu et al., 2021
		EcR-B1	Increased early, reduced later	Gu et al., 2021
		USP	Unchanged	Gu et al., 2021
	A. limnaeus	*VDR-A*	Unchanged	Romney et al., 2018
		VDR-B	Increased	Romney et al., 2018
		RXR	Unchanged	Romney et al., 2018
Rieske oxygenase	*C. elegans*	*daf-36*	Largely unchanged	Rottiers et al., 2006
	Insects	*Nvd*	Reduced[1]	Gu et al., 2021
	A. limnaeus	*LOC106533739*	Reduced	Romney et al., 2018
Cytochrome P450 enzymes	*C. elegans*	*daf-9*	Reduced	Gerisch and Antebi, 2004; Schaedel et al., 2012
	Insects	*Spo*	Reduced[1]	Gu et al., 2021
		Phm	Increased early, reduced later[1]	Gu et al., 2021
		Dib	Unchanged[1]	Gu et al., 2021
		Sad	Unchanged[1]	Gu et al., 2021
		Shd/E20OHase	Reduced[1]	Gu et al., 2021
	A. limnaeus	*vitamin D25-hydroxylase*	Reduced	Romney et al., 2018
		25-hydroxyvitamin D-1a hydroxylase	Reduced	Romney et al., 2018
Phosphatase[2]	Insects	*EPPase*	Reduced	Sonobe and Yamada, 2004; Gu et al., 2021

[1] *The expression pattern of these genes was determined in B. mori (Gu et al., 2021).*
[2] *The ecdysteroid-phosphate phosphatase (EPPase) converts inactive, phosphorylated forms of 20E and its precursors into active forms by removing phosphate groups (Sonobe and Yamada, 2004).*

TABLE 3 | NHR ligands and diapause.

Organism	Active form of ligand	Expression in diapause vs. non-diapause	Effect of ectopic addition of ligand	References
C. elegans	dafachronic acids	Reduced	↓ diapause	Motola et al., 2006; Schaedel et al., 2012
D. melanogaster	20E	Reduced	↓ diapause	Richard et al., 1998, 2001a
B. mori	20E	Reduced	↓ diapause	Makka et al., 2002; Sonobe and Yamada, 2004
A. limnaeus	$1,25(OH)_2D_3$	Reduced	↓ diapause	Romney et al., 2018

genes in *A. limnaeus*: VDR-A and VDR-B. These receptors also bind to target DNA as homodimers or heterodimers, where heterodimerization with RXR is the predominant mode (Carlberg et al., 1993; Carlberg and Campbell, 2013). In *A. limnaeus* embryos the expression of VDR-A and RXR does not change between pre-diapause and pre-escape embryos. However, the expression of VDR-B is increased in pre-diapause embryos relative to those on the escape trajectory (Romney et al., 2018).

Nuclear Hormone Receptor Ligands and Biosynthetic Enzymes and the Regulation of Diapause

As described in the prior section, in the examples given, active NHR signaling promotes non-diapause development. The control of this signaling appears to occur primarily at the level of production of the active ligand. In insects, the bulk of the studies on the role of EcR signaling in diapause have focused on the ligand and the enzymes necessary to produce the active form. The most active EcR ligand is 20-hydroxyecdysone (20E). In *Drosophila*, 20E is synthesized from cholesterol by a series of enzymatic reactions. These reactions begin with cholesterol or plant-derived sterols taken up from the environment, since insects cannot synthesize cholesterol themselves (Niwa and Niwa, 2014). The first step in the pathways is catalyzed by Rieske oxygenase Neverland (Nvd) which converts cholesterol to 7-dehydrocholesterol. Next is a "black box," between 7-dehydrocholesterol and 5β-ketodiol, where the precise reactions are not known. However, the short-chain dehydrogenase Non-molting glossy/Shroud (Nm-g/Sro) and the cytochrome P450 enzymes Spook and Spookier (Spo, Spok) are thought to catalyze reactions within the black box. After that, the rest of the biosynthetic pathway is known, and each step is catalyzed by a series of cytochrome P450 enzymes, including Phantom (Phm), Disembodied (Dib), Shadow (Sad), and Shade (Shd), in that order (Niwa and Niwa, 2014).

To regulate molting, ecdysone is synthesized in the prothoracic glands and then ecdysone is released into the

hemolymph. Ecdysone is then converted into 20E in target tissues around the body (Petryk et al., 2003). However, ecdysone can also be synthesized in the ovaries. In *D. melanogaster*, the strongest evidence for a role for ecdysone signaling in reproductive diapause comes from experiments where injection of diapausing animals with 20E caused recovery, as assessed by the resumption of vitellogenesis (Richard et al., 2001a). Additionally, levels of 20E are low in reproductive diapause animals compared with non-diapause animals. When flies in reproductive diapause are stimulated to recover, the 20E levels rise rapidly (Richard et al., 1998).

Similarly, in *B. mori*, high levels of 20E are found in non-diapause embryos, but not diapause embryos. Instead, diapausing embryos contain high levels of 20E precursors or inactive derivatives. Furthermore, treatment of diapausing embryos with 20E can induce the resumption of development and recovery from diapause (Makka et al., 2002; Sonobe and Yamada, 2004). Therefore, regulation of the production of 20E is necessary to control diapause entry. Because the prothoracic gland has not yet developed in these embryos, the regulation of 20E synthesis differs from the canonical pathway. 20E in embryos is derived from two sources. First, inactive, phosphorylated forms of 20E and its precursors are bound to yolk proteins and maternally packaged into the embryo. Second, 20E is synthesized *de novo*. In both cases, the regulation of enzymes determines whether active 20E is produced. The ecdysteroid-phosphate phosphatase (EPPase) is required to activate the phosphorylated molecules, whereas the enzyme 20-hydroxylase (E20OHase, also called Shade in some species) is required for *de novo* synthesis. The expression of these enzymes is upregulated over time in non-diapause embryos whereas expression remains low during diapause (Sonobe and Yamada, 2004; Maeda et al., 2008; Gu et al., 2021).

Other species with larval and pupal diapause show similar effects whereby increased levels of 20E have been shown to correlate with and/or actively promote non-diapause development. Since EcR signaling is necessary for progression through larval molts and for metamorphosis, it makes sense that removing 20E would allow for developmental arrest. Somewhat surprisingly, however, in some insect species, the role for EcR signaling is reversed, and high levels of 20E are required to maintain diapause. For example, this mode of diapause regulation was shown for the obligate diapause at the end of embryogenesis in the gypsy moth, *Lymantria dispar* (Denlinger et al., 2012; Schiesari and O'Connor, 2013).

In *C. elegans* the first two ligands that activate DAF-12 signaling to be discovered were two 3-Keto-4-Cholestenoic Acids termed Δ^4-dafachronic acid and Δ^7-dafachronic acid (Δ^4-DA and Δ^7-DA) (Motola et al., 2006). The term "dafachronic" comes from the two major roles of DAF-12: the regulation of diapause and the regulation of developmental timing (see section "Developmental Changes Accompanying Diapause in *C. elegans*"). More recent work identified additional DAs, the most prominent being $\Delta^{1,7}$-DA. This study failed to find Δ^4-DA, suggesting that ligand may be less relevant in endogenous contexts (Mahanti et al., 2014).

Fewer steps in the synthesis of DAs are known in detail in comparison to the synthesis of 20E. However, some key enzymes show similar requirements across species. First, the Rieske oxygenase DAF-36 is required for the synthesis of Δ^7-DA. Similar to the role of Nvd in ecdysone synthesis, this enzyme catalyzes the first reaction within this biosynthetic pathway: conversion of cholesterol to 7-dehydrocholesterol (Rottiers et al., 2006; Wollam et al., 2011; Yoshiyama-Yanagawa et al., 2011). Indeed, these enzymes are functionally conserved as addition of *daf-36* to *D. melanogaster* with a mutation in *nvd* rescues the lethal phenotype (Yoshiyama-Yanagawa et al., 2011). The short chain dehydrogenase DHS-16 works further downstream in this pathway. The enzymes involved in the production of other dafachronic acids are unknown. The hydroxysteroid dehydrogenase HSD-1 was proposed to be involved in the production of Δ^4-DA, but subsequent work raises doubts about this notion. At the end of the pathway, the cytochrome P450 enzyme DAF-9 is required to catalyze the final step in biosynthesis of both Δ^4-DA and Δ^7-DA (Antebi, 2015). The expression of *daf-9* is highly regulated during dauer formation, where mildly stressful conditions increase *daf-9* expression, but more severe, dauer-inducing conditions drastically reduce *daf-9* expression, leading to a decrease in DA production and entry into dauer diapause (Gerisch et al., 2001; Gerisch and Antebi, 2004; Schaedel et al., 2012).

In vertebrates, biosynthesis of the active VDR ligand $1,25(OH)_2D_3$ is similar in many respects to the biosynthesis of the invertebrate NHR ligands. Biosynthesis in vertebrates also begins with the conversion of cholesterol to 7-hydroxycholesterol, in this case by the action of the dehydrocholesterol reductase enzyme, DHCR7. Unlike in invertebrates, the next step of the biosynthetic pathway requires UVB light, which enables the conversion of 7-hydroxycholesterol to pre-vitamin D3. Once pre-vitamin D3 is produced, the remainder of the biosynthetic steps are catalyzed by a series of cytochrome P450 enzymes, similar to the invertebrate pathway (Tuckey et al., 2018; Saponaro et al., 2020).

Within the annual fish *A. limnaeus*, expression of the active VDR ligand $1,25(OH)_2D_3$ is reduced in pre-diapause embryos compared to pre-escape embryos. Consistent with this reduction, the expression of several biosynthetic enzymes necessary to produce $1,25(OH)_2D_3$ are also reduced (Romney et al., 2018). This difference in expression is functionally relevant because incubating embryos reared under diapause-inducing conditions with $1,25(OH)_2D_3$ at picomolar concentrations strongly reduced the number of embryos that underwent diapause (Romney et al., 2018). Remarkably, performing a similar experiment with *C. elegans* Δ^4-dafachronic acid also increased the number of embryos following the non-diapause, escape developmental trajectory. This effect was specific because incubating embryos with ligands for the pregnane X, farnesoid X, and liver X receptors, which are closely related to VDR, was ineffective at preventing diapause (Romney et al., 2018). Furthermore, treating *A. limnaeus* embryos grown in non-diapause conditions with dafadine A causes embryos to enter DII. Dafadine A is a compound that inhibits the activity of the cytochrome P450 enzyme DAF-9 and its mammalian counterpart CYP27A1 (vitamin D3 25-hydroxylase) (Luciani et al., 2011).

The diapause-inducing effect of dafadine A in *A. limnaeus* can be countered by addition of $1,25(OH)_2D_3$, consistent with the interpretation that dafadine A promotes diapause by interfering with the production of $1,25(OH)_2D_3$ (Romney et al., 2018). Taken together, these results demonstrate the conserved nature of NHR signaling and its effect on diapause in *A. limnaeus* and *C. elegans*.

Crosstalk Between Hormonal Pathways and the Regulation of Diapause

As described in sections "Insulin/Insulin-Like Growth Factor Signaling and Nuclear Hormone Receptor Signaling," both IIS and NHR pathways regulate development across species. Therefore, there must be interactions between these pathways. Furthermore, additional hormonal pathways contribute to the regulation of diapause. This section provides a brief overview of how those pathways fit together, focusing on the invertebrate systems which lend themselves to in-depth genetic analysis.

Hormonal Pathway Crosstalk in *C. elegans*

Caenorhabditis elegans geneticists have worked out the major regulators of dauer formation in detail. In addition to the IIS and NHR/DAF-12 pathways described in the previous sections, TGFβ signaling is also involved. In brief, TGFβ and IIS pathways operate largely in parallel and both pathways converge to regulate DAF-12 (**Figure 3A**; Baugh and Hu, 2020; Fielenbach and Antebi, 2008).

Favorable environmental cues lead to the production of both DAF-7/TGFβ and multiple insulin-like peptides in sensory neurons (Ren et al., 1996; Schackwitz et al., 1996; Cornils et al., 2011; Murphy and Hu, 2013; Ritter et al., 2013; Fernandes-de-Abreu et al., 2014; Zheng et al., 2018). These signals are released and then received in multiple tissues where DAF-7/TGFβ blocks activity of the downstream DAF-3/SMAD-DAF-5/Ski complex, and insulin signaling blocks the activity of the downstream DAF-16/FOXO transcription factor (Kimura et al., 1997; Lin et al., 1997; Ogg et al., 1997; Patterson et al., 1997; Pierce et al., 2001; da Graca et al., 2004). Both IIS and TGFβ pathways regulate the expression of *daf-9*/CYP, which encodes the last enzyme required to produce dafachronic acid, the ligand for the DAF-12 nuclear hormone receptor (Gerisch et al., 2001; Gerisch and Antebi, 2004; Mak and Ruvkun, 2004; Motola et al., 2006). As described above, ligand-bound DAF-12 promotes continuous (non-dauer diapause) development whereas ligand-free DAF-12 is required for dauer formation (Antebi et al., 1998, 2000; Ludewig et al., 2004). IIS appears to also act in parallel to *daf-12* because when both *daf-2*/IR and *daf-12*/NHR signaling are compromised, larvae arrest development but do not enter dauer. Overexpression of *daf-9* in a *daf-2* mutant background produces a similar arrested phenotype that is different from dauer diapause (Larsen et al., 1995; Gems et al., 1998; Gerisch et al., 2001; Gerisch and Antebi, 2004).

Hormonal Pathway Crosstalk in Insects

In insects, another major regulator of diapause is juvenile hormone (JH). JH is a sesquiterpenoid that is produced in a group of endocrine organs called the corpora allata (CA) in response to the hormone allatotropin (Flatt et al., 2005; Bendena et al., 2020). JH is then secreted and travels through the hemolymph to act on the ovaries, stimulating ovarian development and vitellogenesis. Since reproductive diapause occurs prior to vitellogenesis, it is not surprising that JH is a central regulator of reproductive diapause across insect species (Denlinger et al., 2012).

Juvenile hormone and Insulin/IGF signaling exhibit an interesting and complex relationship in *C. pipiens* (**Figure 3B**). During diapause in *C. pipiens*, JH levels are low, and addition of synthetic JH can induce exit from diapause (Spielman, 1974). Addition of a JH analog is effective at stimulating diapause exit even in IIS mutants, indicating that IIS regulates diapause formation upstream of JH (Sim and Denlinger, 2008, 2009). However, both IIS and JH signaling appear to converge on the same target: negative regulation of FOXO. Negative regulation of FOXO by IIS is expected, but the role of JH is novel. Addition of JH to diapausing animals reduces FOXO levels in the fat body (Sim and Denlinger, 2013b). FOXO appears to be the most direct regulator of diapause genes, including genes that regulate stress-resistance, metabolism, longevity, and other key characteristics of diapause (Sim et al., 2015).

Finally, there is a potential connection to EcR signaling in *C. pipiens* as well. This connection was proposed by Sim and Denlinger and is based on work done in another mosquito species, *Aedes aegypti (A. aegypti)* (Sim and Denlinger, 2013b). In this species, a blood meal enables reproduction by stimulating vitellogenesis. Vitellogenesis requires EcR signaling and the EcR/USP heterodimer directly binds to the promoter of genes encoding yolk proteins in the fat body. JH expression promotes expression of the NHR bFTZ-F1, which in turn recruits the FISC p160/SRC coactivator. These proteins form a complex with EcR and USP at the promoters of target genes and are required for full EcR activation (Zhu et al., 2006). If the same process occurs in *C. pipiens* in the context of diapause, that would forge a connection between the signaling pathways described here.

Similar to the situation in *C. pipiens*, JH levels are reduced in *D. melanogaster* in reproductive diapause. Furthermore, addition of JH to diapausing animals induces recovery from reproductive diapause (Saunders et al., 1990). However, EcR was later proposed to be more directly involved in the regulation of reproductive diapause than JH because levels of ecdysteroids rose more rapidly after stimulation to recover from reproductive diapause, and addition of ecdysteroids to diapausing animals was more effective at terminating reproductive diapause than addition of JH (Richard et al., 1998, 2001b). Unlike in *C. pipiens*, the IIS pathway has been proposed to act downstream of JH and EcR signaling because loss of the insulin receptor substrate protein Chico induces reproductive diapause in an ovary-autonomous manner, suggesting that external signals such as JH and 20E do not affect reproductive diapause in this mutant context. *chico* mutant ovaries did not develop upon transplantation to a wild-type host, whereas wild-type ovaries did develop when transplanted to a *chico* mutant host. Furthermore, *chico* mutants expressed normal levels of JH and ecdysteroids (Richard et al., 2005). Consistent with the notion that IIS is downstream of JH, the addition of a JH inhibitor did affect the expression of *dilp1*, however, the functional consequences of this observation are unclear (Liu et al., 2016).

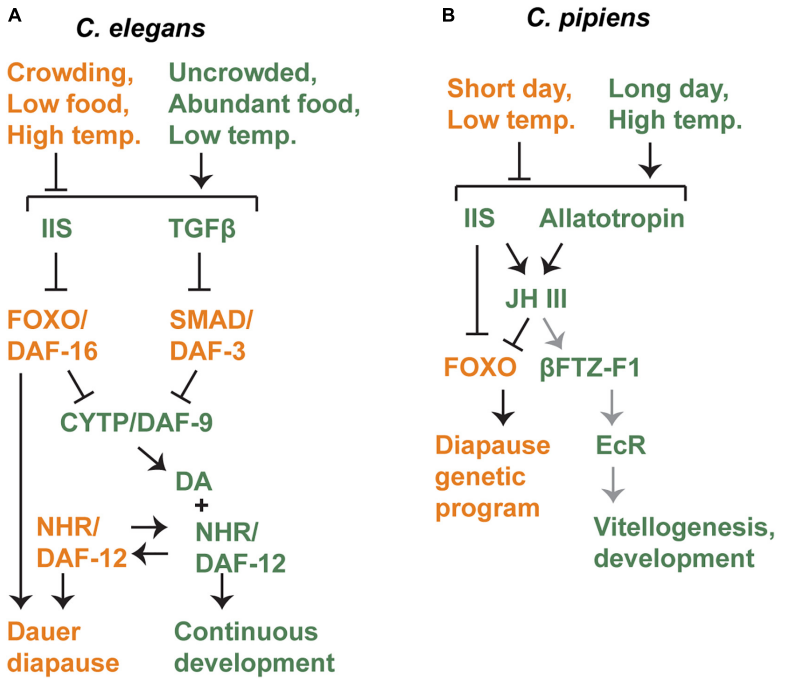


FIGURE 3 | Crosstalk between diapause-regulating pathways in *C. elegans* **(A)** and *C. pipiens* **(B)**. Genetic models outlining the interactions between signaling pathways known to regulate diapause in these two species. Orange components promote diapause and green components promote non-diapause development. In *C. pipiens,* gray arrows indicate interactions that were inferred from a different mosquito species. See text for details.

Diapause in *B. mori* is controlled by a neuropeptide called diapause hormone (DH). This mode of regulation is well studied, though uncommon in other insect species (Denlinger et al., 2012). *B. mori* diapause is transgenerationally programmed (see section "Diapause in Insects: *Drosophila melanogaster*, *Culex pipiens*, and *Bombyx mori*"). Mothers reared in diapause-inducing conditions during their own embryonic development will express DH in the subesophageal ganglion during the pupal stage. DH is released and then received in the ovary via the DH receptor, a G-protein coupled receptor. This stimulus prompts the developing ovaries to induce diapause (Xu et al., 1995b; Yamashita et al., 2001; Homma et al., 2006; Denlinger et al., 2012; Sato et al., 2014). A connection between DH and EcR signaling has been proposed, but experiments testing this connection have yielded few conclusions, such that the relationship between these pathways is still unclear (Xu et al., 1995a; Shiomi et al., 2015). However, MEK/ERK signaling is needed to terminate diapause, and this process appears to occur upstream of EcR signaling (Fujiwara et al., 2006a,b; Schiesari and O'Connor, 2013). Additionally, the IIS pathway has been implicated in diapause in *B. mori,* but it is unclear how this function fits with DH or EcR pathways (Zheng et al., 2016; Gu et al., 2019).

EFFECT OF DIAPAUSE ON DEVELOPMENT

Unlike an immediate response to stress, diapause involves an alternate developmental trajectory, including a preparatory stage prior to diapause, the diapause itself, recovery, and post-diapause development. Each stage can differ from its non-diapause equivalent in terms of morphology, metabolism, and rate of development. Not surprisingly, differences in gene expression often exist between diapause and non-diapause animals at equivalent developmental stages. Developmental pathways must accommodate these changes to allow the normal completion of development as a reproductively mature adult. Across species, the pre-diapause preparatory stage is typically marked by a slower rate of development, metabolic changes and accumulation of lipid stores, and morphological changes. During diapause, development is halted. After diapause, animals must quickly resume growth and development. In *C. elegans,* the same hormonal pathways that regulate diapause appear to also modulate developmental pathways to accommodate diapause, as described below.

Developmental Changes Accompanying Diapause in Annual Fishes

In *A. limnaeus, N. furzeri,* and other annual fishes that undergo facultative DII, visible differences between embryos in the diapause and escape trajectories become apparent around the 18-somite stage. In particular, growth of the head continues more rapidly in escape embryos, and both the length and the width of the pre-DII embryos increase very little until diapause is reached at approximately the 38-somite stage (Podrabsky et al., 2010, 2017; Furness et al., 2015). Other pre-diapause developmental changes include a lack of melanocyte production

and reduced heart rate in pre-DII embryos relative to escape embryos (Podrabsky et al., 2010, 2017; Furness et al., 2015).

The developmental changes that occur prior to DII are accompanied by changes in chromatin structure and gene expression (Toni and Padilla, 2016; Romney and Podrabsky, 2018; Romney et al., 2018; Hu et al., 2020). With respect to development-related gene expression, in *N. furzeri,* genes involved in the Notch, Wnt, FGF, SHH & IHH, TGF, and retinoic acid developmental pathways were all downregulated in pre-DII embryos compared to escape embryos (Hu et al., 2020). In *A. limnaeus*, the expression of microRNAs was examined in pre-DII and escape embryos. Some variants in the miR-10 and miR-430 families were expressed more highly in escape embryos. miR-10 targets Hox genes, whereas miR-430 targets genes involved in the maternal-to-zygotic transition (Romney and Podrabsky, 2018).

Developmental changes during DII have not been described, other than the expected developmental arrest. Upon recovery from DII, cells throughout the embryo re-enter the cell cycle and resume development. Post-DII embryos develop more rapidly than escape embryos in order to catch up in size. By the stage where the embryo covers approximately one half the surface of the yolk, morphological differences between DII and escape embryos are no longer apparent (Podrabsky et al., 2010, 2017; Dolfi et al., 2019). Levels of IGF-I and IGF-II are both increased after DII relative to escape embryos, suggesting that IIS could promote catch-up growth (Woll and Podrabsky, 2017).

Developmental Changes Accompanying Diapause in Insects

In *C. pipiens*, egg follicle length at the onset of diapause is slightly longer than that of non-diapausing animals. However, this length increases rapidly in non-diapause development, whereas during diapause there is no increase for the first 12 days and a very slight increase thereafter (Meuti et al., 2018). mRNA and small RNA sequencing of diapausing and non-diapausing females identified differentially expressed genes encoding proteins and microRNAs. These changes primarily affected genes involved in metabolic processes (Kang et al., 2016; Meuti et al., 2018). These effects are expected given the metabolic changes that occur during diapause. With respect to development, some microRNAs that promote ovarian development were differentially expressed. miR-8-3p and miR-275-3p were downregulated in young, pre-diapause adults, and miR-8-3p, miR-275-3p, and miR-375-3p were all downregulated during diapause compared to non-diapause females (Meuti et al., 2018). In *B. mori*, embryos enter diapause beginning approximately 48 h after oviposition. During the day leading up to diapause, the color of the embryos shifts from pale yellow to reddish (Zhang H. et al., 2017; Gong et al., 2020). mRNA sequencing experiments performed during the pre-diapause pupal stage identified genes involved in the Hippo pathway that regulates organ size. The upstream regulator *lft* was downregulated and the downstream regulator *hth* was upregulated in pre-diapause animals. Genes involved in regulating metabolism were also differentially regulated (Chen et al., 2017).

Developmental Changes Accompanying Diapause in *C. elegans*

Caenorhabditis elegans development is understood in detail, in part due to the invariant cell lineage that has been mapped, so that individual cells can be identified and followed throughout development (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston et al., 1983). The ability to study development at the single-cell level coupled with the powerful genetic tools available in this model organism has resulted in some of the most detailed understanding currently available of the effect of diapause on development. In many cases, these changes are induced by the same hormonal pathways that regulate the diapause decision. In other words, these pathways coordinate the decision to enter diapause with the developmental consequences of diapause. These changes are outlined in this subsection and summarized in **Figure 4**.

As described in section "Diapause in Nematodes: *Caenorhabditis elegans*," *C. elegans* development consists of embryogenesis followed by four larval stages (L1-L4). Dauer diapause occurs immediately after the second larval stage (Cassada and Russell, 1975). Adverse environmental conditions sensed during the first larval stage will induce entry into the alternative, pre-dauer stage called L2d. During L2d, larvae prepare for dauer by increasing lipid stores as they continue to sense their environment. After L2d, larvae can either continue development as L3 stage larvae or molt into dauer diapause, depending on environmental conditions. The L2d stage lasts at least 50% longer than the non-dauer L2 stage (Golden and Riddle, 1984b). There are several developmental events that occur during the second larval stage, and therefore these must occur at a slower pace in L2d larvae, as described below. During dauer, development is paused at the equivalent of an early L3-staged larva (Cassada and Russell, 1975). Progenitor cells that have not yet completed development must retain the capacity to take on normal cell fate in the event of recovery. As described below, this seems to be an active process. Finally, if favorable conditions are found, *C. elegans* dauer larvae undergo a process of recovery and resume development. Post-dauer larvae must complete the developmental events that normally occur in the L3 stage before molting into an L4-staged larva (Cassada and Russell, 1975).

As in insects and annual fishes, chromatin modification and gene expression changes have been identified when comparing pre-dauer, dauer, or post-dauer animals to their developmental equivalents. Differentially expressed genes include protein-coding genes and small RNAs, and in at least some cases effects on developmental pathways have been observed (Liu et al., 2004; Harvey et al., 2009; Hall et al., 2010, 2013; Karp et al., 2011). However, the remainder of this subsection will focus on three developmental contexts and how hormone pathways affect their development in the diapause life history.

Effect of Diapause on Germline and Somatic Gonad Development in *C. elegans*

When L1 larvae hatch from embryos, their germline consists of two cells. In response to Notch signaling, these cells proliferate and expand the germline stem cell population over

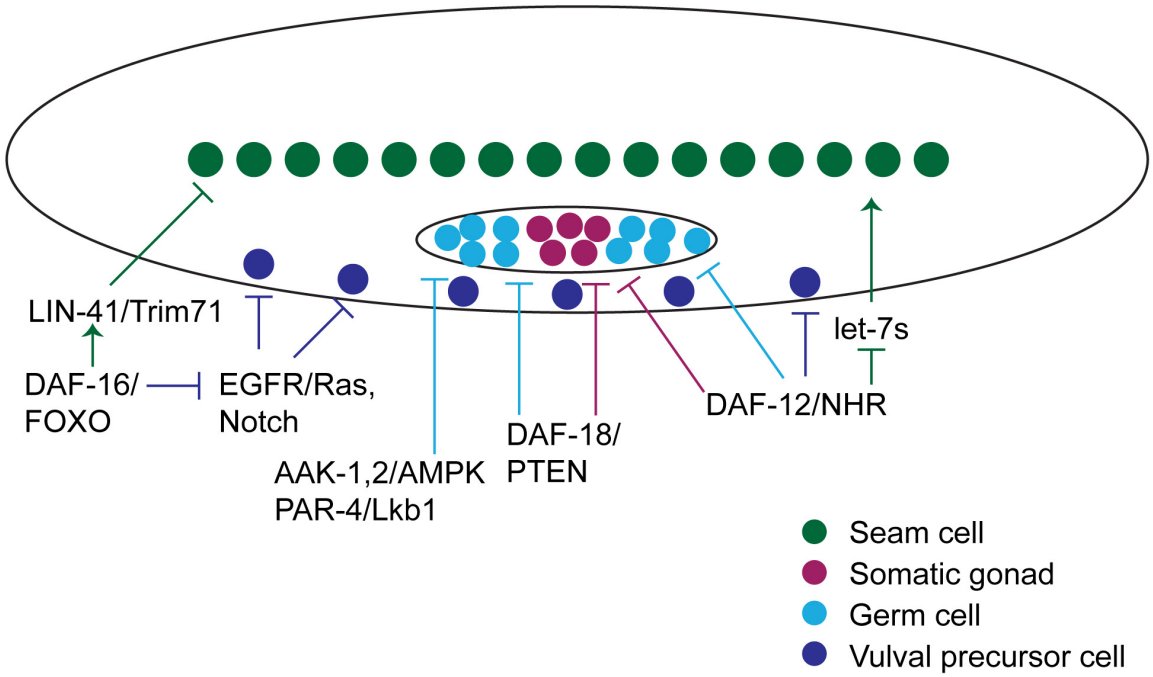


FIGURE 4 | Hormonal pathways regulate development during diapause in *C. elegans*. Graphical summary of the hormonal pathways that interface with developmental pathways to accommodate changes that occur during the diapause developmental trajectory. A diagram of a worm is shown, with the cell types discussed in the text featured. Arrows pointing at cells indicate processes that promote developmental progression, whereas blunt arrows pointing at cells indicate processes that inhibit developmental progression. See text for details.

the first several larval stages. Meiosis does not begin until after dauer would have occurred, during the mid-L3 stage (Hubbard and Schedl, 2019).

During L2d, germ cell proliferation slows as larvae prepare to enter dauer, and proliferation ceases during dauer diapause (Narbonne and Roy, 2006). Reducing the rate of germ cell proliferation in L2d requires several genes within the AMPK and IIS pathways, including genes encoding both subunits of the energy-sensing AMP-activated Protein Kinase (AMPK), called *aak-1* and *aak-2* in worms, the gene encoding the AMPK-activating kinase PAR-4/LKB1, and the gene encoding the PTEN phosphatase DAF-18 (**Figure 4**). Although these genes all affect related pathways, they are each required independently. Furthermore, these pathways function downstream of or in parallel to the Notch pathway that promotes germ cell division in favorable environments (Narbonne and Roy, 2006, 2009; Tenen and Greenwald, 2019).

In addition to its importance in slowing germ cell proliferation in L2d larvae, AMPK is required during and after dauer to regulate small RNA pathways that in turn maintain proper chromatin marks and therefore affect gene expression. Dauer and post-dauer larvae that lack *aak-1* display aberrant gene expression and post-dauer adults are sterile. Remarkably, AMPK activity is required in somatic cells for these functions in germ cells (Kadekar and Roy, 2019).

daf-18/PTEN is unique among the genes listed above in that it is required in the somatic gonad for quiescence in both the germ line and the somatic gonad (Tenen and Greenwald, 2019). Furthermore, *daf-18* is required to maintain developmental arrest during dauer in the somatic gonad progenitor cells, because cell fate markers that indicate developmental decisions that normally occur in the L3 or L4 stage are observed in dauer larvae that lack *daf-18* (Tenen and Greenwald, 2019).

In parallel to AMPK and IIS signaling, the DAF-12 NHR and its co-repressor DIN-1S also inhibit proliferation of germline and somatic gonad cells in the L2d stage (**Figure 4**). In contrast to *aak-1* and *daf-18* that function non-cell-autonomously in somatic cells, *din-1s* is required autonomously in germ cells to prevent germ cell proliferation and in somatic gonad cells to prevent their proliferation (Colella et al., 2016).

Effect of Diapause on Vulval Precursor Development in *C. elegans*

Developmental pathways appear to be not only inactivated but actively re-set during dauer diapause. Some of the best evidence for active re-setting of developmental pathways comes from work examining the vulval precursor cells (VPCs). VPCs are born during the L1 stage and remain multipotent and unspecified until the L3 stage. During the L3 stage these cells respond to a combination of EGFR/Ras and Notch signaling pathways to specify VPCs to one of two vulval cell fates (1° and 2°). The absence of signaling leads to a default non-vulval cell fate (3°) (Sternberg, 2005). In wild-type larvae, 1° cell fate markers are expressed in the presumptive 1° VPC during the molt into dauer diapause, but this specification is erased during dauer. After recovery from dauer, larvae enter

the post-dauer L3 (PDL3) stage that is the developmental equivalent of the L3 stage that occurs during the non-diapause developmental trajectory. VPC specification is again initiated in PDL3 (Karp and Greenwald, 2013).

The re-setting of VPC specification during dauer is more apparent in mutants that cause VPC specification prior to dauer. There are two categories of these mutants. First, loss of the heterochronic gene *lin-28* causes precocious VPC specification during the L2 stage (Ambros and Horvitz, 1984). Larvae lacking *lin-28* that develop through the dauer trajectory show VPC specification during the pre-dauer L2d stage. This specification is erased during dauer, and then re-initiates in VPCs or their descendants in post-dauer larvae (Euling and Ambros, 1996; Karp and Greenwald, 2013). Second, mutants in which EGFR/Ras or LIN-12/Notch signaling have been activated also show VPC specification during the pre-dauer L2d stage. Again, this specification is lost during dauer (Karp and Greenwald, 2013). Therefore, there is an active mechanism that erases any VPC specification that has occurred prior to dauer formation and re-establishes multipotent VPC fate.

Both DAF-12/NHR signaling and DAF-16/FOXO have been implicated in VPC development during dauer (**Figure 4**). Mutations in *daf-12*/NHR*, din-1s,* which encodes a corepressor that binds unliganded DAF-12, or *daf-9*, which encodes an enzyme required for synthesis of the DAF-12 ligand, all result in VPC division and vulval development during dauer (Karp and Greenwald, 2013; Colella et al., 2016). However, this phenotype is suppressed when *daf-12* or *daf-9* mutants are exposed to dauer-inducing conditions (dauer pheromone), suggesting that these genes are not the most proximal regulators of VPC quiescence. By contrast, loss of *daf-16* results in VPC division as well as adoption of 1° and 2° fate markers, even in the presence of dauer pheromone (Karp and Greenwald, 2013). Therefore, during dauer, *daf-16*/FOXO blocks EGFR/Ras and LIN-12/Notch signaling to prevent precocious specification.

Effect of Diapause on Epidermal Seam Cell Development in *C. elegans*

One of the developmental pathways most profoundly affected by diapause is the "heterochronic" pathway that controls stage-specific cell fate, particularly in the stem-cell-like seam cells within the lateral hypodermis. Seam cells divide in a particular pattern and sequence at each larval stage before terminally differentiating at adulthood. The gene network that controls this pattern of stage-specific cell divisions and differentiation is termed the "heterochronic" pathway (Ambros and Horvitz, 1984). These heterochronic genes function as a molecular timer whereby transcription factors and RNA-binding proteins that specify early cell fate are expressed at each larval stage. These early-promoting factors are then downregulated by microRNAs in order to allow progression to the next cell fate (Rougvie and Moss, 2013). A molecular timer would seem to be less useful in an interrupted developmental trajectory, and perhaps for that reason the heterochronic pathway is altered in larvae whose development is interrupted by dauer diapause (Liu and Ambros, 1991). For example, microRNA activity is modulated in L2d and dauer larvae, so that a different set of microRNAs becomes more important in the dauer diapause context (Karp and Ambros, 2012; Ilbay and Ambros, 2019).

DAF-12/NHR plays an important role in coordinating the decision to enter dauer with seam cell fate (**Figure 4**). In diapause-inducing conditions, there is little of the DAF-12 ligand produced and DAF-12 binds to its co-repressor, DIN-1S to promote dauer formation. DAF-12 also directly regulates the transcription of heterochronic microRNAs within the *let-7* family (Bethke et al., 2009). These microRNAs are upregulated in order to promote L3 cell fate. In the lengthened L2d stage, the repressor form of DAF-12 keeps levels of these microRNAs low and L3 fate is not adopted prematurely (Bethke et al., 2009; Hammell et al., 2009). The *let-7* family microRNAs also directly target *daf-12*, forming a feedback loop that helps to coordinate the dauer formation decision with the regulation of seam cell development (Hammell et al., 2009).

As described in section "Effect of Diapause on Vulval Precursor Development in *C. elegans*," VPC fate appears to be re-set during dauer. A similar type of re-setting appears to occur in lateral hypodermal cells, including the seam cells. This type of re-setting was initially inferred based on the observation that the seam cell lineage defects that occur in certain heterochronic mutants were corrected in larvae that developed through the diapause trajectory (Liu and Ambros, 1991). More recently, a role for *daf-16*/FOXO was discovered in these cells. *daf-16*/FOXO is required in dauer larvae to prevent the precocious expression of collagens that are normally enriched in adults. *daf-16*/FOXO appears to act partially via known heterochronic genes and partially via a novel mechanism. Specifically, *daf-16*/FOXO opposes expression of the *lin-41*/TRIM71 RNA-binding protein during dauer to block adult cell fate. However, *lin-41*/TRIM71 does not act via its canonical target, the LIN-29 transcription factor, indicating a novel mechanism at play during dauer (Wirick et al., 2021). Thus, *daf-16*/FOXO coordinates the diapause developmental trajectory with multiple developmental events (**Figure 4**).

CONCLUSION

Diapause is a commonly used mechanism to help animals in the wild increase their chances of survival and reproduction in changing environmental conditions. Since diapause occurs at the level of the organism, individual cells and tissues must respond to diapause-inducing cues in a consistent manner. Hormonal pathways function to relay these cues across the organism, coordinating the developmental trajectory according to current or predicted environmental conditions. Many of the pathways that regulate diapause also regulate growth and development in non-diapause contexts. One possibility is that negative regulation of these growth-promoting pathways is a common molecular mechanism that enabled the evolution of diapause in different species.

The IIS and NHR hormonal pathways are involved in the diapause decision across animal phyla. IIS appears to function universally as an anti-diapause pathway. The IIS pathway has two evolutionarily conserved features that would seem

to make this pathway particularly well-suited to this role. First, IIS positively regulates growth and metabolism in a variety of contexts. Second, production of insulin and related ligands is regulated by environmental cues, including nutritional status (Oldham and Hafen, 2003; Tothova and Gilliland, 2007; Murphy and Hu, 2013).

Nuclear hormone receptor signaling also commonly opposes diapause, but this direction is not universal among insect species. Class II NHRs are also well-positioned to serve as environmentally responsive regulators of diapause, given their function as molecular switches between transcriptional repressors and activators (Mangelsdorf et al., 1995). This switch function allows them to promote growth when ligand is present and inhibit growth when ligand is absent. In insects EcR signaling is required for each molt, making negative regulation of EcR signaling a straightforward way to pause developmental progression. It is interesting that among the large and diverse family of NHRs, the NHRs involved in regulating diapause across phyla are closely related, such that addition of *C. elegans* ligands or inhibitors can control diapause in the annual fish *A. limnaeus* (Romney et al., 2018). These observations indicate a high degree of conservation at the molecular level between these two species.

In addition to IIS and NHR pathways, other hormonal and non-hormonal pathways that regulate growth and development are also involved in the diapause decision in each species. Some examples of these pathways are juvenile hormone in insects and growth-factor-mediated signaling in *C. elegans* and insects (Fielenbach and Antebi, 2008; Denlinger et al., 2012; Schiesari and O'Connor, 2013). It makes sense that the use of juvenile hormone to regulate diapause occurs only in insects, since juvenile hormone does not regulate growth and development in nematodes or fishes. However, in other cases it is unclear why some pathways regulate diapause only in particular species. The differences may relate to the specific role of such pathways in growth and development in that species, or how readily those pathways could integrate with diapause-inducing machinery.

In *C. elegans,* the same hormonal pathways that regulate the decision to enter diapause are also critical regulators of the alterations to developmental programs that occur to accommodate the pre-diapause preparatory phase, the interruption to development that occurs during diapause, and the post-diapause phase where development is rejoined. In the future it will be interesting to discover the extent to which these pathways also regulate development in insects and annual fishes that undergo diapause.

AUTHOR CONTRIBUTIONS

XK wrote this manuscript in its entirety.

FUNDING

XK was supported by NSF CAREER award 1652283.

ACKNOWLEDGMENTS

I would like to thank members of my lab for helpful discussions.

REFERENCES

Ailion, M., and Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in *Caenorhabditis elegans*. *Genetics* 165, 127–144.

Ambros, V., and Horvitz, H. R. (1984). Heterochronic mutants of the nematode *Caenorhabditis elegans*. *Science* 226, 409–416.

Anduaga, A. M., Nagy, D., Costa, R., and Kyriacou, C. P. (2018). Diapause in *Drosophila melanogaster* – Photoperiodicity, cold tolerance and metabolites. *J. Insect. Physiol.* 105, 46–53. doi: 10.1016/j.jinsphys.2018.01.003

Antebi, A. (2015). Nuclear receptor signal transduction in *C. elegans*. *WormBook* 1–49. doi: 10.1895/wormbook.1.64.2

Antebi, A., Culotti, J. G., and Hedgecock, E. M. (1998). daf-12 regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. *Development* 125, 1191–1205.

Antebi, A., Yeh, W. H., Tait, D., Hedgecock, E. M., and Riddle, D. L. (2000). daf-12 encodes a nuclear receptor that regulates the Dauer diapause and developmental age in *C. elegans*. *Genes Dev.* 14, 1512–1527. doi: 10.1101/gad.14.12.1512

Arezo, M. J., Papa, N. G., Berois, N., Clivio, G., Montagne, J., and De la Piedra, S. (2017). Annual killifish adaptations to ephemeral environments: diapause I in two austrolebias species. *Dev. Dyn.* 246, 848–857. doi: 10.1002/dvdy.24580

Arunkumar, K. P., Metta, M., and Nagaraju, J. (2006). Molecular phylogeny of silkmoths reveals the origin of domesticated silkmoth, *Bombyx mori* from Chinese *Bombyx mandarina* and paternal inheritance of *Antheraea proylei* mitochondrial DNA. *Mol. Phylogenet. Evol.* 40, 419–427. doi: 10.1016/j.ympev.2006.02.023

Avery, L. (2014). A model of the effect of uncertainty on the *C. elegans* L2/L2d decision. *PLoS One* 9:e100580. doi: 10.1371/journal.pone.0100580

Barberà, M., Cañas-Cañas, R., and Martínez-Torres, D. (2019). Insulin-like peptides involved in photoperiodism in the aphid *Acyrthosiphon pisum*. *Insect. Biochem. Mol.* 112:103185. doi: 10.1016/j.ibmb.2019.103185

Baugh, L. R., and Hu, P. J. (2020). Starvation responses throughout the *Caenorhabditis elegans* life cycle. *Genetics* 216, 837–878. doi: 10.1534/genetics.120.303565

Bendena, W. G., Hui, J. H. L., Chin-Sang, I., and Tobe, S. S. (2020). Neuropeptide and microRNA regulators of juvenile hormone production. *Gen. Comp. Endocr.* 295:113507. doi: 10.1016/j.ygcen.2020.113507

Bethke, A., Fielenbach, N., Wang, Z., Mangelsdorf, D. J., and Antebi, A. (2009). Nuclear hormone receptor regulation of microRNAs controls developmental progression. *Science* 324, 95–98. doi: 10.1126/science.1164899

Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Juo, P., Hu, L. S., et al. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96, 857–868. doi: 10.1016/s0092-8674(00)80595-4

Butcher, R. A., Fujita, M., Schroeder, F. C., and Clardy, J. (2007). Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. *Nat. Chem. Biol.* 3, 420–422. doi: 10.1038/nchembio.2007.3

Byerly, L., Cassada, R. C., and Russell, R. L. (1976). The life cycle of the nematode Caenorhabditis elegans. I. Wild-type growth and reproduction. *Dev. Biol.* 51, 23–33.

Cambron, L. D., Yocum, G. D., Yeater, K. M., and Greenlee, K. J. (2021). Overwintering conditions impact insulin pathway gene expression in diapausing *Megachile rotundata*. *Comp. Biochem. Physiol. Mol. Integr. Physiol.* 256:110937. doi: 10.1016/j.cbpa.2021.110937

Carlberg, C., Bendik, I., Wyss, A., Meier, E., Sturzenbecker, L. J., Grippo, J. F., et al. (1993). Two nuclear signalling pathways for vitamin D. *Nature* 361, 657–660. doi: 10.1038/361657a0

Carlberg, C., and Campbell, M. J. (2013). Vitamin D receptor signaling mechanisms: integrated actions of a well-defined transcription factor. *Steroids* 78, 127–136. doi: 10.1016/j.steroids.2012.10.019

Cassada, R. C., and Russell, R. L. (1975). The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 46, 326–342.

Chen, H., Wu, G., Zhou, H., Dai, X., Steeghs, N. W. F., Dong, X., et al. (2021). Hormonal regulation of reproductive diapause that occurs in the year-round mass rearing of *Bombus terrestris* queens. *J. Proteome Res.* 20, 2240–2250. doi: 10.1021/acs.jproteome.0c00776

Chen, Y., Jiang, T., Zhu, J., Xie, Y., Tan, Z., Chen, Y., et al. (2017). Transcriptome sequencing reveals potential mechanisms of diapause preparation in bivoltine silkworm *Bombyx mori* (Lepidoptera: Bombycidae). *Comp. Biochem. Physiol. D Genomics Proteomics* 24, 68–78. doi: 10.1016/j.cbd.2017.07.003

Colella, E., Li, S., and Roy, R. (2016). Developmental and cell cycle quiescence is mediated by the nuclear hormone receptor coregulator DIN-1S in the *Caenorhabditis elegans* dauer larva. *Genetics* 203, 1763–1776. doi: 10.1534/genetics.116.191858

Cornils, A., Gloeck, M., Chen, Z., Zhang, Y., and Alcedo, J. (2011). Specific insulin-like peptides encode sensory information to regulate distinct developmental processes. *Development* 138, 1183–1193. doi: 10.1242/dev.060905

Crook, M. (2013). The dauer hypothesis and the evolution of parasitism: 20 years on and still going strong. *Int. J. Parasitol.* 44, 1–8. doi: 10.1016/j.ijpara.2013.08.004

Czech, M. P. (1989). Signal transmission by the insulin-like growth factors. *Cell* 59, 235–238. doi: 10.1016/0092-8674(89)90281-x

da Graca, L. S., Zimmerman, K. K., Mitchell, M. C., Kozhan-Gorodetska, M., Sekiewicz, K., Morales, Y., et al. (2004). DAF-5 is a Ski oncoprotein homolog that functions in a neuronal TGF beta pathway to regulate *C. elegans* dauer development. *Development* 131, 435–446. doi: 10.1242/dev.00922

David, J. R., and Capy, P. (1988). Genetic variation of *Drosophila melanogaster* natural populations. *Trends Genet.* 4, 106–111. doi: 10.1016/0168-9525(88)90098-4

Denlinger, D. L., and Armbruster, P. A. (2013). Mosquito diapause. *Annu. Rev. Entomol.* 59, 73–93. doi: 10.1146/annurev-ento-011613-162023

Denlinger, D. L., Yocum, G. D., and Rinehart, J. P. (2012). "Hormonal control of diapause," in *Insect Endocrinology*, ed. L. I. Gilbert (Amsterdam: Elsevier), 430–463. doi: 10.1016/b978-0-12-384749-2.10010-x

Diniz, D. F. A., de Albuquerque, C. M. R., Oliva, L. O., Melo-Santos, M. A. V., de, and Ayres, C. F. J. (2017). Diapause and quiescence: dormancy mechanisms that contribute to the geographical expansion of mosquitoes and their evolutionary success. *Parasit. Vectors* 10:310. doi: 10.1186/s13071-017-2235-0

Dolfi, L., Ripa, R., Antebi, A., Valenzano, D. R., and Cellerino, A. (2019). Cell cycle dynamics during diapause entry and exit in an annual killifish revealed by FUCCI technology. *Evodevo* 10:29. doi: 10.1186/s13227-019-0142-5

Eldridge, B. F. (1966). Environmental control of ovarian development in mosquitoes of the *Culex pipiens* complex. *Science* 151, 826–828. doi: 10.1126/science.151.3712.826

Ellis, R. E., and Lin, S.-Y. (2014). The evolutionary origins and consequences of self-fertility in nematodes. *F1000Prime Rep.* 6:62. doi: 10.12703/p6-62

Emerson, K. J., Uyemura, A. M., McDaniel, K. L., Schmidt, P. S., Bradshaw, W. E., and Holzapfel, C. M. (2009). Environmental control of ovarian dormancy in natural populations of *Drosophila melanogaster*. *J. Comp. Physiol.* 195, 825–829. doi: 10.1007/s00359-009-0460-5

Euling, S., and Ambros, V. (1996). Reversal of cell fate determination in *Caenorhabditis elegans* vulval development. *Development* 122, 2507–2515.

Félix, M.-A., and Duveau, F. (2012). Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol.* 10:59. doi: 10.1186/1741-7007-10-59

Fernandes-de-Abreu, D. A., Caballero, A., Fardel, P., Stroustrup, N., Chen, Z., Lee, K., et al. (2014). An insulin-to-insulin regulatory network orchestrates phenotypic specificity in development and physiology. *PLoS Genet.* 10:e1004225. doi: 10.1371/journal.pgen.1004225

Fielenbach, N., and Antebi, A. (2008). *C. elegans* dauer formation and the molecular basis of plasticity. *Genes Dev.* 22, 2149–2165. doi: 10.1101/gad.1701508

Flatt, T., Tu, M., and Tatar, M. (2005). Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. *Bioessays* 27, 999–1010. doi: 10.1002/bies.20290

Frézal, L., and Félix, M.-A. (2015). *C. elegans* outside the Petri dish. *eLife* 4:e05849. doi: 10.7554/elife.05849

Fujiwara, Y., Shindome, C., Takeda, M., and Shiomi, K. (2006a). The roles of ERK and P38 MAPK signaling cascades on embryonic diapause initiation and termination of the silkworm, *Bombyx mori*. *Insect Biochem. Mol.* 36, 47–53. doi: 10.1016/j.ibmb.2005.10.005

Fujiwara, Y., Tanaka, Y., Iwata, K., Rubio, R. O., Yaginuma, T., Yamashita, O., et al. (2006b). ERK/MAPK regulates ecdysteroid and sorbitol metabolism for embryonic diapause termination in the silkworm. *Bombyx mori*. *J. Insect. Physiol.* 52, 569–575. doi: 10.1016/j.jinsphys.2006.02.004

Furdui, E. M., Mărghitaş, L. A., Dezmirean, D. S., Paşca, I., Pop, I. F., Erler, S., et al. (2014). Genetic characterization of *Bombyx mori* (Lepidoptera: Bombycidae) breeding and hybrid lines with different geographic origins. *J. Insect. Sci.* 14:211. doi: 10.1093/jisesa/ieu073

Furness, A. I., Reznick, D. N., Springer, M. S., and Meredith, R. W. (2015). Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. *Proc. R. Soc. B Biol. Sci.* 282:20142189. doi: 10.1098/rspb.2014.2189

Gao, L., Yuan, Z., Zhou, T., Yang, Y., Gao, D., Dunham, R., et al. (2019). FOXO genes in channel catfish and their response after bacterial infection. *Dev. Comp. Immunol.* 97, 38–44. doi: 10.1016/j.dci.2019.03.010

Gems, D., Sutton, A. J., Sundermeyer, M. L., Albert, P. S., King, K. V., Edgley, M. L., et al. (1998). Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* 150, 129–155.

Gerisch, B., and Antebi, A. (2004). Hormonal signals produced by DAF-9/cytochrome P450 regulate *C. elegans* dauer diapause in response to environmental cues. *Development* 131, 1765–1776. doi: 10.1242/dev.01068

Gerisch, B., Weitzel, C., Kober-Eisermann, C., Rottiers, V., and Antebi, A. (2001). A hormonal signaling pathway influencing *C. elegans* metabolism, reproductive development, and life span. *Dev. Cell* 1, 841–851.

Golden, J. W., and Riddle, D. L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. *Science* 218, 578–580.

Golden, J. W., and Riddle, D. L. (1984a). A *Caenorhabditis elegans* dauer-inducing pheromone and an antagonistic component of the food supply. *J. Chem. Ecol.* 10, 1265–1280. doi: 10.1007/bf00988553

Golden, J. W., and Riddle, D. L. (1984b). The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev. Biol.* 102, 368–378.

Gong, J., Zheng, X., Zhao, S., Yang, L., Xue, Z., Fan, Z., et al. (2020). Early molecular events during onset of diapause in silkworm eggs revealed by transcriptome analysis. *Int. J. Mol. Sci.* 21:6180. doi: 10.3390/ijms21176180

Gottlieb, S., and Ruvkun, G. (1994). daf-2, daf-16 and *daf-23*: genetically interacting genes controlling dauer formation in *Caenorhabditis elegans*. *Genetics* 137, 107–120.

Gu, S., Chen, C., and Lin, P. (2021). Changes in expressions of ecdysteroidogenic enzyme and ecdysteroid signaling genes in relation to *Bombyx* embryonic development. *J. Exp Zool. Ecol. Integr. Physiol.* 335, 477–488. doi: 10.1002/jez.2466

Gu, S.-H., Lin, P.-L., and Hsieh, H.-Y. (2019). Bombyxin/Akt signaling in relation to the embryonic diapause process of the silkworm, *Bombyx mori*. *J. Insect Physiol.* 116, 32–40. doi: 10.1016/j.jinsphys.2019.04.007

Hall, S. E., Beverly, M., Russ, C., Nusbaum, C., and Sengupta, P. (2010). A cellular memory of developmental history generates phenotypic diversity in *C. elegans*. *Curr. Biol. CB* 20, 149–155. doi: 10.1016/j.cub.2009.11.035

Hall, S. E., Chirn, G. W., Lau, N. C., and Sengupta, P. (2013). RNAi pathways contribute to developmental history-dependent phenotypic plasticity in *C. elegans*. *RNA* 19, 306–319. doi: 10.1261/rna.036418.112

Hammell, C. M., Karp, X., and Ambros, V. (2009). A feedback circuit involving let-7-family miRNAs and DAF-12 integrates environmental signals and developmental timing in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18668–18673. doi: 10.1073/pnas.0908131106

Hand, S. C., Denlinger, D. L., Podrabsky, J. E., and Roy, R. (2016). Mechanisms of animal diapause: recent developments from nematodes, crustaceans, insects,

and fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 310, R1193–R1211. doi: 10.1152/ajpregu.00250.2015

Hao, K., Jarwar, A. R., Ullah, H., Tu, X., Nong, X., and Zhang, Z. (2019). Transcriptome sequencing reveals potential mechanisms of the maternal effect on egg diapause induction of *Locusta migratoria*. *Int. J. Mol. Sci.* 20:1974. doi: 10.3390/ijms20081974

Harvey, S. C., Barker, G. L., Shorto, A., and Viney, M. E. (2009). Natural variation in gene expression in the early development of dauer larvae of *Caenorhabditis elegans*. *BMC Genomics* 10:325. doi: 10.1186/1471-2164-10-325

Hochbaum, D., Zhang, Y., Stuckenholz, C., Labhart, P., Alexiadis, V., Martin, R., et al. (2011). DAF-12 regulates a connected network of genes to ensure robust developmental decisions. *PLoS Genet.* 7:e1002179. doi: 10.1371/journal.pgen.1002179.g009

Homma, T., Watanabe, K., Tsurumaru, S., Kataoka, H., Imai, K., Kamba, M., et al. (2006). G protein-coupled receptor for diapause hormone, an inducer of *Bombyx* embryonic diapause. *Biochem. Biophys. Res. Co* 344, 386–393. doi: 10.1016/j.bbrc.2006.03.085

Hsu, S. Y., Nakabayashi, K., Nishi, S., Kumagai, J., Kudo, M., Sherwood, O. D., et al. (2002). Activation of orphan receptors by the hormone relaxin. *Science* 295, 671–674. doi: 10.1126/science.1065654

Hu, C.-K., Wang, W., Brind'Amour, J., Singh, P. P., Reeves, G. A., Lorincz, M. C., et al. (2020). Vertebrate diapause preserves organisms long term through Polycomb complex members. *Science* 367, 870–874. doi: 10.1126/science.aaw2601

Hubbard, E. J. A., and Schedl, T. (2019). Biology of the *Caenorhabditis elegans* germline stem cell system. *Genetics* 213, 1145–1188. doi: 10.1534/genetics.119.300238

Ilbay, O., and Ambros, V. (2019). Pheromones and nutritional signals regulate the developmental reliance on let-7 family microRNAs in *C. elegans*. *Curr. Biol.* 29, 1735.e–1745.e. doi: 10.1016/j.cub.2019.04.034

Jeong, P.-Y., Jung, M., Yim, Y.-H., Kim, H., Park, M., Hong, E., et al. (2005). Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* 433, 541–545. doi: 10.1038/nature03201

Jones, J. I., and Clemmons, D. R. (1995). Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.* 16, 3–34. doi: 10.1210/edrv-16-1-3

Kadekar, P., and Roy, R. (2019). AMPK regulates germline stem cell quiescence and integrity through an endogenous small RNA pathway. *PLoS Biol.* 17:e3000309. doi: 10.1371/journal.pbio.3000309

Kang, D. S., Cotten, M. A., Denlinger, D. L., and Sim, C. (2016). Comparative transcriptomics reveals key gene expression differences between diapausing and non-diapausing adults *of Culex pipiens*. *PLoS One* 11:e0154892. doi: 10.1371/journal.pone.0154892

Kaplan, F., Srinivasan, J., Mahanti, P., Ajredini, R., Durak, O., Nimalendran, R., et al. (2011). Ascaroside expression in *Caenorhabditis elegans* is strongly dependent on diet and developmental stage. *PLoS One* 6:e17804. doi: 10.1371/journal.pone.0017804

Karp, X., and Ambros, V. (2012). Dauer larva quiescence alters the circuitry of microRNA pathways regulating cell fate progression in *C. elegans*. *Development* 139, 2177–2186. doi: 10.1242/dev.075986

Karp, X., and Greenwald, I. (2013). Control of cell-fate plasticity and maintenance of multipotency by DAF-16/FoxO in quiescent *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2181–2186. doi: 10.1073/pnas.1222377110

Karp, X., Hammell, M., Ow, M. C., and Ambros, V. (2011). Effect of life history on microRNA expression during *C. elegans* development. *RNA* 17, 639–651. doi: 10.1261/rna.2310111

Kimble, J., and Hirsh, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* 70, 396–417.

Kimura, K. D., Riddle, D. L., and Ruvkun, G. (2011). The *C. elegans* DAF-2 insulin-like receptor is abundantly expressed in the nervous system and regulated by nutritional status. *Cold Spring Harb. Symp. Quant. Biol.* 76, 113–120. doi: 10.1101/sqb.2011.76.010660

Kimura, K. D., Tissenbaum, H. A., Liu, Y., and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277, 942–946.

Klass, M., and Hirsh, D. (1976). Non-ageing developmental variant of *Caenorhabditis elegans*. *Nature* 260, 523–525.

Klass, M. R. (1983). A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. *Mech. Ageing Dev.* 22, 279–286.

Koelle, M. R., Talbot, W. S., Segraves, W. A., Bender, M. T., Cherbas, P., and Hogness, D. S. (1991). The *Drosophila EcR* gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. *Cell* 67, 59–77. doi: 10.1016/0092-8674(91)90572-g

Kops, G. J. P. L., de Ruiter, N. D., Vries-Smits, A. M. M. D., Powell, D. R., Bos, J. L., Burgering, B. M., et al. (1999). Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* 398, 630–634. doi: 10.1038/19328

Kubrak, O. I., Kuèerová, L., Theopold, U., and Nässel, D. R. (2014). The sleeping beauty: how reproductive diapause affects hormone signaling, metabolism, immune response and somatic maintenance in *Drosophila melanogaster*. *PloS One* 9:e113051. doi: 10.1371/journal.pone.0113051

Kubrak, O. I., Kučerová, L., Theopold, U., Nylin, S., and Nässel, D. R. (2016). Characterization of reproductive dormancy in male *Drosophila melanogaster*. *Front. Physiol.* 7:572. doi: 10.3389/fphys.2016.00572

Larsen, P. L., Albert, P. S., and Riddle, D. L. (1995). Genes that regulate both development and longevity in *Caenorhabditis elegans*. *Genetics* 139, 1567–1583. doi: 10.1093/genetics/139.4.1567

Lau, M. M., Stewart, C. E., Liu, Z., Bhatt, H., Rotwein, P., and Stewart, C. L. (1994). Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Gene Dev.* 8, 2953–2963. doi: 10.1101/gad.8.24.2953

Lee, H., Choi, M., Lee, D., Kim, H., Hwang, H., Kim, H., et al. (2012). Nictation, a dispersal behavior of the nematode *Caenorhabditis elegans*, is regulated by IL2 neurons. *Nat. Neurosci.* 15, 107–112. doi: 10.1038/nn.2975

Levels, P. J., van Tits, L. J. H., and Denucé, J. M. (1986). The effect of the presence of adult fishes, gonad homogenates, and embryo homogenates on the dispersion-reaggregation phase during early embryonic development of the annual fish *Nothobranchius korthausae*. *J. Exp. Zool.* 240, 259–264. doi: 10.1002/jez.1402400212

Ley, P. D. (2006). A quick tour of nematode diversity and the backbone of nematode phylogeny. *Wormbook* 1–8. doi: 10.1895/wormbook.1.41.1

Li, W., Kennedy, S. G., and Ruvkun, G. (2003). *daf-28* encodes a *C. elegans* insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. *Genes Dev.* 17, 844–858. doi: 10.1101/gad.1066503

Lin, K., Dorman, J. B., Rodan, A., and Kenyon, C. (1997). daf-16: an HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* 278, 1319–1322. doi: 10.1126/science.278.5341.1319

Lin, K., Hsin, H., Libina, N., and Kenyon, C. (2001). Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat. Genet.* 28, 139–145. doi: 10.1038/88850

Liu, T., Zimmerman, K. K., and Patterson, G. I. (2004). Regulation of signaling genes by TGFbeta during entry into dauer diapause in *C. elegans*. *BMC Dev. Biol.* 4:11. doi: 10.1186/1471-213x-4-11

Liu, Y., Liao, S., Veenstra, J. A., and Nässel, D. R. (2016). *Drosophila* insulin-like peptide 1 (DILP1) is transiently expressed during non-feeding stages and reproductive dormancy. *Sci. Rep. UK* 6:26620. doi: 10.1038/srep26620

Liu, Z., and Ambros, V. (1991). Alternative temporal control systems for hypodermal cell differentiation in *Caenorhabditis elegans*. *Nature* 350, 162–165. doi: 10.1038/350162a0

Luciani, G. M., Magomedova, L., Puckrin, R., Urbanus, M. L., Wallace, I. M., Giaever, G., et al. (2011). Dafadine inhibits DAF-9 to promote dauer formation and longevity of *Caenorhabditis elegans*. *Nat. Chem. Biol.* 7, 891–893. doi: 10.1038/nchembio.698

Ludewig, A. H., Kober-Eisermann, C., Weitzel, C., Bethke, A., Neubert, K., Gerisch, B., et al. (2004). A novel nuclear receptor/coregulator complex controls *C. elegans* lipid metabolism, larval development, and aging. *Genes Dev.* 18, 2120–2133. doi: 10.1101/gad.312604

Ludewig, A. H., and Schroeder, F. C. (2013). Ascaroside signaling in *C. elegans*. *WormBook* 1–22. doi: 10.1895/wormbook.1.155.1

Maeda, S., Nakashima, A., Yamada, R., Hara, N., Fujimoto, Y., Ito, Y., et al. (2008). Molecular cloning of ecdysone 20-hydroxylase and expression pattern of the enzyme during embryonic development of silkworm *Bombyx mori*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 149, 507–516. doi: 10.1016/j.cbpb.2007.11.015

Mahanti, P., Bose, N., Bethke, A., Judkins, J. C., Wollam, J., Dumas, K. J., et al. (2014). Comparative metabolomics reveals endogenous ligands of DAF-12, a nuclear hormone receptor, regulating *C. elegans* development and lifespan. *Cell Metab.* 19, 73–83. doi: 10.1016/j.cmet.2013.11.024

Mak, H. Y., and Ruvkun, G. (2004). Intercellular signaling of reproductive development by the *C. elegans* DAF-9 cytochrome P450. *Development* 131, 1777–1786. doi: 10.1242/dev.01069

Makka, T., Seino, A., Tomita, S., Fujiwara, H., and Sonobe, H. (2002). A possible role of 20-hydroxyecdysone in embryonic development of the silkworm *Bombyx mori*. *Arch. Insect Biochem.* 51, 111–120. doi: 10.1002/arch.10055

Malone, E. A., Inoue, T., and Thomas, J. H. (1996). Genetic analysis of the roles of *daf-28* and *age-1* in regulating *Caenorhabditis elegans* dauer formation. *Genetics* 143, 1193–1205.

Mangelsdorf, D. J., and Evans, R. M. (1995). The RXR heterodimers and orphan receptors. *Cell* 83, 841–850. doi: 10.1016/0092-8674(95)90200-7

Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., et al. (1995). The nuclear receptor superfamily: the second decade. *Cell* 83, 835–839. doi: 10.1016/0092-8674(95)90199-x

Manning, B. D., and Cantley, L. C. (2007). AKT/PKB signaling: navigating downstream. *Cell* 129, 1261–1274. doi: 10.1016/j.cell.2007.06.009

Mansilla, A., Martín, F. A., Martín, D., and Ferrús, A. (2016). Ligand-independent requirements of steroid receptors EcR and USP for cell survival. *Cell Death Differ.* 23, 405–416. doi: 10.1038/cdd.2015.108

Martin, K., and Podrabsky, J. E. (2017). Hit pause: developmental arrest in annual killifishes and their close relatives. *Dev. Dyn.* 246, 858–866. doi: 10.1002/dvdy.24507

Meuti, M. E., Bautista-Jimenez, R., and Reynolds, J. A. (2018). Evidence that microRNAs are part of the molecular toolkit regulating adult reproductive diapause in the mosquito, *Culex pipiens*. *PLoS One* 13:e0203015. doi: 10.1371/journal.pone.0203015

Morris, J. Z., Tissenbaum, H. A., and Ruvkun, G. (1996). A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* 382, 536–539. doi: 10.1038/382536a0

Motola, D. L., Cummins, C. L., Rottiers, V., Sharma, K. K., Li, T., Li, Y., et al. (2006). Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. *Cell* 124, 1209–1223. doi: 10.1016/j.cell.2006.01.037

Murphy, C. T., and Hu, P. J. (2013). Insulin/insulin-like growth factor signaling in *C. elegans*. *WormBook* 1–43.

Nagy, D., Andreatta, G., Bastianello, S., Anduaga, A. M., Mazzotta, G., Kyriacou, C. P., et al. (2018). A semi-natural approach for studying seasonal diapause in *Drosophila melanogaster* reveals robust photoperiodicity. *J. Biol. Rhythm* 33, 117–125. doi: 10.1177/0748730417754116

Narbonne, P., and Roy, R. (2006). Inhibition of germline proliferation during C. elegans dauer development requires PTEN. LKB1 and AMPK signalling. *Development* 133, 611–619. doi: 10.1242/dev.02232

Narbonne, P., and Roy, R. (2009). *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. *Nature* 457, 210–214. doi: 10.1038/nature07536

Nika, L., Gibson, T., Konkus, R., and Karp, X. (2016). Fluorescent beads are a versatile tool for staging *Caenorhabditis elegans* in different life histories. *G3* 6, 1923–1933. doi: 10.1534/g3.116.030163

Niwa, R., and Niwa, Y. S. (2014). Enzymes for ecdysteroid biosynthesis: their biological functions in insects and beyond. *Biosci. Biotechnol. Biochem.* 78, 1283–1292. doi: 10.1080/09168451.2014.942250

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., Tissenbaum, H. A., et al. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389, 994–999. doi: 10.1038/40194

Oh, S. W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R. J., and Tissenbaum, H. A. (2005). JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc. Natl. Acad. Sci. U.S.A.* 102, 4494–4499. doi: 10.1073/pnas.0500749102

Olademehin, O. P., Liu, C., Rimal, B., Adegboyega, N. F., Chen, F., Sim, C., et al. (2020). Dsi-RNA knockdown of genes regulated by Foxo reduces glycogen and lipid accumulations in diapausing *Culex pipiens*. *Sci. Rep. UK* 10:17201. doi: 10.1038/s41598-020-74292-6

Oldham, S., and Hafen, E. (2003). Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control. *Trends Cell Biol.* 13, 79–85. doi: 10.1016/s0962-8924(02)00042-9

Oro, A. E., McKeown, M., and Evans, R. M. (1990). Relationship between the product of the *Drosophila* ultraspiracle locus and the vertebrate retinoid X receptor. *Nature* 347, 298–301. doi: 10.1038/347298a0

Patterson, G. I., Koweek, A., Wong, A., Liu, Y., and Ruvkun, G. (1997). The DAF-3 Smad protein antagonizes TGF-beta -related receptor signaling in the *Caenorhabditis elegans* dauer pathway. *Genes Dev.* 11, 2679–2690. doi: 10.1101/gad.11.20.2679

Petryk, A., Warren, J. T., Marqués, G., Jarcho, M. P., Gilbert, L. I., Kahler, J., et al. (2003). Shade is the *Drosophila* P450 enzyme that mediates the hydroxylation of ecdysone to the steroid insect molting hormone 20-hydroxyecdysone. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13773–13778. doi: 10.1073/pnas.2336088100

Pierce, S. B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S. A., Buchman, A. R., et al. (2001). Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev.* 15, 672–686. doi: 10.1101/gad.867301

Podrabsky, J., Riggs, C., and Wagner, J. (2016a). "Tolerance of environmental stress," in *Annual Fishes Life History Strategy, Diversity, and Evolution*, eds N. Berois, G. Garcia, and R. D. Sá (Boca Raton, FL: CRC Press, Taylor & Francis), 159–184. doi: 10.1201/b19016-13

Podrabsky, J., Romney, A., and Culpepper, K. (2016b). "Alternative developmental pathways," in *Annual Fishes Life History Strategy, Diversity, and Evolution*, eds N. Berois, G. Garcia, and R. D. Sá (Boca Raton, FL: CRC Press, Taylor and Francis), 63–74. doi: 10.1201/b19016-6

Podrabsky, J. E., Garrett, I. D. F., and Kohl, Z. F. (2010). Alternative developmental pathways associated with diapause regulated by temperature and maternal influences in embryos of the annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* 213, 3280–3288. doi: 10.1242/jeb.045906

Podrabsky, J. E., Riggs, C. L., Romney, A. L., Woll, S. C., Wagner, J. T., Culpepper, K. M., et al. (2017). Embryonic development of the annual killifish *Austrofundulus limnaeus*: an emerging model for ecological and evolutionary developmental biology research and instruction. *Dev. Dyn.* 246, 779–801. doi: 10.1002/dvdy.24513

Polačik, M., Vrtílek, M., Reichard, M., Žák, J., Blažek, R., and Podrabsky, J. (2021). Embryo ecology: developmental synchrony and asynchrony in the embryonic development of wild annual fish populations. *Ecol. Evol.* 11, 4945–4956. doi: 10.1002/ece3.7402

Reed, E., and Wallace, H. (1965). Leaping locomotion by an insect-parasitic nematode. *Nature* 206, 210–211. doi: 10.1038/206210a0

Reichard, M., and Polačik, M. (2019). *Nothobranchius furzeri*, an "instant" fish from an ephemeral habitat. *Elife* 8:e41548. doi: 10.7554/elife.41548

Ren, P., Lim, C.-S., Johnsen, R., Albert, P. S., Pilgrim, D., and Riddle, D. L. (1996). Control of *C. elegans* larval development by neuronal expression of a TGF-β homolog. *Science* 274, 1389–1391. doi: 10.1126/science.274.5291.1389

Richard, D. S., Gilbert, M., Crum, B., Hollinshead, D. M., Schelble, S., and Scheswohl, D. (2001a). Yolk protein endocytosis by oocytes in *Drosophila melanogaster*: immunofluorescent localization of clathrin, adaptin and the yolk protein receptor. *J. Insect Physiol.* 47, 715–723. doi: 10.1016/s0022-1910(00)00165-7

Richard, D. S., Jones, J. M., Barbarito, M. R., Cerula, S., Detweiler, J. P., Fisher, S. J., et al. (2001b). Vitellogenesis in diapausing and mutant *Drosophila melanogaster*: further evidence for the relative roles of ecdysteroids and juvenile hormones. *J. Insect. Physiol.* 47, 905–913. doi: 10.1016/s0022-1910(01)00063-4

Richard, D. S., Rybczynski, R., Wilson, T. G., Wang, Y., Wayne, M. L., Zhou, Y., et al. (2005). Insulin signaling is necessary for vitellogenesis in *Drosophila melanogaster* independent of the roles of juvenile hormone and ecdysteroids: female sterility of the *chico1* insulin signaling mutation is autonomous to the ovary. *J. Insect Physiol.* 51, 455–464. doi: 10.1016/j.jinsphys.2004.12.013

Richard, D. S., Watkins, N. L., Serafin, R. B., and Gilbert, L. I. (1998). Ecdysteroids regulate yolk protein uptake by *Drosophila melanogaster* oocytes. *J. Insect Physiol.* 44, 637–644. doi: 10.1016/s0022-1910(98)00020-1

Riddle, D. L. (1977). A genetic pathway for dauer larva formation in *Caenorhabditis elegans*. *Stad. Genet. Symp.* 9, 101–120.

Riddle, D. L., Swanson, M. M., and Albert, P. S. (1981). Interacting genes in nematode dauer larva formation. *Nature* 290, 668–671.

Ritter, A. D., Shen, Y., Bass, J. F., Jeyaraj, S., Deplancke, B., Mukhopadhyay, A., et al. (2013). Complex expression dynamics and robustness in *C. elegans* insulin networks. *Genome Res.* 23, 954–965. doi: 10.1101/gr.150466.112

Robich, R. M., and Denlinger, D. L. (2005). Diapause in the mosquito *Culex pipiens* evokes a metabolic switch from blood feeding to sugar gluttony. *Proc. Natl. Acad. Sci. U.S.A.* 102, 15912–15917. doi: 10.1073/pnas.0507958102

Romney, A. L. T., Davis, E. M., Corona, M. M., Wagner, J. T., and Podrabsky, J. E. (2018). Temperature-dependent vitamin D signaling regulates developmental trajectory associated with diapause in an annual killifish. *Proc. Natl. Acad. Sci. U.S.A.* 115, 12763–12768. doi: 10.1073/pnas.1804590115

Romney, A. L. T., and Podrabsky, J. E. (2018). Small noncoding RNA profiles along alternative developmental trajectories in an annual killifish. *Sci. Rep.* 8:13364. doi: 10.1038/s41598-018-31466-7

Rottiers, V., Motola, D. L., Gerisch, B., Cummins, C. L., Nishiwaki, K., Mangelsdorf, D. J., et al. (2006). Hormonal control of *C. elegans* dauer formation and life Span by a Rieske-like oxygenase. *Dev. Cell* 10, 473–482. doi: 10.1016/j.devcel.2006.02.008

Rougvie, A. E., and Moss, E. G. (2013). Developmental transitions in *C. elegans* larval stages. *Curr. Top. Dev. Biol.* 105, 153–180. doi: 10.1016/b978-0-12-396968-2.00006-3

Rowiński, P. K., Sowersby, W., Näslund, J., Eckerström-Liedholm, S., Gotthard, K., and Rogell, B. (2021). Variation in developmental rates is not linked to environmental unpredictability in annual killifishes. *Ecol. Evol.* 11, 8027–8037. doi: 10.1002/ece3.7632

Sanburg, L. L., and Larsen, J. R. (1973). Effect of photoperiod and temperature on ovarian development in *Culex pipiens pipiens*. *J. Insect Physiol.* 19, 1173–1190. doi: 10.1016/0022-1910(73)90202-3

Saponaro, F., Saba, A., and Zucchi, R. (2020). An update on vitamin D metabolism. *Int. J. Mol. Sci.* 21:6573. doi: 10.3390/ijms21186573

Sato, A., Sokabe, T., Kashio, M., Yasukochi, Y., Tominaga, M., and Shiomi, K. (2014). Embryonic thermosensitive TRPA1 determines transgenerational diapause phenotype of the silkworm, *Bombyx mori*. *Proc. Natl. Acad. Sci. U.S.A.* 111, E1249–E1255. doi: 10.1073/pnas.1322134111

Saunders, D. S., and Gilbert, L. I. (1990). Regulation of ovarian diapause in *Drosophila melanogaster* by photoperiod and moderately low temperature. *J. Insect Physiol.* 36, 195–200. doi: 10.1016/0022-1910(90)90122-v

Saunders, D. S., Henrich, V. C., and Gilbert, L. I. (1989). Induction of diapause in *Drosophila melanogaster*: photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. *Proc. Natl. Acad. Sci. U.S.A.* 86, 3748–3752. doi: 10.1073/pnas.86.10.3748

Saunders, D. S., Richard, D. S., Applebaum, S. W., Ma, M., and Gilbert, L. I. (1990). Photoperiodic diapause in *Drosophila melanogaster* involves a block to the juvenile hormone regulation of ovarian maturation. *Gen. Comp. Endocr.* 79, 174–184. doi: 10.1016/0016-6480(90)90102-r

Schackwitz, W. S., Inoue, T., and Thomas, J. H. (1996). Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. *Neuron* 17, 719–728. doi: 10.1016/s0896-6273(00)80203-2

Schaedel, O. N., Gerisch, B., Antebi, A., and Sternberg, P. W. (2012). Hormonal signal amplification mediates environmental conditions during development and controls an irreversible commitment to adulthood. *PLoS Biol.* 10:e1001306. doi: 10.1371/journal.pbio.1001306

Schiesari, L., Andreatta, G., Kyriacou, C. P., O'Connor, M. B., and Costa, R. (2016). The insulin-like proteins dILPs-2/5 determine diapause inducibility in *Drosophila*. *PLoS One* 11:e0163680. doi: 10.1371/journal.pone.0163680

Schiesari, L., and O'Connor, M. B. (2013). Diapause: delaying the developmental clock in response to a changing environment. *Curr. Top. Dev. Biol.* 105, 213–246. doi: 10.1016/b978-0-12-396968-2.00008-7

Schmidt, P. S., Matzkin, L., Ippolito, M., and Eanes, W. F. (2005a). Geographic variation in diapause incidence, life-history traits, and climactic adaptation in *Drosophila melanogaster*. *Evolution* 59, 1721–1732. doi: 10.1111/j.0014-3820.2005.tb01821.x

Schmidt, P. S., Paaby, A. B., and Heschel, M. S. (2005b). Genetic variance for diapause expression and associated life histories in *Drosophila melanogaster*. *Evolution* 59, 2616–2625. doi: 10.1111/j.0014-3820.2005.tb00974.x

Schmidt, P. S., and Paaby, A. B. (2008). Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. *Evolution* 62, 1204–1215. doi: 10.1111/j.1558-5646.2008.00351.x

Shiomi, K., Takasu, Y., Kunii, M., Tsuchiya, R., Mukaida, M., Kobayashi, M., et al. (2015). Disruption of diapause induction by TALEN-based gene mutagenesis in relation to a unique neuropeptide signaling pathway in *Bombyx*. *Sci. Rep.* 5:15566. doi: 10.1038/srep15566

Shostak, Y., Gilst, M. R. V., Antebi, A., and Yamamoto, K. R. (2004). Identification of *C. elegans* DAF-12-binding sites, response elements, and target genes. *Genes Dev.* 18, 2529–2544. doi: 10.1101/gad.1218504

Sim, C., and Denlinger, D. L. (2008). Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6777–6781. doi: 10.1073/pnas.0802067105

Sim, C., and Denlinger, D. L. (2009). A shut-down in expression of an insulin-like peptide, ILP-1, halts ovarian maturation during the overwintering diapause of the mosquito *Culex pipiens*. *Insect Mol. Biol.* 18, 325–332. doi: 10.1111/j.1365-2583.2009.00872.x

Sim, C., and Denlinger, D. L. (2013a). Insulin signaling and the regulation of insect diapause. *Front. Physiol.* 4:189. doi: 10.3389/fphys.2013.00189

Sim, C., and Denlinger, D. L. (2013b). Juvenile hormone III suppresses forkhead of transcription factor in the fat body and reduces fat accumulation in the diapausing mosquito, *Culex pipiens*. *Insect Mol. Biol.* 22, 1–11. doi: 10.1111/j.1365-2583.2012.01166.x

Sim, C., Kang, D. S., Kim, S., Bai, X., and Denlinger, D. L. (2015). Identification of FOXO targets that generate diverse features of the diapause phenotype in the mosquito *Culex pipiens*. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3811–3816. doi: 10.1073/pnas.1502751112

Sonobe, H., and Yamada, R. (2004). Ecdysteroids during early embryonic development in silkworm *Bombyx mori*: metabolism and functions. *Zool. Sci.* 21, 503–516. doi: 10.2108/zsj.21.503

Spiebnan, A., and Wong, J. (1973). Studies on autogeny in natural populations of *Culex pipiens*. III. Midsummer preparation for hibernation in anautogenous populations. *J. Med. Entomol.* 10, 319–324. doi: 10.1093/jmedent/10.4.319

Spielman, A. (1974). Effect of synthetic juvenile hormone on ovarian diapause of *Culex pipiens* mosquitoes. *J. Med. Entomol.* 11, 223–225. doi: 10.1093/jmedent/11.2.223

Sternberg, P. W. (2005). Vulval development. *WormBook* 1–28. doi: 10.1895/wormbook.1.6.1

Stiernagle, T. (2006). Maintenance of *C. elegans*. *WormBook* 1–11. doi: 10.1895/wormbook.1.101.1

Sulston, J. E., and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* 56, 110–156.

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100, 64–119.

Talbot, W. S., Swyryd, E. A., and Hogness, D. S. (1993). *Drosophila* tissues with different metamorphic responses to ecdysone express different ecdysone receptor isoforms. *Cell* 73, 1323–1337. doi: 10.1016/0092-8674(93)90359-x

Tatar, M., Chien, S. A., and Priest, N. K. (2001). Negligible senescence during reproductive dormancy in *Drosophila melanogaster*. *Am. Nat.* 158, 248–258. doi: 10.1086/321320

Tenen, C. C., and Greenwald, I. (2019). Cell non-autonomous function of daf-18/PTEN in the somatic gonad coordinates somatic gonad and germline development in *C. elegans* dauer larvae. *Curr. Biol.* 29, 1064.e–1072.e. doi: 10.1016/j.cub.2019.01.076

Tepper, R. G., Ashraf, J., Kaletsky, R., Kleemann, G., Murphy, C. T., and Bussemaker, H. J. (2013). PQM-1 complements DAF-16 as a key transcriptional regulator of DAF-2-mediated development and longevity. *Cell* 154, 676–690. doi: 10.1016/j.cell.2013.07.006

Thomas, H. E., Stunnenberg, H. G., and Stewart, A. F. (1993). Heterodimerization of the *Drosophila* ecdysone receptor with retinoid X receptor and ultraspiracle. *Nature* 362, 471–475. doi: 10.1038/362471a0

Toni, L. S., and Padilla, P. A. (2016). Developmentally arrested *Austrofundulus limnaeus* embryos have changes in post-translational modifications of histone H3. *J. Exp. Biol.* 219, 544–552. doi: 10.1242/jeb.131862

Tothova, Z., and Gilliland, D. G. (2007). FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. *Cell Stem Cell* 1, 140–152. doi: 10.1016/j.stem.2007.07.017

Tuckey, R. C., Cheng, C. Y. S., and Slominski, A. T. (2018). The serum vitamin D metabolome: what we know and what is still to discover. *J. Steroid Biochem. Mol. Biol.* 186, 4–21. doi: 10.1016/j.jsbmb.2018.09.003

Vincent, A. M., and Feldman, E. L. (2002). Control of cell survival by IGF signaling pathways. *Growth Horm. IGF Res.* 12, 193–197. doi: 10.1016/s1096-6374(02)00017-5

Vowels, J. J., and Thomas, J. H. (1992). Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. *Genetics* 130, 105–123.

Williams, K. D., Busto, M., Suster, M. L., So, A. K.-C., Ben-Shahar, Y., Leevers, S. J., et al. (2006). Natural variation in *Drosophila melanogaster* diapause due to the insulin-regulated PI3-kinase. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15911–15915. doi: 10.1073/pnas.0604592103

Wirick, M. J., Cale, A. R., Smith, I. T., Alessi, A. F., Starostik, M. R., Cuko, L., et al. (2021). *daf-16*/FOXO blocks adult cell fate in *Caenorhabditis elegans* dauer larvae via *lin-41*/TRIM71. *bioRxiv* [Preprint] doi: 10.1101/2021.06.30.450637

Woll, S. C., and Podrabsky, J. E. (2017). Insulin-like growth factor signaling regulates developmental trajectory associated with diapause in embryos of the annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* 220:jeb.151373. doi: 10.1242/jeb.151373

Wollam, J., Magomedova, L., Magner, D. B., Shen, Y., Rottiers, V., Motola, D. L., et al. (2011). The Rieske oxygenase DAF-36 functions as a cholesterol 7-desaturase in steroidogenic pathways governing longevity. *Aging Cell* 10, 879–884. doi: 10.1111/j.1474-9726.2011.00733.x

Wourms, J. P. (1972). The developmental biology of annual fishes. III. Pre-embryonic and embryonic diapause of in the eggs of annual fishes. *J. Exp. Zool.* 182, 389–414. doi: 10.1002/jez.1401820310

Xu, W.-H., Sato, Y., Ikeda, M., and Yamashita, O. (1995a). Molecular characterization of the gene encoding the precursor protein of diapause hormone and pheromone biosynthesis activating neuropeptide (DH-PBAN) of the silkworm, *Bombyx mori* and its distribution in some insects. *Biochim. Biophys. Acta BBA Gene Struct. Expr.* 1261, 83–89. doi: 10.1016/0167-4781(94)00238-x

Xu, W.-H., Sato, Y., Ikeda, M., and Yamashita, O. (1995b). Stage-dependent and temperature-controlled expression of the gene encoding the precursor protein of diapause hormone and pheromone biosynthesis activating neuropeptide in the silkworm, *Bombyx mori*. *J. Biol. Chem.* 270, 3804–3808. doi: 10.1074/jbc.270.8.3804

Yamashita, O., Shiomi, K., Ishida, Y., Katagiri, N., and Niimi, T. (2001). "Insights for future studies on embryonic diapause promoted by molecular analyses of diapause hormone and its action in *Bombyx mori*," in *Insect Timing: Circadian Rhythmicity to Seasonality*, eds D. L. Denlinger, J. Giebultowicz, and D. S. Saunders (Amsterdam: Elsevier Science BV), 145–153. doi: 10.1016/b978-044450608-5/50044-1

Yao, T.-P., Segraves, W. A., Oro, A. E., McKeown, M., and Evans, R. M. (1992). *Drosophila* ultraspiracle modulates ecdysone receptor function via heterodimer formation. *Cell* 71, 63–72. doi: 10.1016/0092-8674(92)90266-f

Yoshiyama-Yanagawa, T., Enya, S., Shimada-Niwa, Y., Yaguchi, S., Haramoto, Y., Matsuya, T., et al. (2011). The conserved Rieske oxygenase DAF-36/Neverland Is a novel cholesterol-metabolizing enzyme. *J. Biol. Chem.* 286, 25756–25762. doi: 10.1074/jbc.m111.244384

Zhang, H., Lin, Y., Shen, G., Tan, X., Lei, C., Long, W., et al. (2017). Pigmentary analysis of eggs of the silkworm *Bombyx mori*. *J. Insect. Physiol.* 101, 142–150. doi: 10.1016/j.jinsphys.2017.07.013

Zhang, X.-S., Wang, T., Lin, X.-W., Denlinger, D. L., and Xu, W.-H. (2017). Reactive oxygen species extend insect life span using components of the insulin-signaling pathway. *Proc. Natl. Acad. Sci. U.S.A.* 114, E7832–E7840. doi: 10.1073/pnas.1711042114

Zheng, S., Chiu, H., Boudreau, J., Papanicolaou, T., Bendena, W., and Chin-Sang, I. (2018). A functional study of all 40 *C. elegans* insulin-like peptides. *J. Biol. Chem.* 293, 16912–16922. doi: 10.1074/jbc.ra118.004542

Zheng, X., Gong, Y., Kumar, D., Chen, F., Kuan, S., Liang, Z., et al. (2016). Effect of BBX-B8 overexpression on development, body weight, silk protein synthesis and egg diapause of *Bombyx mori*. *Transgenic Res.* 25, 507–516. doi: 10.1007/s11248-016-9947-3

Zhu, J., Chen, L., Sun, G., and Raikhel, A. S. (2006). The competence factor βFtz-F1 potentiates ecdysone receptor activity via recruiting a p160/SRC coactivator. *Mol. Cell Biol.* 26, 9402–9412. doi: 10.1128/mcb.01318-06

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

REVIEW
published: 04 October 2021
doi: 10.3389/fevo.2021.735487

Interdependence of Thyroid and Corticosteroid Signaling in Vertebrate Developmental Transitions

*Karine Rousseau[1], Sylvie Dufour[1] and Laurent M. Sachs[2]**

[1] UMR BOREA, CNRS, Muséum National d'Histoire Naturelle, Sorbonne Université, Paris, France, [2] UMR PhyMA, CNRS, Muséum National d'Histoire Naturelle, Paris, France

Post-embryonic acute developmental processes mainly allow the transition from one life stage in a specific ecological niche to the next life stage in a different ecological niche. Metamorphosis, an emblematic type of these post-embryonic developmental processes, has occurred repeatedly and independently in various phylogenetic groups throughout metazoan evolution, such as in cnidarian, insects, molluscs, tunicates, or vertebrates. This review will focus on metamorphoses and developmental transitions in vertebrates, including typical larval metamorphosis in anuran amphibians, larval and secondary metamorphoses in teleost fishes, egg hatching in sauropsids and birth in mammals. Two neuroendocrine axes, the hypothalamic-pituitary-thyroid and the hypothalamic-pituitary-adrenal/interrenal axes, are central players in the regulation of these life transitions. The review will address the molecular and functional evolution of these axes and their interactions. Mechanisms of integration of internal and environmental cues, and activation of these neuroendocrine axes represent key questions in an "eco-evo-devo" perspective of metamorphosis. The roles played by developmental transitions in the innovation, adaptation, and plasticity of life cycles throughout vertebrates will be discussed. In the current context of global climate change and habitat destruction, the review will also address the impact of environmental factors, such as global warming and endocrine disruptors on hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenal/interrenal axes, and regulation of developmental transitions.

Keywords: thyroid hormones, corticosteroids, hormonal crosstalk, metamorphosis, development, environment, stress

OPEN ACCESS

Edited by:
Sarah Kelly McMenamin, Boston College, United States

Reviewed by:
Juan Ignacio Fernandino, CONICET Institute of Biotechnological Research (IIB-INTECH), Argentina
Ivan Manzini, University of Giessen, Germany

****Correspondence:***
Laurent M. Sachs
sachs@mnhn.fr

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *02 July 2021*
Accepted: *13 September 2021*
Published: *04 October 2021*

Citation:
Rousseau K, Dufour S and Sachs LM (2021) Interdependence of Thyroid and Corticosteroid Signaling in Vertebrate Developmental Transitions. Front. Ecol. Evol. 9:735487. doi: 10.3389/fevo.2021.735487

INTRODUCTION

The hypothalamic-pituitary-thyroid (HPT) axis is the neuroendocrine thyrotropic axis, which literally acts ("trope") on the thyroid gland ("thyro"). In a simplified way, it is composed of brain thyrotropin-releasing hormone (TRH) neurons, pituitary thyrotropin (TSH) cells, and peripheral thyroid hormones (TH), thyroxine (T_4) and triiodothyronine (T_3). TRH acts *via* its receptor

(TRH-R) expressed by the pituitary thyrotropes, and stimulates the synthesis and release of pituitary TSH. TSH binds its receptor (TSH-R) expressed by the thyroid gland to induce the production and release of T_4. At target tissues, T_4 is transformed into T_3 by monodeiodinases, and both bind to TH nuclear receptor, TR. TH can act on various tissues and they negatively feedback on the brain (hypothalamic TRH)/pituitary (TSH) axis (Zoeller et al., 2007).

The hypothalamic-pituitary-adrenal axis (HPA), in mammals and sauropsids, and hypothalamic-pituitary-interrenal (HPI), in amphibians and teleosts, is the neuroendocrine corticotropic axis responsible for the response to stress in all vertebrates (Gorissen and Flik, 2016). The neurohormone, corticotropin-releasing hormone (CRH), controls the production and release of corticotropin (also named adrenocorticotropic hormone, ACTH), at the pituitary level. ACTH acts to control the production and release of corticosteroids (CS) from adrenal cortex cells in amniotes or interrenal cells in amphibians and teleosts. CS can act on various tissues and they negatively feedback on the brain (hypothalamic CRH) / pituitary (ACTH) corticotropic axis (Bernier et al., 2009; Faught et al., 2016; Gorissen and Flik, 2016) *via* specific receptors.

The molecular components of the HPT and HPA/HPI axes have been subject to whole genome duplication (WGD) events during evolution. Two events of WGD, referred to as 1R and 2R for first and second rounds of WGD, occurred in ancestral vertebrates (Dehal and Boore, 2005). An additional WGD event occurred in ancestral teleosts, referred to as 3R for third round of WGD or as teleost specific WGD (Meyer and Schartl, 1999). A further WGD occurred more recently in some teleost groups, such as salmonids (Lien et al., 2016) and some carps (Wang et al., 2012). This event is referred to as 4R (fourth round of WGD). The WGD were at the origin of the diversification of the functions of the HPT and HPA/HPI axis.

One of the actions controlled by the HPT and HPA/HPI axis in vertebrates is the developmental body change coming from a need to adapt to a remarkable change in habitat during birth, hatching or post-embryonic development events. Dramatic changes occur in various metazoa such as cnidaria, insects, crustacean, molluscs, tunicates, and vertebrates. These events have been collectively termed as metamorphosis from the Greek meta- (change) and morph (form). Metamorphosis has a major role in the structure of complex life cycles encompassing different ecophases. In vertebrates, different developmental modes are observed. Amniotes, namely mammals, and sauropsids (birds and reptiles), as well as some amphibians, go through direct development and present a more mature stage at birth/hatching, with an earlier ontogeny of the HPA axis. Non-amniotes, comprising various fish and amphibians, go through indirect development with metamorphosis and present a more immature stage at hatching with a later ontogeny of the HPT and HPA/HPI axis. All the post-embryonic developmental changes are hormonally controlled processes by TH (Paris and Laudet, 2008), but recent studies confer to CS an increasingly prominent role (Sachs and Buchholz, 2017). These TH and CS actions allow to distinguish this period from other major late developmental events such as puberty, which is under the control of sexual steroids.

The post-embryonic developmental transitions are also sensitive to diverse biotic and abiotic factors that can profoundly influence behavior, morphology, growth, development, and sometimes survival. Vertebrates respond to the exposome by modulating the production of CS and TH (**Figure 1**). The adaptive response allows to adjust the timing of the developmental program, but with a trade-off between risk and benefit.

MOLECULAR AND FUNCTIONAL EVOLUTION OF THE HYPOTHALAMIC-PITUITARY-THYROID AND THE HYPOTHALAMIC-PITUITARY-ADRENAL/INTERRENAL AXES AND THEIR INTERACTIONS

Hypothalamic-Pituitary-Thyroid Axis

Thyrotropin-Releasing Hormone and Its Receptors

The hypothalamic-pituitary-thyroid (HPT) axis is the neuroendocrine thyrotropic axis (**Figure 2**). After indirect evidence of the existence of a thyrotropin-releasing factor in dogs (Shibusawa et al., 1955), a fraction, purified from bovine or ovine hypothalamic extracts, was found to stimulate the release of TSH (Schreiber et al., 1961; Guillemin et al., 1962, 1963). The TRH gene encodes multiple identical repeats of the three amino-acid (pyroglutamic acid-histidine-proline) TRH sequence (Galas et al., 2009). This is an example of intragenic duplication which allows an amplification of the synthesis and production of this neurohormone.

TRH-R are members of membrane class A G-protein coupled receptors (GPCR). Two subtypes, TRH-R1 and TRH-R2, have been characterized in mammals. A third one, TRH-R3, has been identified in birds (Li et al., 2020), amphibians (Bidaud et al., 2002), reptiles (Li et al., 2020), and teleosts (Mekuchi et al., 2011; Saito et al., 2011; Li et al., 2020). In birds, only TRH-R1 and TRH-R3 have been identified so far (Li et al., 2020). Four subtypes have been cloned in sockeye salmon *Oncorhynchus nerka* (TRH-R1, TRH-R2a, TRH-R2b, and TRH-R3; Saito et al., 2011), and in medaka *Oryzias latipes* (TRH-R1a, TRH-R1b, TRH-R2, and TRH-R3; Mekuchi et al., 2011). As in medaka (Mekuchi et al., 2011), duplicated TRH-R1a and b are found in many teleosts including zebrafish *Danio rerio*, and might be the result of 3R, even if only one TRH-R1 is identified in sockeye salmon (Saito et al., 2011). The duplicated TRH-R2s present in sockeye salmon may be due to the 4R; TRH-R2b is truncated (Saito et al., 2011). According to the recent hypothesis of Li et al. (2020) on the evolutionary history of TRH-Rs, multiple gene loss events might have occurred during evolution, including TRH-R2 loss in birds, and many mammals, as well as TRH-R3 loss in mammals. Interestingly, pituitary TRH-R3 expression was recently shown to elevate as metamorphosis progressed in bullfrog tadpole (Nakano et al., 2018). It would be interesting to investigate whether the possible involvement of

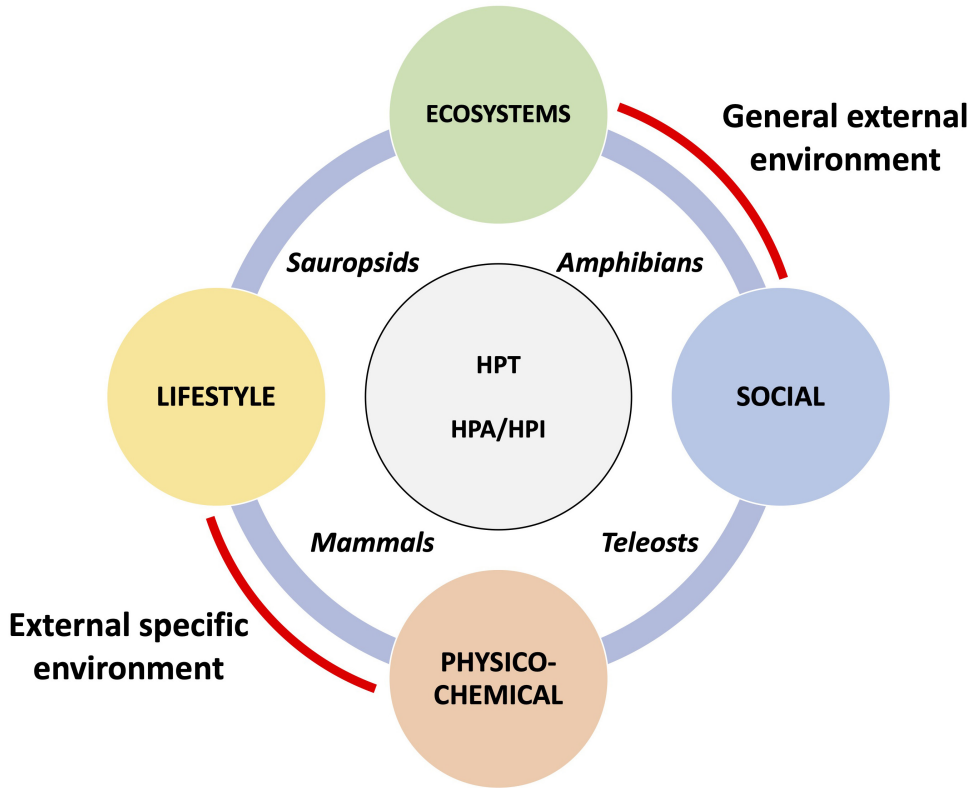


FIGURE 1 | The exposome targets the HPT and HPA/HPI axe in vertebrates. The four components of the exposome (ecosystems, lifestyle, social, and physico-chemical expositions) may affect developmental transitions in vertebrates, including teleosts, amphibians, sauropsids, and mammals, by acting in part at the level of the HPT and HPA/HPI axes.

TRH-R3 in amphibian metamorphosis is also observed in other major life history transitions, such as teleost metamorphoses and sauropsid hatching.

Thyrotropin and Its Receptors

TSH is a glycoprotein hormone composed of two subunits, the alpha subunit (Gpα) common to gonadotropins, and the hormone-specific TSHβ subunit, which is paralogous to the luteinising hormone (LH) and follicle-stimulating hormone (FSH) β subunits. The genes encoding the β subunits of the two gonadotropins LH, FSH and of TSH are paralogous genes that likely arose from the two successive 1R/2R in ancestral vertebrates. A single TSHβ is present in mammals and other tetrapods, as well as in actinopterygians, and is considered as the "classical" TSH. Its β subunit is now named TSHβ1 since a second TSHβ, called TSHβ2, has been identified in some representative species of early vertebrates such as in chondrichthyans and basal sarcopterygians (Maugars et al., 2014). These data suggest that the gene coding for *tshβ2* was lost twice independently during osteichthyan evolution, in a tetrapod ancestor and in an actinopterygian ancestor. Indeed *tshβ2* could not be retrieved in the genome of an holostean (spotted gar *Lepisosteus oculatus*) nor any teleosts, supporting an early loss of *tshβ2* in the actinopterygian lineage, leading to inheritance of only *tshβ1* by the teleost lineage (Maugars et al., 2014). Two *tshβ* paralogs were found in teleosts, resulting from the 3R duplication of *tshβ1* (Maugars et al., 2014; Fleming et al., 2019), and named *tshβ1a* and *tshβ1b* (Fleming et al., 2019). Recently, up to three *tshβ1* paralogs were identified in *Oncorhynchus* species, due to the conservation of the duplicated 4R-paralogs of *tshβ1a (tshβ1aα and tshβ1aβ)* while only the two *tshβ1* paralogs issued from 3R (*tshβ1a* and *tshβ1b*) are present in Atlantic salmon *Salmo salar* as in other teleosts (Fleming et al., 2019). Interestingly, the two *tshβ* paralogs identified in Atlantic salmon are expressed in different pituitary cells (Fleming et al., 2019): *tshβ1a* in the anterior adenohypophysis, and *tshβ1b* in cells near to the pituitary stalk, cells comparable to the *pars tuberalis* TSH cells involved in seasonal physiology and behavior in birds (Yoshimura, 2013) and mammals (Dardente et al., 2019).

TSH-R is present at the surface of the thyroid follicle cells in the thyroid gland. It belongs to the class A GPCR receptors and is paralogous to the receptors for gonadotropins. In teleosts, due to the 3R, duplicated receptors *tshra* and *tshrb* have been characterized (Maugars et al., 2014). TSH-R are not only expressed in the thyroid gland, but also in a variety of other tissues (Williams, 2011). Of particular interest is the presence of *tshr* in the brain and the gonads, where they mediate TSH retrograde action on brain deiodinase and direct regulatory effect on reproduction, in birds and mammals (Nakane and Yoshimura, 2019).

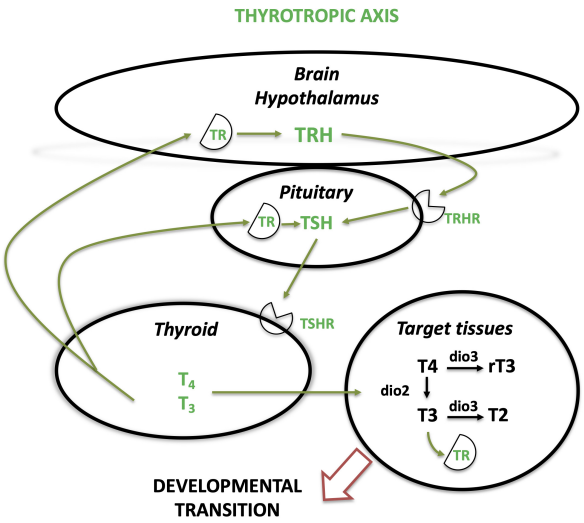


FIGURE 2 | Thyrotropic neuroendocrine axis in vertebrates. The figure displays the main actors of the thyrotropic (hypothalamic-pituitary-thyroid, HPT) axis. dio, deiodinase; T_4, thyroxine; T_3, tri-iodothyronine; rT_3, reverse triiodothyronine; T_2, diiodothyronine; TR, thyroid hormone receptor; TRH, thyrotropin releasing hormone; TRHR, thyrotropin releasing hormone receptor; TSH, thyrotropin; TSHR, thyrotropin receptor.

Thyroid Hormones and Their Receptors

Two main forms of TH are found in vertebrates, T_4 with four iodinated tyrosine residues (3,3′,5,5′), the hormonal precursor produced by the thyroid gland, and T_3 with three iodinated tyrosine residues (3,3′,5), the active hormone which binds to TR with a 10-fold higher affinity compared to T_4 (Holzer et al., 2017). T_4 is produced, by thyrocytes under TSH control, from thyroglobulin, a large dimeric protein containing many tyrosine residues.

TR belong to the nuclear receptor superfamily. Two homologous receptors, TRα and TRβ, are present in tetrapods (Sap et al., 1986; Weinberger et al., 1986; Thompson et al., 1987; Brooks et al., 1989; Helbing et al., 2006; Kanaho et al., 2006). The 3R led to two duplicated *trα* paralogs and two duplicated *trβ* paralogs in basal teleosts, such as conger and eel. So far, four *tr* cDNAs have been cloned in Japanese flounder: two α types and two β types (Yamano et al., 1994; Yamano and Inui, 1995). In some other teleosts, such as zebrafish, two *trα* genes but a single *trβ* gene have been retrieved, suggesting that one of the *trβ* duplicated paralog would have been lost in the course of teleost evolution (Lazcano and Orozco, 2018). *Xenopus laevis* possesses two distinctive genes for each *tr* likely due to its genome polyploidisation (Yaoita et al., 1990). In tetrapods, alternative splicing of TR leads to various protein isoforms with different binding activities and biological functions. In mammals, two TRα variants (α*1* and α*2*) are thus generated from a single gene (Izumo and Mahdavi, 1988). Similarly, in addition to the 3R-duplicated genes, alternative splicing may lead to a large variety of TR isoforms in teleosts, with species-specific variations (Marchand et al., 2001).

Deiodinases

Deiodination of TH corresponds to the removal of iodine from their outer or inner rings, and is performed by iodothyronine deiodinase enzymes in the thyroid, peripheral or central target tissues. By regulating the bioavailability of T_4 and T_3, they thus control their action (Bianco et al., 2002; Orozco and Valverde-R, 2005; Darras and Van Herck, 2012; Orozco et al., 2012; Steegborn and Schweizer, 2020). The first deiodinase to be cloned was *dio1* from rat (Berry et al., 1991), then *dio3* was cloned from *Xenopus laevis* (St. Germain et al., 1994), and *dio2* from *Rana catesbeiana* (Davey et al., 1995). So far, these three types of iodothyronine deiodinases, DIO1, DIO2, and DIO3, have been identified in most vertebrates (Bianco et al., 2002; Darras and Van Herck, 2012; Orozco et al., 2012). DIO1 is a low selectivity enzyme with both outer and inner ring deiodinase activity. DIO2 is an outer ring deiodinase, which removes an iodine residue from T_4 and gives active T_3. DIO3 is an inner ring deiodinase, which inactivates T_4 and T_3 in reverse T_3 (rT_3 or 3,3′,5′-T_3), and diiodothyronine (T_2 or 3,3′-T_2), respectively. Orozco et al. (2012) reviewed the evolutionary history of deiodinase family and point out that *dio1* is the oldest vertebrate deiodinase gene and *dio2* the most recent one. Some teleosts possess 3R-duplicated *dio2* and *dio3* genes (Orozco et al., 2012; Alves et al., 2017). The widespread and differential tissue distribution for the three types of deiodinase in vertebrates has been reviewed previously (Darras and Van Herck, 2012; Orozco et al., 2012). In the Atlantic salmon, up to six deiodinase paralogs have been characterized, one *dio1*, two *dio2* (*dio2a* and *dio2b*) and three *dio3* (*dio3a1*, *dio3a2,* and *dio3b*) (Lorgen et al., 2015). A single *dio2* gene is present in Northern pike *Esox lucius* (Rondeau et al., 2014), which suggests that the two *dio2* paralogs of the Atlantic salmon arose recently from the salmonid-specific WGD, 4R (Lorgen et al., 2015). *Dio3a1*/*3a2* likely also resulted from 4R (Lorgen et al., 2015). Functional divergence of the two salmon *dio2* paralogs is reported: while gill *dio2a* expression is induced by seawater exposure (Lorgen et al., 2015), *dio2b* expression in the brain is sensitive to photoperiod during smoltification (Lorgen et al., 2015; Irachi et al., 2021).

Hypothalamic-Pituitary-Adrenal/Interrenal

Corticotropin-Releasing Hormone and Its Receptors

The hypothalamic-pituitary-adrenal axis (HPA), in mammals and sauropsids, and hypothalamic-pituitary-interrenal (HPI), in amphibians and teleosts, is the neuroendocrine corticotropic axis (**Figure 3**). In 1955, a substance, present in extracts of mammalian hypothalamus and able to stimulate ACTH secretion *in vitro*, was named corticotropin-releasing factor (CRF) (Guillemin and Rosenberg, 1955; Saffran et al., 1955). CRF (or CRH) was first isolated from sheep hypothalamus (Vale et al., 1981) and identified in all vertebrates thereafter (Lovejoy et al., 2014; On et al., 2019). Together with urotensin I (UI) in teleosts, sauvagine (SVG) in amphibians, and urocortins (Ucn) in mammals, it forms a large family of peptides, the CRH/urocortin family. As CRH, urotensin I, sauvagine and urocortins have roles in the regulation of ACTH and MSH, as well as energy metabolism and reproduction (Lovejoy and Balment, 1999). It

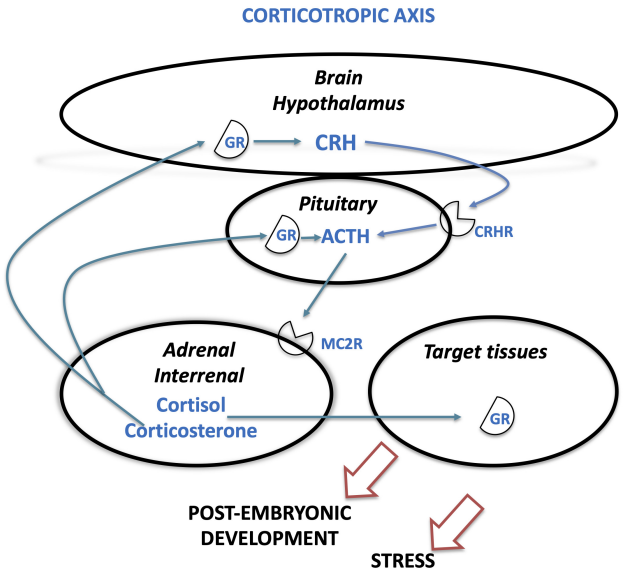


FIGURE 3 | Corticotropic neuroendocrine axis in vertebrates. The figure displays the main actors of the corticotropic (hypothalamic-pituitary-adrenal/interrenal, HPA/HPI) axis. ACTH, adrenocorticotropin; CRH, corticotropin-releasing hormone; CRHR, corticotropin-releasing hormone receptor; GR, glucocorticoid receptor; MC2R, melanocortin receptor 2.

was first suggested that two ancestral *crh/ucn1* and *ucn2/ucn3* genes likely arose by specific gene duplication before vertebrate WGD events (Hwang et al., 2013), as a single crh-like gene is identified in amphioxus and tunicates (On et al., 2019). Both ancestral genes were duplicated twice in ancestral vertebrates *via* 1R and 2R, followed by some paralog losses, leading to up to 5 genes (*crh1, crh2, ucn1* coming from ancestral *crh/ucn1*; *ucn2, ucn3* issued from ancestral *ucn2/ucn3*) in extant representative species of some vertebrate lineages such as chondrichthyans, holosteans and actinistians (Cardoso et al., 2016; On et al., 2019). Teleost specific 3R resulted in the duplication of *crh1* into two paralogs *crh1a* and *crh1b* conserved in many species (Grone and Maruska, 2015; Cardoso et al., 2016). *Crh2* may have been lost in recent teleosts (Cardoso et al., 2016), while one 3R-*crh2* paralog has been conserved in basal groups of teleosts such as European eel (Maugars et al., 2016).

In tetrapods, CRH has been shown to act *via* two GPCRs, corticotropin-releasing hormone receptors (CRHR), CRHR1, and CRHR2, which belong to the class 2 subfamily B1 of secretin-like GPCR superfamily (Lovejoy et al., 2014). *Crhr1* was duplicated by 3R into two paralogs (*crhr1a* and *crhr1b*) which were conserved in many extant teleosts, while one of 3R-duplicated *crhr2* paralogs would have been lost (Cardoso et al., 2016). A third CRHR, CRHR3, identified in the catfish, *Ameiurus nebulosus* (Arai et al., 2001), highly similar to catfish CRHR1, likely results from gene-specific duplication, that may also occurred in various teleosts (Lovejoy et al., 2014; Cardoso et al., 2016).

Proopiomelanocortin-Derived Peptides and Their Receptors

The main pituitary hormone involved in the HPA/HPI axis is ACTH. ACTH is derived from tissue-specific post-translational processing of its precursor, the proopiomelanocortin (POMC); POMC also gives rise to melanocyte stimulating hormone (MSH), and β-endorphin (β-END). POMC forms the opioid/orphanin gene family together with proenkephalin, prodynorphin and proorphanin (Dores et al., 2002; Sundström et al., 2010). ACTH and MSH, called melanocortins (MC), act *via* MC receptors (MCR), while β-END acts *via* opioid receptor. Teleost 3R gave rise to *pomc* gene duplicates: *pomc*-α (*a* or *A*) and *pomc*-β (*b* or *B*), with *pomc*-β having lost a functional β-endorphin (De Souza et al., 2005). Further independent gene-specific duplications during teleost evolution resulted in duplicates of *pomc*-α, such as in sea bream *Sparus aurata* (Cardoso et al., 2011) and *Astatotilapia burtoni* (Harris et al., 2014).

Melanocortin receptors (MCR) belong to the rhodopsin class A13 family of GPCRs. In tetrapods, five MCR genes have been identified: *mc1r* to *mc5r* (Cortés et al., 2014; Dores et al., 2014). In teleost fish, probably due to 3R, the number of receptors increases up to six in zebrafish, which has two MC5R paralogs (Västermark and Schiöth, 2011), while pufferfish *Fugu rubripes* has only four, with no *mc3r* and only one copy of *mc5r* (Logan et al., 2003). Concerning the ligand selectivity of MCRs, all of the paralogous MCRs can be activated by both ACTH and MSH in extant cartilaginous fishes, while in extant teleosts and tetrapods, only ACTH can activate MC2R (Cortés et al., 2014; Dores et al., 2014). In mammals, the MCRs have distinct tissue expression (Cone, 2006; Dores et al., 2014): *mc1r* is mainly expressed in melanocytes; *mc2r* in adrenal cortex; *mc3r* and *mc4r* in the brain; and *mc5r* in a variety of exocrine glands, such as sebaceous, lacrimal and preputial glands. In concordance with their expression sites, each MCR has its proper function: MC1R is involved in skin and hair pigmentation; MC2R in adrenal steroidogenesis and stress response; MC3R and MC4R in energy homeostasis; and MC5R in exocrine gland secretion. These features can also be observed in non-mammalian vertebrates, with some differences. Of particular interest, *mc5r* is co-expressed with *mc2r* in the interrenal of *Xenopus tropicalis* (Dores and Garcia, 2015) and several teleosts [rainbow trout *Oncorhynchus mykiss* (Haitina et al., 2004; Aluru and Vijayan, 2008); common carp *Cyprinus carpio* (Metz et al., 2005); barfin flounder *Verasper moseri* (Kobayashi et al., 2011)], as well as in the chicken adrenal (Takeuchi and Takahashi, 1998), suggesting a possible role of MC5R, in addition to MC2R, in the regulation of HPI axis in these non-mammalian vertebrates.

Corticosteroids and Their Receptors

Corticosteroids (CS) are steroid hormones, divided into glucocorticoids (GC), and mineralocorticoids (MC). In mammals and sauropsids, CS are synthetized by the adrenal gland. In amphibians and teleosts, CS are synthetized by the interrenal gland, a tissue embedded inside the anterior part of the kidney (head kidney) and homologous to the adrenal cortex of the adrenal gland in mammals (Chester-Jones, 1987). Cortisol

is the primary GC in most mammals and teleosts, while it is corticosterone in birds, reptiles, amphibians and many rodents (Mommsen et al., 1999; Aerts, 2018). Both GC can co-exist in most vertebrates. Aldosterone, which is the principal MC in mammals (Gilmour, 2005), is lacking in all teleosts studied so far. In teleosts, it is generally accepted that cortisol plays both GC and MC roles (McCormick, 2001; McCormick et al., 2008).

GC and MC receptors (respectively, GR and MR) belong to the nuclear receptor superfamily. 2R resulted in the emergence of GR and MR, present in all extant vertebrates. In teleosts, 3R gave rise to duplicated *gr* and *mr* (Bury, 2017). Zebrafish is the only known teleost to have conserved only one of the two *gr* paralogs (Schaaf et al., 2008). The two *mr* paralogs are present in a basal teleost, the European eel *Anguilla anguilla* (Lafont et al., 2014), while only one *mr* paralog has been conserved in the other extant teleosts studied so far (Baker and Katsu, 2019).

METAMORPHOSES AND OTHER DEVELOPMENTAL TRANSITIONS IN VERTEBRATES AND THEIR CONTROL BY THYROID HORMONES AND CORTICOSTEROIDS

Larval Metamorphosis in Anuran Amphibians

Involvement of Thyroid Hormones

After hatching and a period of growth, the tadpole undergoes a rapid and irreversible physiological, anatomical and environmental transition marking the transition from the larval state to the adult state, the metamorphosis. Gudernatch observed that the administration of extracts of the thyroid gland (and only this organ) *via* the water induced tadpole premature metamorphosis (Gudernatch, 1912). Following TH measurements (Leloup and Buscaglia, 1977), the elucidation of the roles and mechanisms of action of TH in vertebrates has thus benefited enormously from amphibian model (Grimaldi et al., 2013; Buchholz, 2017; Sachs and Buchholz, 2017). The activity of TH is regulated locally by deiodinases (DIO) and cytosolic proteins (Shi et al., 1994). In particular, the expression of DIO2 (enzyme activating TH) is high in tissues undergoing metamorphosis, while the expression of inactivating deiodinase (DIO3) is high in tissues before and after metamorphosis (Leloup et al., 1981; Galton, 1989).

Another level of TH signaling control is linked to the expression profiles of thyroid hormone receptors (TR) (Yaoita and Brown, 1990). A dual role of thyroid signaling was proposed (Sachs et al., 2000; Grimaldi et al., 2013; Buchholz and Shi, 2018). Thus, before metamorphosis, the relative absence of TH and the expression of TRα contribute to repressing target genes allowing tadpole growth. During the climax of metamorphosis, the high concentration of TH concomitant with the expression of TRα and TRβ contributes to the activation of the transcriptional program leading to tadpole transformation. The two TR isoforms have also different roles during metamorphosis. TRα was shown to be more involved in cell proliferation while TRβ in cell differentiation and apoptosis (Furlow and Neff, 2006; Denver et al., 2009). TRα is required for TH-dependent neurogenesis (Wen et al., 2019). The autoinduction of TRβ is one of the earliest responses to the thyroid signal (Yaoita and Brown, 1990) because *trβ* is a direct TH-target gene (Ranjan et al., 1994; Bilesimo et al., 2011).

Transgenic studies in *Xenopus laevis* revealed that TR are necessary and sufficient for mediating the effects of T_3 during metamorphosis (Buchholz et al., 2003, 2004). However, recent gene knockout studies in *Xenopus tropicalis* showed that TR are not required for most metamorphic transformations, although tadpoles lacking TR die in the middle of metamorphosis (Shi, 2021). Removal of TR enables premature metamorphosis of several adult tissues (Buchholz and Shi, 2018), likely due to the absence of T_3-inducible gene repression. This result supports the role of unliganded TR to avoid precocious metamorphosis for proper tadpole growth. Finally, removal of TR prevents the disappearance of tadpole-specific tissues (Shibata et al., 2020, 2021).

Involvement of Corticosteroids

Glucocorticoids (GC) are also essential during anuran metamorphosis. In *Xenopus laevis* tadpoles, corticosterone concentration is high during pre-metamorphosis, decreases during pro-metamorphosis and increases again during metamorphic climax (Jaudet and Hatey, 1984; Jolivet-Jaudet and Leloup-Hâtey, 1986; Kloas et al., 1997; Glennemeier and Denver, 2002). However, the actions of GC on anuran development are difficult to dissect because of their involvement in many biological processes. Thus, GC inhibit growth, both in pre- and pro-metamorphic tadpoles and the administration of exogenous GC to pre-metamorphic tadpoles inhibits the emergence of lower limbs (Kobayashi, 1958). However, the use of drugs which antagonize the molecular action of GC or prevent the production of GC by the adrenals, inhibits metamorphosis (Kikuyama et al., 1982).

During metamorphosis, GC act through glucocorticoid receptors (GR) that have dynamic and tissue-specific expression profiles (Krain and Denver, 2004). In the tail, GR expression follows the concentration profile of GC, but in the gut and brain the number of transcripts were constant during pro-metamorphosis, and slightly decreased or increased, respectively, during climax. GR knockout in *Xenopus* leads to complete abrogation of the corticosterone-responsive gene induction by exogenous hormone (Sterner et al., 2020). In addition, tadpoles lacking GR developed faster than wild-type sibling until forelimb emergence. Then, they developed more slowly and died at the climax of metamorphosis, indicating that GR is required for metamorphosis and control the developmental rate. The essential requirement of the HPA axis was confirmed with the gene-editing disruption of the *pomc* gene (Sterner et al., 2020). Mutant tadpoles had a reduced level of plasma corticosterone at metamorphosis, as well as lower expression of the corticosterone-responsive genes. Last but not least, these tadpoles had reduced rates of growth and development that finally led to death, late during metamorphosis at the time of tail resorption.

Interactions Between Thyroid Hormones and Corticosteroids

Although TH are the trigger for metamorphosis and are required throughout this process (Sachs and Buchholz, 2017), their actions strongly intersect with those of CS (Sachs and Buchholz, 2019). Stressors such as predation or pond desiccation can be experienced by tadpoles and cause activation of the HPI axis (Glennemeier and Denver, 2002). In the tadpole, the synthesis and release of TSH by the anterior pituitary is under the control of CRH (Denver, 1993, 2021), as in other non-mammalian vertebrates (De Groef et al., 2006). When injected to prometamorphic *Bufo arenarum* larvae, CRH is able to accelerate metamorphosis, likely by direct action on both TSH and ACTH pituitary cells (Miranda et al., 2000). This dual role of CRH allows the tadpole to modulate the rate of metamorphosis in response to environmental stimuli (Denver, 1997). CS are closely linked to metamorphosis by delaying or accelerating its rate of progression. Elevation in circulating CS during the pre-metamorphic stage slows tadpole growth (Denver, 2017). However, during pro-metamorphosis, tadpoles increase the production of TH and CS in response to environmental stress, and are therefore able to accelerate metamorphosis, which can allow the animal to escape a deteriorating larval habitat by transitioning to the next life history stage (Denver, 1997, 2009; Denver et al., 2009; Kulkarni and Buchholz, 2014). Remarkably, CS are capable of triggering metamorphosis in *Buffo boreas* (Hayes et al., 1993). This action is probably due to the endogenous levels of TH with which GC are able to act synergistically, but insufficient, on their own, to induce the metamorphosis.

How does the crosstalk between TH and CS occur? CS are known to promote the expression of activating deiodinases and repress the expression of inactivating deiodinases, thus contributing to the accumulation of active T_3 in target tissues. This model has long been the basis for explaining the potentiating effect of GC on metamorphosis (Galton, 1990). Furthermore, GC can also act in synergy with TH via cross-regulation at the level of their respective nuclear receptors. GC are able to increase the binding capacity of T_3 in cell nucleus (Suzuki and Kikuyama, 1983) and the amount of *tr*β transcripts in the tail, brain and intestine (Bonett et al., 2010). Conversely, the expression of GR seems to be partly controlled by TH depending on the tissues observed: while in the brain and intestine, treatment with T_3 induces a decrease in the expression of GR, the opposite occurs in the tail (Krain and Denver, 2004). These results suggest that CS are able to increase the sensitivity to TH in some tissues, thus accelerating metamorphosis. These actions are synergistic with low concentrations of TH (Bonett et al., 2010). Brown et al. (2014) reported similar hormonal synergy during larval development in fish.

Other genes expressed during metamorphosis are synergistically regulated by TH and CS (Kulkarni and Buchholz, 2012; Bagamasbad et al., 2015), some through direct transcriptional regulation by liganded TR and GR. One of the best studied examples of this type of regulation is the transcription factor krüppel-like factor 9 (Klf9), which is directly and synergistically regulated by TH and CS *via* an ultra-conserved super enhancer located upstream of the transcription start site (Bonett et al., 2009; Bagamasbad et al., 2015). Klf9 strongly enhances TRβ autoinduction in tadpole tissues, among other roles modulating gene transcription and hormone action during metamorphosis (Hu et al., 2016). Other genes show synergistic regulation by TH and CS in tadpole tissues, but whether their protein products function to modulate the rate of metamorphosis requires further study (Kulkarni and Buchholz, 2012, 2014).

Finally, the recent results with tadpoles lacking *pomc* reinforce the inseparable link between TH and GC at metamorphosis (Sterner et al., 2020). Tadpoles lacking *pomc* had reduced expression levels of TH-responsive genes such as *klf9* and *tr*β. Even more significant, mutant death at metamorphosis is rescued by exogenous TH, suggesting a strong entanglement of the signaling by both hormones.

First/Primary and Secondary Metamorphoses in Teleost Fishes

In fishes, two types of metamorphoses can be observed. Both types involve various morphological, physiological and behavioral modifications that preadapt the animal to life in a new environmental niche/habitat, but occur at different life-stages. First/primary metamorphosis typically occurs in elopomorphs and pleuronectiforms, during larval stage and is so-called larval metamorphosis. Secondary metamorphosis occurs in juveniles of some diadromic migratory teleosts and involves less drastic morphological changes. This is the case of smoltification in anadromous salmonids, which allows the transition from a juvenile ecophase in freshwater to the next ecophase in the ocean.

Larval Metamorphosis in Teleosts: The Example of Flatfish

Beside the striking larval metamorphosis in eels and flatfishes, studies also report developmental changes controlled by TH during larval to juvenile transition in various teleosts such as grouper *Epinephelus coioides* (de Jesus et al., 1998), seabream (Campinho et al., 2010), gobiid *Sicyopterus lagocephalus* (Taillebois et al., 2011), or clownfish *Amphiprion percula* (Salis et al., 2021). Our review will focus on the well-known pleuronectiform/flatfish metamorphosis. Larval metamorphosis with the migration of one eye to the opposite side of the head is unparalleled in vertebrate development (Inui and Miwa, 2012). Spectacular morphological changes are accompanied by behavioral (pelagic to benthic life and locomotion) and physiological (such as development of gastrointestinal tract to adapt to the novel food resources in a new habitat) changes.

Involvement of Thyroid Hormones

In the Japanese flounder *Paralichthys olivaceus*, larval tissue T_4 and T_3 concentration increases gradually during pro-metamorphosis, and rises sharply at the beginning of climax, reaching highest level at climax (Miwa et al., 1988; Tanangonan et al., 1989; Tagawa et al., 1990; de Jesus et al., 1991). A similar surge of tissue TH concentration at metamorphic climax is reported in spotted halibut, *Verasper variegatus* (Hotta et al., 2001a,b), Atlantic halibut *Hippoglossus hippoglossus*

(Einarsdóttir et al., 2006), and summer flounder, *Paralichthys dentatus* (Schreiber and Specker, 1998).

Treatments of flatfish by TH or anti-thyroid drug have demonstrated the involvement of TH in many metamorphic changes (Inui and Miwa, 2012). One of the most striking examples concerns skeleton development (shortening of fin rays, eye migration and asymmetry). The elongated fin rays at the anterior end of the dorsal fin shorten to reach similar length to other fin rays. Manipulation of T_4 availability disrupts pre-metamorphic Japanese flounder larvae shortening of elongated fin rays (Inui and Miwa, 1985; Miwa and Inui, 1987; de Jesus et al., 1990). Administration of T_4 to pre-metamorphic larvae accelerates eye migration (Inui and Miwa, 1985; Miwa and Inui, 1987; Solbakken et al., 1999). In addition, Schreiber and Specker showed a stage-specific developmental response to TH, with a more pronounced effect in the induction of eye migration at earlier stages (Schreiber and Specker, 1998). Campinho et al. (2018) gave the first evidence of a TH-responsive asymmetric center located in the anterior head region that is correlated with asymmetric development during metamorphosis of flatfish.

In Japanese flounder, expression of *trαA* and *trβs* (*trβ1* and *trβ2*) genes increase rapidly at metamorphic climax; while expression of *trαA* peaks at climax and decreases thereafter (like T_4 content), expression of *trβs* peak at post-climax and remains high in metamorphosed juveniles; in contrast, expression of *trαB* remains low throughout larval development (Yamano and Miwa, 1998). In the turbot, *Scophthalmus maximus*, *trα*, but not *trβ*, expression is up-regulated at metamorphic climax (Marchand et al., 2004). In contrast, studies in two other flatfishes, the halibut (Galay-Burgos et al., 2008) and the Senegalese sole *Solea senegalensis* (Isorna et al., 2009; Manchado et al., 2009), describe a situation similar to that in amphibian with only *trβ* expression showing a peak during metamorphosis.

DIO2 activity and *dio2* expression increase during sole metamorphosis, while DIO3 activity and *dio3* expression decline at mid-late metamorphic period (Isorna et al., 2009). These developmental profiles of deiodinases coincide with the rise of TH levels observed. In this species, there is an asymmetric expression of *dio2* and *TRβ* in the head, which coincides with the head region where asymmetric development of bone and brain occurs (Campinho et al., 2018). In the Japanese flounder, *dio1* is expressed in liver from pro-metamorphosis to early climax, while *dio2* is expressed in limited regions of the eyes, tectum and skeletal muscle from pro-metamorphosis to post-climax, and *dio3* in skeletal muscle and gastric gland blastemas at metamorphic climax (Itoh et al., 2010).

Involvement of Corticosteroids

Data support a role of CS in larval flatfish metamorphosis. Whole body cortisol concentration in Japanese flounder increases during pro-metamorphic stage, reaching a peak level at climax, and decreases thereafter to about half of the maximal level (de Jesus et al., 1991). *In vitro* cortisol treatment of cultured dorsal ray fins from Japanese flounder is not effective alone on inducing metamorphic-like changes such as resorption of the fin rays (de Jesus et al., 1990). In contrast, *in vivo* cortisol treatment *via* water of pro-metamorphic flounder larvae for 15 days is not able to trigger some of the changes observed during metamorphosis, like settling (benthic) behavior and eye migration, but does induce shortening of second fin ray (de Jesus et al., 1990). Administration of cortisol *via* water to spotted halibut increases the occurrence of ambicolored juveniles by inducing the development of adult type pigment cells on the blind side of fishes (Yamada et al., 2011). In cortisol-implanted juvenile Senegalese sole, hepatic and renal DIO2 activities are enhanced, suggesting that CS can be key regulator of extrathyroidal T_3 production in this species (Arjona et al., 2011). This regulation is likely to occur also during sole larval metamorphosis.

Interactions Between Thyroid Hormones and Corticosteroids

Few data are available concerning the interactions between TH and GC in the control of teleost larval metamorphosis. In the Japanese flounder, a permissive effect of cortisol on thyroid hormone action is observed during metamorphosis (de Jesus et al., 1990). Indeed, cortisol alone does not affect the shortening of fin rays *in vitro*, whereas when added together with T_4 or T_3, it enhances its rate compared to T_4 or T_3 alone. This permissive effect of cortisol on TH action was not reported *in vivo* (de Jesus et al., 1990). Combined T_4 and cortisol treatment does not induce either synergistic effects on settling behavior and eye migration, compared to T_4 treatment alone (de Jesus et al., 1990). The authors concluded that it may be due to sufficient production of endogenous cortisol by larval interrenal. Future studies should aim at investigating the mechanisms of CS/TH interactions in teleost metamorphoses as deciphered in amphibians.

Secondary Metamorphosis in Teleosts: Example of Smoltification in Salmonids

Smoltification is the transition of sedentary juvenile parr into downstream migratory smolt, which will pursue its growth phase in the ocean. Smoltification gathers many changes, morphological ones, such as body silvering and fin darkening, behavioral ones, with swimming activity in open space, formation of schools and downstream migration, and physiological ones related to adaptation to seawater and imprinting (Hoar, 1976, 1988; Boeuf, 1993; McCormick, 2012; Rousseau et al., 2012).

Involvement of Thyroid Hormones

Even if some authors do not consider salmonid smoltification as a metamorphosis (Bishop et al., 2006), this transformation from parr to smolt involves major changes and is necessary for the fish to reach its next habitat and survive in a new environment. Moreover, many parallel features exist between flatfish metamorphosis and salmonid smoltification, as reviewed by Björnsson et al. (2012). Lastly, control by TH is crucial in smoltification.

In salmonids, a rise of plasma T_4 and/or T_3 levels is observed at the time of smoltification (McCormick et al., 2009; Björnsson et al., 2012). Manipulation of TH levels by administration of T_4, T_3 or anti-thyroid drugs supports a major role of TH in many smoltification-related morphological, behavioral and physiological changes, such as metabolism, olfaction, change in visual pigments, swimming behavior and downstream migration (McBride et al., 1982; Rousseau et al., 2012). The examples of TH-induced changes, given here, are necessary for avoiding

predation and for adaptation to a new environment, seawater (SW). A pioneer study showed that intramuscular injection of mammalian thyroid extract or TSH induces silvery smolt stage in rainbow trout (Robertson, 1949). Following studies confirmed that exogenous TH (Miwa and Inui, 1983, 1985; Ikuta et al., 1985; Coughlin et al., 2001) and TSH (rainbow trout: Premdas and Eales, 1976) induce body silvering. The silvering of the body is due to the deposition in the skin of purines, hypoxanthine and guanine, the level of which is increased by T_4 treatment in masu salmon *O. masou* (Ura et al., 1994). Early evidence reported the involvement of TH in salinity preference: TSH-treated coho salmon *O. kisutch* shows a change from freshwater (FW) to SW-preference, while the contrary is observed in pink salmon *O. gorbuscha* treated by an anti-thyroid drug (Baggerman, 1963). Similarly, T_4 treatment increases the salinity preference in coho salmon (Iwata et al., 1990). In Atlantic salmon, T_3 increases SW survival (Saunders et al., 1985).

Interestingly, a large peak of expression of *tshβ1b* during Atlantic salmon smoltification, at the time of the initiation of the downstream migration, has recently been demonstrated, with no change in the expression of the paralog *tshβ1a*, suggesting the involvement of *tshβ1b* at smoltification, possibly in the initiation of migration (Fleming et al., 2019). In addition, the expression of pituitary *tshβ1b*, and not *tshβ1a*, is induced by long day-photoperiod (16h of light and 8h of dark) in Atlantic salmon (Irachi et al., 2021).

Expression of TR has been detected in all the tissues involved in smoltification-related changes (Marchand et al., 2001; Jones et al., 2002; Raine et al., 2005; Harada et al., 2008). Expressions of *trα* and *trβ* show no drastic change between the different stages of smoltification in brain, liver, eyeball, and skin in coho salmon (Harada et al., 2008). In contrast, the olfactory epithelium of masu salmon presents more T3 binding sites at smolt than at parr and pre-smolt stages, suggesting that olfactory tissues may be particularly sensitive to TH, related to a possible role in imprinting of natal stream odors in order to migrate back there to reproduce (Kudo et al., 1994). DIO3 activities are enhanced at smoltification in liver (Eales et al., 1993; Sweeting et al., 1994; Specker et al., 2000) and gill (Sweeting et al., 1994). The expression of one of the 4R-paralog, *dio2b*, increases in the brain in zones of cell proliferation during smoltification in response to photoperiod (Lorgen et al., 2015; Irachi et al., 2021).

Involvement of Corticosteroids

The cells of interrenal tissue undergo hypertrophy during smoltification (for review: (Specker, 1982). Pituitary corticotropic cells are also activated during smoltification in Atlantic salmon (Olivereau, 1975). Plasma cortisol levels are low in winter, increase during spring at the time of smoltification and decline from July to September (Langhorne and Simpson, 1981, 1986; Specker and Schreck, 1982; Barton et al., 1985; Virtanen and Soivio, 1985; Young et al., 1989; Nagae et al., 1994; Sundell et al., 2003). Maximal *in vitro* responsiveness of interrenal tissue to ACTH is observed in April in coho salmon (Young, 1986).

As for TH, we will concentrate on the effects of HPI manipulation on pigmentation and osmoregulation. Injection of ACTH in Atlantic salmon can induce darkening of dorsal, pectoral and caudal fins, but neither ACTH nor cortisol have an effect on body silvering (Langdon et al., 1984). Prolonged cortisol treatment in pre-smolt salmons increase Na^+/K^+-ATPase activity in the gill (Richman et al., 1987; Madsen, 1990) and in gut (Madsen, 1990; Veillette and Young, 2005). *In vitro*, cortisol treatment maintains Na^+/K^+-ATPase activity, which declines in controls, in tissue culture of FW-adapted sockeye salmon intestine (Veillette and Young, 2005). Injections of FW-Atlantic salmon with cortisol increase the expression of claudins (tight-junction proteins) involved in the remodeling of the gill in response to salinity changes (Tipsmark et al., 2009). Using primary cultures of Atlantic salmon gill tissue, a stimulatory effect of cortisol is observed on the expression of claudins; an effect blocked by GR antagonist, mifepristone (or RU486), suggesting the involvement of a glucocorticoid type receptor (GR) (Tipsmark et al., 2009).

An increase in gill GR concentration and *gr* expression is reported during smoltification in all studied salmonid species (McLeese et al., 1994; Shrimpton and Randall, 1994; Shrimpton, 1996; Mazurais et al., 1998; Shrimpton and McCormick, 1998, 2003; Mizuno et al., 2001; Kiilerich et al., 2007). This increase occurs before the increase in plasma cortisol, which could explain the increased responsiveness of gill tissue to cortisol observed in early spring in coho and Atlantic salmon (McCormick et al., 1991) and also demonstrated *in vitro* in rainbow trout (Shrimpton and McCormick, 1999).

Interactions of Thyroid Hormones and Corticosteroids

Studies report interactions between HPT and HPI during smoltification. For example, TH can increase CRH neurogenesis during smoltification in Atlantic salmon (Ebbesson et al., 2011), suggesting a positive effect of TH on HPI. Cortisol treatment can lower plasma T_3 (but not plasma T_4) in coho salmon (Vijayan and Leatherland, 1989), during smoltification (Redding et al., 1991). The increases of *tr* mRNA and TR protein expressions and of plasma TH levels, observed during transfer from FW to SW, are lower in cortisol-injected smolt sockeye salmon compared to controls (Shin et al., 2014), suggesting some antagonistic/negative effects of cortisol on TH action.

Egg Hatching in Sauropsids

Thyroid hormones have an important role in fish egg hatching (tilapia: Reddy and Lam, 1991; Walpita et al., 2007; zebrafish: Heijlen et al., 2014; for review: Brown et al., 2014), but it will not be considered in this review, as egg hatching in fish implies less drastic changes of environmental conditions, as compared to sauropsids. In contrast, hatching in oviparous sauropsids, such as birds and reptiles, is characterized by a transition from a "protected" aqueous environment to a terrestrial environment exposed to desiccation and predation.

Egg Hatching in Birds

Involvement of Thyroid Hormones

Considering the implication of TH in egg hatching, one must differentiate precocial from altricial birds (McNabb, 2006; De Groef et al., 2013). Precocial species (e.g., chicken *Gallus domesticus*, Japanese quail *Coturnix japonica*, bobwhite

quail *Colinus virginianus*, turkey *Melleagris gallopavo,* mallard Pekin duck *Anas platyrhynchos*, goose *Anser anser*) have youngs that are immediately mobile and independent after hatching. In contrast, youngs of altricial birds (e.g., European starling *Sturnus vulgaris*, Ring dove *Streptopelia risorii*, red-winged blackbird *Agelaius phoeniceus*, great tit *Parus major*) need to be fed and thermoregulated by their parents in the nest (Starck and Ricklefs, 1998).

A gradual increase in plasma TH concentrations of the embryos to a peak is observed in precocial birds during the peri-hatch period (for review McNabb, 2006). In contrast, in altricial birds, circulating concentrations of TH are low during embryonic life and at hatching, and only increase after hatching (McNabb et al., 1984; Silverin and Rudas, 1996; Výboh et al., 1996; Olson et al., 1999).

Manipulation of egg TH levels influences avian hatching time. Injection of antithyroid drugs in the yolk sac of chick embryos retards hatching, and T_4 fully neutralizes these effects (Grossowicz, 1946; Adams and Bull, 1949; Romanoff and Laufer, 1956; Sinha et al., 1959; Balaban and Hill, 1971; Haba et al., 2011). In turkey, T_4 or T_3 injected to fertile eggs at Day 0 of incubation depresses hatchability, while improving it when administered at Day 25 of incubation out of a 28-day incubation period (Christensen, 1985). In Pekin duck, injection of T_3 results in earlier hatching, while injection of antithyroid drug delays hatching (Sirsat and Dzialowski, 2020). In Japanese quail, injection of T_4 alone or with T_3 doubles hatching success (Sarraude et al., 2020). Similarly, in a type 2 semi-altricial development model, the rock pigeon *Columba livia*, injection of a mixture of both T_3 and T_4 early results in higher hatching success but unchanged hatching time (Hsu et al., 2017).

In chicken, a differential temporal expression pattern of the three TR is observed. TRα mRNAs are detected as early as gastrula stage and throughout development in many embryonic tissues (Forrest et al., 1990). TRβ0 mRNAs appear later in embryonic life and are restricted to brain, eye, lung, and yolk sac (Forrest et al., 1990). TRβ2, a N-terminal variant of TRβ0, is predominantly expressed in retina (Sjöberg et al., 1992). The minimal generation of T3 until just before hatching is primarily due to the presence of DIO3 in chicken embryo liver (Galton and Hiebert, 1987). Hepatic DIO1 activity increases up to hatching and decreases thereafter, while hepatic DIO3 activity increases during embryogenesis, then decreases before hatching to remain low at post-hatching (Darras et al., 1992; Reyns et al., 2003). Dynamics of expression in deiodinases in the choroid plexus of the developing chicken brain have been reported: *dio1* and *dio2* mRNA levels increase over time to reach a peak around hatching, while *dio3* expression is high before hatching and decreases at hatching to re-increase up to 1 day post-hatching (Van Herck et al., 2015).

Involvement of Corticosteroids

An increase of avian embryonic GC is observed around the time of hatching in plasma (Wise and Frye, 1973; Kalliecharan and Hall, 1974; Siegel and Gould, 1976; Marie, 1981; Scott et al., 1981; Tanabe, 1982; Tanabe et al., 1983, 1986; Wentworth and Hussein, 1985; Porter et al., 2007), in adrenal glands (Tanabe et al., 1983, 1986), and in feces (Frigerio et al., 2001), and in adrenocortical cells in culture (Carsia et al., 1987).

Most studies investigating the effects of exogenous treatment of embryos with corticosterone in birds look at the postnatal growth and behavior linked to stress response (Rubolini et al., 2005; Saino et al., 2005; Freire et al., 2006; Hayward et al., 2006; Janczak et al., 2006; Henriksen et al., 2013; Zimmer et al., 2013; Weber et al., 2018). However, a few looked at the effects of GC treatment on hatching. Injection of corticosterone in the yolk sac 2 days prior to hatching shortens incubation time and increases hatchability in turkey (Wentworth and Hussein, 1985). In contrast, dipping fertile eggs in corticosterone prevents hatching in chicken (Mashaly, 1991). Elevation of plasma corticosterone levels by mean of synthetic GC (dexamethasone) injection during the final stages of incubation in chicken embryos increases or shortens incubation period when administered, on Day 16 and Day 18, respectively (Tona et al., 2007). In Japanese quail, incubation period is shortened in eggs laid by corticosterone-implanted hens (Schmidt et al., 2009). Chicken lung becomes sensitive to corticosterone prior hatching (Hylka and Doneen, 1983). Corticosterone treatment triggers prehatching stimulation of surfactant phospholipid synthesis (Hylka and Doneen, 1983). The first two studies reporting the cloning and developmental expression of pituitary GR in bird (chicken) gave different results: While Kwok et al. (2007) demonstrate constant expression of GR during embryonic period, other authors show an increase of GR mRNA levels (Porter et al., 2007).

Interactions Between Thyroid Hormones and Corticosteroids

Creating hypothyroidism in chick embryos induces a cortical atrophy and medulla hypertrophy in adrenal gland (Kingsbury et al., 1955). Injection of cortisol onto the allantoic membrane of 17 or 16-days old chicken embryos induces the same premature maturational changes in T_3 and T_4 metabolism observed naturally on 19–20-days old embryos, meaning a decrease of DIO3 preserving the T_3 formed, and an increase of DIO2 sparing T_4 (Borges et al., 1980). The administration of either dexamethasone or corticosterone, or ACTH to chicken embryos increase plasma T_3 concentrations and hepatic DIO2 activity (Decuypere et al., 1983) and increase plasma T_3 levels, decreased plasma T_4, decreased hepatic DIO3 activity and increased hepatic DIO1 activity (Darras et al., 1996).

Egg Hatching in Reptiles

Involvement of Thyroid Hormones

An increase in TH correlates with hatching in saltwater crocodile *Crocodylus porosus* (Shepherdley et al., 2002). In the grass snake *Natrix natrix*, the activity of the embryonic thyroid exhibits the features of a fully active gland at the time of hatching (Rupik, 2011). Injection of antithyroid drug into embryos of the snapping turtle *Chelydra serpentina* gives enlarged thyroid gland and delays hatching time (Dimond, 1954). Eggs of red-eared turtle, *Trachemys scripta*, treated with T_3, have shortened incubation duration (Sun et al., 2016). Embryos from Murray River short-necked turtle, *Emydura macquarii*, exposed to T_3, hatch earlier than untreated ones (McGlashan et al., 2017). Treatment of

saltwater crocodile embryos with T_3 increases DIO1 activity in liver and DIO2 in kidney (Shepherdley et al., 2002). Treatment with T_3 stimulates the secretion of phosphatidylcholine (PC, the major component of pulmonary surfactant) in sea turtle, *Chelonia mydas* (Sullivan et al., 2001). In saltwater crocodile, pre-treatment with T_3 increases surfactant phospholipids in lung (Sullivan et al., 2002a).

Involvement of Corticosteroids

An increase of corticosterone is observed from the last third of incubation to hatching in American alligator *Alligator mississippiensis* (Medler and Lance, 1998; Jennings et al., 2000). In contrast, a decrease in corticosterone is observed at hatching in saltwater crocodile (Shepherdley et al., 2002). Yolk corticosterone concentrations peak near the time of hatching in the tree lizard, *Urosaurus ornatus* (Jennings et al., 2000) and in sea turtles (Owens and Morris, 1985). In the tree lizard, treatment with corticosterone accelerates egg hatching (Weiss et al., 2007). Treatment with dexamethasone of saltwater crocodile embryos decreases DIO1 activity in kidney, DIO2 activity in liver and in kidney and DIO3 activity in liver (Shepherdley et al., 2002). Dexamethasone alone increases phospholipids from pulmonary surfactant in sea turtle when administered *in ovo* during late incubation (Sullivan et al., 2001). It also stimulates the secretion of phosphatidylcholine *in vitro* in saltwater crocodile (Sullivan et al., 2002a) and in lizard bearded dragon *Pogona vitticeps* (Sullivan et al., 2002b). In Chinese alligator *Alligator sinensis*, variations of the mRNA levels for *gr* in kidney, liver, and heart during embryonic development suggest a potential role for GC in tissue maturation before hatching (Izaz et al., 2021).

Interactions Between Thyroid Hormones and Corticosteroids

Treatment with dexamethasone and T_3 of saltwater crocodile embryos has effects on deiodinase activities: decrease of DIO1 activity in kidney, decrease of DIO2, and DIO3 activities in liver (Shepherdley et al., 2002). In the sea turtle *Chelonia mydas*, the secretion of phosphatidylcholine is stimulated by a combination of T_3 and dexamethasone before hatching (Sullivan et al., 2001). Similar results were obtained in the saltwater crocodile (Sullivan et al., 2002a) and in the lizard *Pogona vitticeps* (Sullivan et al., 2002b). All these data demonstrate the involvement of both TH and GC in the regulation of surfactant maturation in reptiles (Sullivan et al., 2003). Sullivan et al. (2003) reviewed the control of pulmonary surfactant and stated that dexamethasone and T_3 are crucial stimulators of surfactant production during embryonic development throughout evolution.

Birth in Mammals

Involvement of Thyroid Hormones

During most of the gestation in human, T_4 is converted in rT_3, while toward term, developmental changes in tissue deiodinase activity occur, leading to preferential deiodination of T4 to T3 (instead of rT_3) and rise in plasma T_3 concentration near term (Forhead and Fowden, 2014). In contrast, in the rat, circulating T_3 concentrations do not increase before birth (Dubois and Dussault, 1977; Harris et al., 1978; Lamers et al., 1986). Comparison of hormonal profiles during the perinatal period in two closely related murine species with distinct modes of development (altricial vs. precocial), the rat (altricial), and the spiny mouse *Acomys cahirinus* (precocial) allowed to show a correlation between perinatal increase in T_3 levels and precocial timing of birth (Lamers et al., 1986).

TH have a major regulatory role on fetal growth (Forhead and Fowden, 2014), and maturation of the central nervous system (Patel et al., 2011; Stenzel and Huttner, 2013). Furthermore, maternal hypothyroidism is associated with increased rates of respiratory distress syndrome in new-born (Casey et al., 2005; Männistö et al., 2013) and cardiorespiratory disorder (Rousseau et al., 2019). In various mammalian species, TH change the synthesis of the components of surfactant (Ballard et al., 1984; Das et al., 1984; Warburton et al., 1988; Gilbert et al., 2001; Van Tuyl et al., 2004). TH are also involved in the intestinal structural development (Trahair and Sangild, 1997; Sirakov and Plateroti, 2011; Sirakov et al., 2014). Specific TRα1 loss-of-function leads to low development and impaired activity of murine intestinal stem cells in culture (Godart et al., 2021). In addition, *in vivo* treatment confirms the positive action of T_3 on intestinal crypt cell proliferation and demonstrates its key action in modulating the number of stem cells, the expression of their specific markers and the commitment of progenitors into lineage-specific differentiation (Godart et al., 2021).

Involvement of Corticosteroids

An increase of plasma fetal GC is detected near parturition in many mammals (Alexander et al., 1968; Mulay et al., 1973; Lamers et al., 1986; Silver and Fowden, 1989; Yoon et al., 1998). The involvement of HPA axis in the timing of birth is first evidenced in ovines (Van Rensburg, 1967; Barnes et al., 1977). Furthermore, premature delivery is induced by injection of ACTH or corticosteroids into the ovine fetus (Van Rensburg, 1967; Halliday and Buttle, 1968; Liggins, 1968, 1969) as well as in fetal piglet (Bosc, 1973). More recently, the use of antalarmin, a CRH-R type I antagonist, can delay the onset of parturition in sheep, which suggests that CRH is involved in the induction of parturition in this species (Chan et al., 1998).

The many roles of GC on organ maturation have been the subjects of recent reviews (Fowden and Forhead, 2015; Fowden et al., 2016; Jellyman et al., 2020). As for TH, we will concentrate on GC involvement in lung and gastro-intestinal tract (GIT) maturations, which are crucial to cope with the change of environment (from aquatic to terrestrial) and of nutrition (from parenteral to enteral), respectively. In lambs born prematurely by infusion of dexamethasone, partial aeration of the lungs is noted, suggesting an accelerated appearance of surfactant activity (Liggins, 1969). Infusion of ACTH into one of twin pairs of lamb fetuses accelerates the morphological changes, that would normally occur in the lung, before birth (Sundell et al., 1979). GC treatment changes the synthesis of the phospholipid and protein components of surfactant (Warburton et al., 1988; Gilbert et al., 2001). At birth, there is a shift from mainly parenteral nutrition in the fetus (via the placenta) to enteral nutrition in the neonate, and the GIT has to prepare during gestation for coping with this change. This prenatal development of GIT is regulated by GC (Trahair and Sangild, 1997; Sangild et al., 2000). After bilateral

adrenalectomy of fetal sheep, growth of mucosal structures and villus height are reduced in the small intestine (Trahair et al., 1987a). After infusion of cortisol, no change in enterocyte morphology is detected, but the proportion of crypt cells and the migration of enterocytes are increased (Trahair et al., 1987b). In addition, cortisol influences the prenatal development of gastric acid and gastrin secretion, and of GIT hydrolase activities in both the fetal pig and sheep (Trahair and Sangild, 1997; Sangild et al., 2000).

Interactions Between Thyroid Hormones and Corticosteroids

Before birth, TH and GC synergize for the maturation of various organs, especially the lung, the liver and the brain. This synergism is partly linked to the fact that GC induce local deiodinase expression and activities resulting in the increase of circulating T_3 and thus T_3 bioavailability (Forhead et al., 2006; Fowden and Forhead, 2009). A synergism of TRH/T_3 and cortisol has been reported on lung maturation in fetal sheep, as infusion of cortisol and TRH (Liggins et al., 1988) or T_3 (Schellenberg et al., 1988; Warburton et al., 1988) increase the distensibility and the stability of the lung. In explant culture of human fetal lung, a supra-additive response in the synthesis of surfactant is observed in the presence of both dexamethasone and T_3 (Gonzalez et al., 1986).

T_4 injection to rats induces a precocious appearance of pepsinogen in the oxyntic gland mucosa and increases acid secretion, while in propylthiouracil-induced hypothyroid pups, pepsinogen content and basal acid secretion are low (Tseng and Johnson, 1986). However, T_4 has no such effects in adrenalectomized rats, while corticosterone is able to increase pepsinogen content and basal acid secretion in the absence of normal levels of TH (Tseng and Johnson, 1986).

Comparison of the Involvement and the Interdependence Between Thyroid and Corticosteroid Signaling in Vertebrate Developmental Transitions

In all the major developmental transitions described here, a common synergistic activation of thyrotropic and corticotropic axes is observed, leading to synchronized increase of TH and CS production and release (**Figure 4**). Besides this hormonal activation, increased expression of receptors allows tissues to become more responsive to TH and CS in time (**Figure 4**) to induce morphological, behavioral and physiological changes when needed, meaning just before the animal has to adapt to its new environment and its new way of life. As in all the mechanisms underlying major biological processes, they are conserved along evolution. Thus, the involvement of both TH and CS signaling is encountered in fish and amphibian metamorphic processes, as well as in egg hatching in sauropsids and birth in mammals (**Table 1**).

In non-mammalian vertebrates, the neurohormone, corticotropin-releasing hormone (CRH), appears to be potentially a coordinator of activation of both thyrotropic and corticotropic axes, as it is able to simulate thyrotropin production and release as much as corticotropin ones. CRH may thus be involved in the simultaneous activation of these neuroendocrine axes at the time of developmental transitions in non-mammalian vertebrates, such as fish and amphibian metamorphoses or sauropsid egg hatching (**Table 1**). It is not the case in mammals, in which the thyrotropic and corticotropic axes are controlled centrally by different hormones, respectively, TRH and CRH. Another difference among vertebrate developmental transitions described in this review may lay in the degree of involvement (triggering vs. permissive) of the two signaling systems and their interdependence. Except perhaps during birth for mammals, corticosteroid signaling seems to be more permissive than crucial, in the way it allows the thyroid signaling to be even more efficient, by elevating the activating deiodinase expression and activities, as well as the expression of TH receptors. Another difference may be likely due to neofunctionalization of gene paralogs observed after WGD. Duplication of some genes, notably in teleosts, may lead to redistribution of function. For example, the involvement of *tshβ1b* at smoltification in Atlantic salmon, instead of the classical *tshβ* in other vertebrate developmental transitions, or also the relative importance and differential role of the various thyroid hormone receptors between flatfish and amphibian larval metamorphoses.

IMPACT OF ENVIRONMENTAL FACTORS ON POST-EMBRYONIC TRANSITIONS

Climate Change

Water Warming for Population Strictly Dependent on an Aquatic Habitat

Teleost fish as ectothermic vertebrates show complex responses to temperature variation (Pinsky et al., 2019). Temperature has in teleost fishes a direct effect on oxygen delivery, cardiovascular function, muscle function, food conversion, mitochondria efficiency, and biochemical reaction rates (Little et al., 2020). Fish physiology is thus likely to be affected by increases in average temperature and temperature variability leading to energy metabolism changes with impact on growth and locomotion. All these physiological processes have been associated with thyroid function.

T_3 regulates thermal acclimation in zebrafish (Little et al., 2013) with the decrease of metabolism, skeletal muscle function and swimming performance in hypothyroid cold-acclimated (18°C) but not hypothyroid warm-acclimated (28°C) animals. The interactions between temperature levels, thyroid conditions and swimming performance are also linked to performance of the heart and oxygen transport (Little and Seebacher, 2014). In the context of global warming, the low sensitivity to TH at warm temperature is a concern because it may reduce the capacity of zebrafish to match this environmental change. Furthermore, in medaka, exposure until hatching to warm temperatures (32°C) increases the activation of TH biosynthesis, via TSH (Castañeda-Cortés et al., 2020), as well as the activation of the stress axis, via CRH (Castañeda-Cortés et al., 2019).

Rainbow trout is a typical cold-water fish species for which seasonally warmer water correlates with a decrease in fry survival

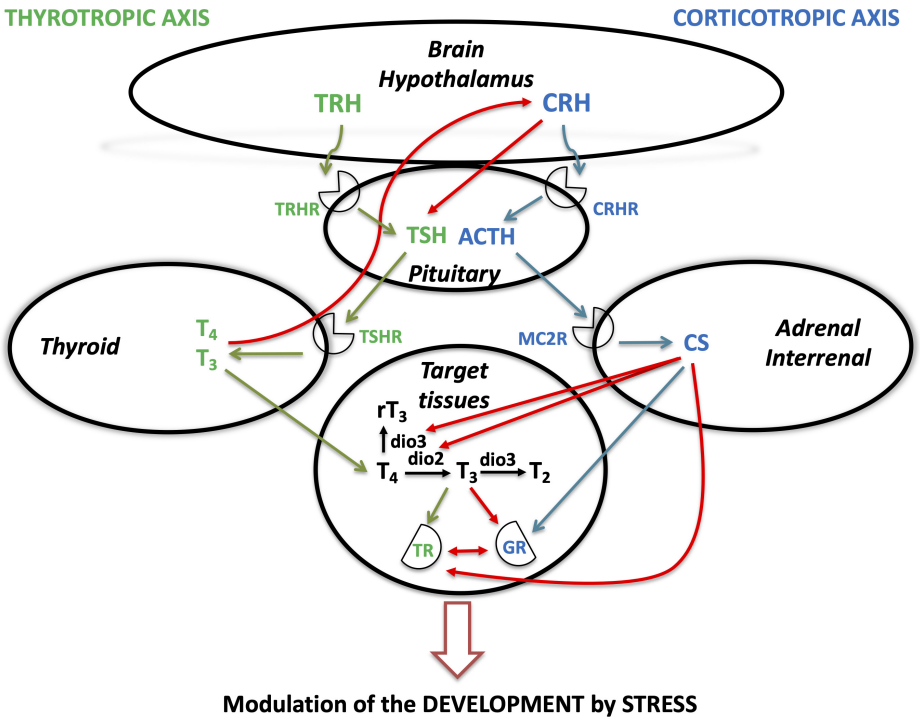


FIGURE 4 | Interactions between thyrotropic and corticotropic axes during vertebrate developmental transitions. The figure illustrates current knowledge on the interactions between the thyrotropic (HPT) and corticotropic (HPA/HPI) axes, during various developmental transitions (amphibian and teleost metamorphoses, sauropsid egg hatching and mammalian birth). ACTH, adrenocorticotropin; CRH, corticotropin-releasing hormone; CRHR, corticotropin-releasing hormone receptor; CS, corticosteroids; dio, deiodinase; GR, glucocorticoid receptor; MC2R, melanocortin receptor 2; MR, mineralocorticoid receptor; T_4, thyroxine; T_3, triiodothyronine; rT_3, reverse triiodothyronine; T_2, diiodothyronine; TR, thyroid hormone receptor; TRH, thyrotropin releasing hormone; TRHR, thyrotropin releasing hormone receptor; TSH, thyrotropin; TSHR, thyrotropin receptor.

TABLE 1 | Comparison of the interactions between HPT and HPA axes during vertebrate developmental transitions.

	Vertebrates	Amphibian	Teleost	Sauropsid	Mammal
	Developmental period	**Metamorphosis**	**Metamorphosis**	**Egg hatching**	**Birth**
HPT axis on HPA/I axis	TH on CRH	+	+	+	+
	TH on CS availability	?	?	?	+
	TH on GR	+	+	+	+
HPA/I axis on HPT axis	CRH on TSH	+	+	+	-
	CS on TH availability	+	+	+	+
	CS on TR	+	+	+	+

and an increase of T_3 and T_4 concentrations (Giroux and Schlenk, 2021). Transcriptome analysis of liver tissues from adult rainbow trout under heat stress (24°C) and control conditions (18°C) identifies the differential expression of 428 long non coding RNA some of which can be involved in maintenance of homeostasis or adaptation to stress (Quan et al., 2020). In Atlantic salmon, elevated temperature increases T_4 nocturnal levels (Nisenbaum et al., 2020). Thus, future climate changes can induce lower salinity tolerance and accordingly result in poor survival in seawater after smoltification.

To show the wide diversity of the links between global warming and TH, we will give three more examples. First, in the mosquitofish *Gambusia holbrooki* the thermal acclimation is mediated by TH and leads to decreased mitochondrial efficiency, metabolic rates, and swimming performance (Le Roy and Seebacher, 2020). Second, in the desert fish Amargosa pupfish *Cyprinodon nevadensis amargosae*, temperature contributes to altered TH levels and morphological development (Lema et al., 2016). We can thus expect that many desert fishes will be vulnerable to rising temperatures because they occupy habitats with already high temperatures. Finally, increased temperature induces a decrease of TH levels in convict surgeonfish *Acanthurus triostegus* (Besson et al., 2020). The effect on TH levels correlates with affected sensory development leading to higher predation.

Chronic temperature increases (persisting for a long time) are also known to induce acute stress responses in fishes

(Alfonso et al., 2020). Global warming can cause water freshening from increased freshwater inputs. In Antarctic spiny plunderfish *Harpagifer antarcticus*, salinities decrease leads to high plasma cortisol levels compare to normal salinity (Vargas-Chacoff et al., 2021). This has implications for fish species that have evolved in stable environmental conditions like in the Antarctic. In a totally different environment, climate-induced bleaching of coral reefs alters anemonefish hormonal stress, resulting in decreased reproductive hormones and severely impacted reproduction (Beldade et al., 2017). Finally, in juvenile brown trout, *Salmo trutta*, water temperature affects cortisol and GR mRNA levels (Filipsson et al., 2020).

Aquatic and Terrestrial Life Cycle: Warming Leads to Double Penalty

Amphibians are another ectodermic taxon with complex interactions between costs and benefits of life in the aquatic and terrestrial environments. They are undergoing a precipitous decline linked with environmental pressures including climate change. Many amphibian species exploit temporary or even ephemeral aquatic habitats for reproduction. Survival from desiccation of pond drying is reached by maximizing larval growth and acceleration of development, including precocious metamorphosis.

The western spadefoot toad tadpoles, *Pelobates cultripes*, usually have long larval period, but it can shorten it in response to pond drying (Gomez-Mestre et al., 2013). Developmental acceleration correlates with increased corticosterone and TH levels as well as increased TRβ levels to increase metamorphic process. Anuran tadpole shows thus phenotypic plasticity in age and size at metamorphosis as a response to temperature variation (Ruthsatz et al., 2020). In Arizona tiger salamander *Ambystoma tigrinum*, the small increase in temperature tends also to decrease the time to metamorphosis and results in a worse body condition (Park et al., 2016). Plasticity in rates of growth and development is beneficial to allow a more rapid transition into the juvenile stage where rates of mortality are lower. However, early metamorphosis leads to juveniles with smaller size correlated with low survival and locomotor performance (Székely et al., 2020). Pond drying may also lead to the loss of the capacity to respond to desiccation following decreased food availability (Enriquez-Urzelai et al., 2013), liver damage with upregulation of DIO2 and TRα transcript levels while there is a decrease of TRβ (Chen et al., 2021), or increased oxidative damage during metamorphosis (Petrović et al., 2021).

TH and GC level variations underlie differences in the timing of metamorphosis. In response to pond drying, western spadefoot toad and New Mexico spadefoot toad *Spea multiplicata* that have long larval periods and large size at metamorphosis, accelerate metamorphosis and elevate whole-body content of TH and corticosterone (Kulkarni et al., 2017). In contrast, in Couch's spadefoot toad *Scaphiopus couchii* that has a short larval period, whole-body TH and corticosterone content are high during metamorphosis and weakly affected by pond drying. Thus, species exhibiting less plasticity will be more sensitive to environmental changes. The TH and CS level variations to accelerate metamorphosis during habitat desiccation may originate from elevated CRH (Denver, 1997). CRH can be a transducer of environmental stimuli to modulate the rate of metamorphosis *via* the endocrine response. Interestingly, CRH has also been shown to control the term of pregnancy in mammals. Maternal plasma CRH concentrations are elevated early in pregnancy in those patients destined to deliver preterm, and are lower in patients destined to deliver post-dates (Challis and Hooper, 1989; McLean et al., 1995).

Climate Changes and Terrestrial Biodiversity

Temperature increase alters avian phenotypes such as advanced reproduction, migration schedules and individual appearance. Bird ability to maintain a stable body temperature in a wide range of thermal environments is regulated *via* endocrine pathways including TH and with a lesser extent CS (Ruuskanen et al., 2021). The knowledge on endocrine regulation of thermogenesis concerns mainly poultry, but poorly describe for environmental temperature variation. Offspring development are dependent of females via hormones deposited in eggs. Ambient temperature changes affect the hormone levels in the yolk including TH and CS. More specifically, T_4 levels were negatively correlated with ambient temperature in great tits *Parus major* (Ruuskanen et al., 2016). However, in wild pied flycatchers *Ficedula hypoleuca*, there is no evidence for context-dependent effects of prenatal TH related to postnatal temperature on growth, survival, and plasma TH levels (Hsu et al., 2020), suggesting species differences or unknown confounding effects.

In reptiles and turtles, temperature effect on TH and GC plasma levels is controversial being either absent, positive or negative. Highlighting only a few examples, in the alligator lizards *Elgaria coerulea* and *Elgaria multicarinata*, corticosterone levels increase with temperature (Telemeco and Addis, 2014) and in the South-American tegu lizard *Salvator merianae*, T_3, T_4, and corticosterone levels show a positive relationship with body temperature (Zena et al., 2020). Similar correlation between body temperature and plasma corticosterone is observed in the eastern fence lizards *Sceloporus undulatus*, under laboratory conditions, but not in the field (Racic et al., 2020). In contrast, in the common lizard *Zootoca vivipara*, baseline corticosterone levels decrease with increasing thermal conditions (Dupoué et al., 2018).

In mammals, we will only highlight two of the numerous examples. First, the transition between late gestation and early lactation is a developmental window sensitive to warming. Dairy cows experience stress due to the high energy and nutrient requirements of the fetus and the mammary gland. In early lactation, the decline of TH levels is more pronounced in cow maintained at 28°C compared to the one maintained at 15°C (Weitzel et al., 2017). The second example is an Artic mammal, which has highly evolved to these extreme environments and its capacity to adapt to this change may be limited. The polar bear is emblematic to this environment. It was shown that CS binding capacity of plasma CS binding globulin increases in polar bears as a consequence of climate warming (Boonstra et al., 2020). Evidence is still lacking to have a complete view of the interactions between GC and TH in response to global warming.

Endocrine Disruptors

Thyroid Function Disruption

As previously mentioned, TH orchestrates metamorphosis, brain development, and metabolism representing a potential target for endocrine disruptor chemicals/compounds (EDC). Although the molecular mechanisms for TH disruption is still unknown for most of the EDC, several affect TH synthesis, transport or metabolism and downstream effects (Thambirajah et al., 2019). One of the main concerns is disruption during early neurogenesis, which may affect several TH actions such as proliferation, differentiation, migration, synaptogenesis and myelination in the developing nervous system (Préau et al., 2015).

Because anuran metamorphosis is strictly dependent on TH, amphibians represent sensitive models for the detection and mechanistic elucidation of TH disrupting activities (thyroid histology, metamorphosis progression). EDC impact wild anurans and contribute to population declines. Some pesticides and biocides may interfere with TH signaling during nervous system development (Leemans et al., 2019; Trudeau et al., 2020) or metamorphosis timing (Orton and Tyler, 2015). Last but not least, exposition of *Xenopus* tadpoles to the benzo[a]pyrene leads to delayed metamorphosis and sexual maturity with transgenerational disruption of metabolism and population decline (Usal et al., 2021).

In zebrafish, exposure during the 7 first days of development to 25 known TH disrupting compounds leads to morphological defects and variations of transcripts involved in the HPT axis (Spaan et al., 2019). In another teleost fish, the metamorphosing convict surgeonfish *Acanthurus triostegus*, high doses of the pesticide chlorpyrifos induce defects by decreasing TH levels impacting olfactory, visual and mechano-sensory structure development (Besson et al., 2020). Interestingly, similar phenotypes were observed following temperature increase, highlighting the profound threat anthropogenic stressors pose to fish communities. To highlight the EDC mode of action, liver transcriptomic responses in yellow perch *Perca flavescens* population show variation of genes transcripts related to reproduction, retinol, iron, TH, oxidative stress, lipid metabolism, and immune functions between strongly impacted population vs. less impacted groups (Defo et al., 2018).

In birds as in teleost fishes and amphibians, because GC regulate metabolism, ability to modulate corticosterone in response to stressor is essential to face a wide array of environmental challenges. EDC contamination was shown to impair this GC role. Methylmercury exposure reduces stress-induced GC response in zebra finches, *Taeniogypia guttata* (Moore et al., 2014). TH function is also EDC target. The rural nestling peregrine falcon *Falco peregrinus* has significantly lower circulating concentrations of TH compared to urban nestlings where flame retardants are environmental contaminants that accumulate in predatory birds (Fernie et al., 2017).

EDC also affect TH signaling in mammals. Considering the large amount of recent data available, only a few examples will be given here. In mice, exposure to the flame-retardant, tetrabromo bisphenol A, modulates hypothalamic set-points controlling metabolic responses by targeting TR regulation of *trh* and *mc4r* (Decherf et al., 2010). In the polar bear *Ursus maritimus*, an heavily polluted organism for which some EDC are banned for decades (Routti et al., 2019), some EDC, such as organochlorine and perfluoroalkyl, are negatively correlated with plasma TH levels (Bourgeon et al., 2017). In wild chimpanzees, contamination by polluted drinking waters containing pesticides (Krief et al., 2017) and bisphenols (Krief et al., 2020) has been reported, and the water samples exhibit TH disrupting activities (Spirhanzlova et al., 2019). Experimental exposure of *Xenopus laevis* tadpoles to a mixture of 15 chemicals at concentrations reported in human amniotic fluid induces variation of behavior, of TH-responsive gene expression and of nervous system development (Fini et al., 2017). Considering the conservation of TH function across vertebrates, one may speculate on such impacts of amniotic fluid chemicals on human fetal brain development.

Environmental and health concerns remain particularly challenging when addressing thyroid hormone axis disruption. Only few chemicals have been tested, the number of test methods is limited and the thyroid system is complex. The development of specific chemical safety testing is required (Browne et al., 2020) with special attention to neurological development (Gilbert et al., 2020; Kortenkamp et al., 2020; O'Shaughnessy and Gilbert, 2020). Attention is also paid to break down the wall between mammalian and non-mammalian vertebrate regulatory testing (Couderq et al., 2020; Holbech et al., 2020). In addition to phenotypic or histological end-points, the use of molecular assays (transcripts, proteins or metabolites) will be more sensitive and most of all will allow detection before adverse effects occur (Fini et al., 2007; Kulkarni and Buchholz, 2013). Development of non-destructive (without euthanasia) biomarkers that provide causal link between the presence of a chemical and an ecological effect are also needed to allow the study of vulnerable populations.

Corticosteroid Function Disruption

The adrenal has been neglected in endocrine disruption regulatory testing strategy while the negative consequences of adrenocortical dysfunction during development have been recognized (Hinson and Raven, 2006; Harvey, 2016). Upstream of the biological effect, an activity mimicking that of GC was measured in surface waters (Schriks et al., 2013). Hydrocortisone was detected but could not explain a significant fraction of the observed GR activity.

GC synthesis pathway is also a target. Different environmental toxicants, including phthalate esters together with bisphenols and their analogs pose deleterious effects on the biosynthesis of GC *in vitro* (Ahmad et al., 2017; Verma et al., 2018). These observations were confirmed *in vivo* using nitrate, a major anthropogenic contaminant in the FW environment. In three-spined stickleback *Gasterosteus aculeatus* L., dissolved inorganic N directly exerts a disruptive influence on the function of the stress axis and GC levels, supporting concerns that nitrate is an EDC (Pottinger, 2017). GC disruption was linked to adverse effect in zebrafish exposed to methylparaben, widely used as antimicrobial preservative. In addition to increase in

cortisol levels, animals show alteration of heart rate and hatching percentage as well as signs of anxiety-like behavior (Luzeena Raja et al., 2019).

Finally, because persistent organic pollutants can interact with GR, EDC mixtures were screened for GR translocation and GR transactivation in an *in vitro* assay (Wilson et al., 2016). EDC mixtures did not induce GR translocation in nucleus nor produce an agonist response in the GR transcriptional assay. However, in the presence of cortisol, some chemicals were found to decrease GR transcriptional activity.

Endocrine Disruptor Chemicals/Compounds and Climate Change: A Worst Combination

Climate change and exposure to EDCs are currently two of the most serious anthropogenic threats to biodiversity and ecosystems. Moreover, the ecological threats posed by EDC are further exacerbated by changing environmental conditions such as temperature, pond drying, food restriction, pH and ultraviolet radiation.

In aquatic environment, global warming may lead to temperature increase associated with either salinity increase due to evaporation or salinity decrease due to ice melting. One study addresses the combinatorial effect of temperature, salinity and diuron, an herbicide and antifouling agent with EDC property (Moreira et al., 2018). Combinatorial treatment of juveniles of the estuarine fish, the inland silverside *Menidia beryllina*, affects growth and hormonal levels. T_3 increases in all of them, while T_4 increases at low temperature and low salinity and decreases at low temperature and high salinity, in agreement with DIO2 expression. EDC, acidification and other contaminants can also perturb smolt development, resulting in poor survival after salinity increase (Björnsson et al., 2011). In the San Francisco Bay, the rainbow trout experiences warmer waters and saltwater intrusion (Giroux and Schlenk, 2021) leading to fry decrease in survival associated with TH increase. Our last example in teleost fishes highlights the interaction between temperature and perchlorate, a well-known thyroid disruptor (Lee et al., 2014). Overall, the results suggest that perchlorate affects thyroid function and reproduction, and these adverse effects are worsened under high temperature.

As previously described in amphibians, a rapid transition into the juvenile stage is beneficial in stress conditions. Pond desiccation is associated to a decrease in dissolved oxygen and an increase in nitrogen levels for which anthropogenic sources such as chemical fertilizers can be heavily suspected. Ammonium nitrate or hypoxic conditions leads to survival decrease of the Natterjack toad *Bufo calamita* tadpoles (Ortiz-Santaliestra and Marco, 2015). When both stressors were combined, the lethal effects were additive and include malformations. Global warming lead also to predator-prey relationship modifications decreasing survival rates of tadpoles (Jara et al., 2019). In addition, the Bullfrog sensitivity to temperature leads to corticosterone hormonal changes that correlate with immunosuppression (Lima et al., 2020) and increases sensitivity to emerging infectious diseases in the concept of OneHealth. Urodela amphibians are also affected. Compared to a single exposure, Arizona tiger salamander larvae subjected to both temperature increase and perchlorate treatment further decrease their rate of development. These cotreated salamander larvae also present a significant smaller body mass and worse body condition.

Finally, Artic biodiversity is concerned by this double threat. EDC were measured in Arctic marine mammals and seabirds. The most pronounced relationships with endocrine function have been reported with the thyroid hormone system, the sex steroid hormones and cortisol (Jenssen, 2006). Because these endocrine systems are essential for completing life cycles and enabling animals to respond adequately to environmental stress, EDC may interfere with adaptations to increased stress situations.

CONCLUSION

We have highlighted the roles of TH- and CS-regulated developmental transitions in the innovation, adaptation and plasticity of life cycles in vertebrates. In one of his review, Buchholz discusses the similarities between birth in mammals and metamorphosis in frog with a focus on their regulation by TH and GC (Buchholz, 2015). He reminds us that "both frogs and mammals undergo a life history transition from aquatic (water and amniotic fluid) to terrestrial habitat," involving for the most striking examples, air-breathing thanks to lung maturation and maturation of the intestine to cope with the transition to a new food source. Similar TH and CS concentration profiles are observed in certain birds and reptiles at hatching and during molting, as well as in certain teleost fishes at hatching and during larval metamorphosis and smoltification (Wada, 2008). The multiple cross-talks between HPT and HPA/HPI axes represent critical mechanisms by which vertebrates modulate their perinatal/post-embryonic development as well as their responses to a changing environment (**Figure 4**).

The exposome includes stressors with, for some, potential mortality risk by their long-term effects. Vertebrate respond to the risk/benefit trade-off by modulating the production of hormones by the stress and thyroid neuroendocrine axes. In this context, global warming leads to increases in average temperature and temperature variability. Considered as a threat for biodiversity (Radchuk et al., 2019), global warming is a well-known reprotoxicity risk (Parisi and Guerriero, 2019). However, the properties of TH and CS suggest their fundamental roles in thermal acclimation (de Bruijn and Romero, 2018), and evolution of ectothermy/endothermy (Little, 2021). Changes in hormone sensitivity at warm temperatures could mean that increasing temperatures will reduce the capacity of animals to regulate their physiologies to match demands. Aquatic and terrestrial environments are also increasingly contaminated by anthropogenic sources. The contaminants include pharmaceuticals, personal care products, and industrial and agricultural chemicals. Many of these chemicals have the potential to disrupt endocrine function that may lead to disease or

foundations to disease later in life as well as disease transmission to descendants (Gore et al., 2015). The adverse effects including metabolic diseases, decreased capacity of reproduction, hormone-sensitive cancers, thyroid disorders, and neurological defects may contribute to population decline. The difficulties tie in the circumstances EDC act, including non-monotonic dose-responses, low-dose effects, and developmental vulnerability. Possible roles for the neuroendocrine stress axis in mediating these developmental responses requires further investigation.

AUTHOR CONTRIBUTIONS

All Authors contribute equally to the redaction of the review.

FUNDING

LS work was supported by "ERGO," a European Union's Horizon 2020 Research and Innovation program, under grant agreement no. 825753. The authors are supported by the "Centre National de la Recherche Scientifique" and the "Muséum National d'Histoire Naturelle."

ACKNOWLEDGMENTS

We thank Robert Denver (Department of Molecular, Cellular and Developmental Biology and Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, United States) for critical reading of the manuscript, and Dr. A. Virmani for proofreading the manuscript.

REFERENCES

Adams, A., and Bull, A. (1949). The effects of antithyroid drugs of chick embryos. *Anat. Rec.* 104, 421–443.

Aerts, J. (2018). Quantification of a glucocorticoid profile in non-pooled samples is pivotal in stress research across vertebrates. *Front. Endocrinol. (Lausanne)* 9:635. doi: 10.3389/fendo.2018.00635

Ahmad, S., Khan, M. F., Parvez, S., Akhtar, M., and Raisuddin, S. (2017). Molecular docking reveals the potential of phthalate esters to inhibit the enzymes of the glucocorticoid biosynthesis pathway. *J. Appl. Toxicol.* 37, 265–277. doi: 10.1002/jat.3355

Alexander, D. P., Britton, H. G., James, V. H., Nixon, D. A., Parker, R. A., Wintour, E. M., et al. (1968). Steroid secretion by the adrenal gland of foetal and neonatal sheep. *J. Endocrinol.* 40, 1–13. doi: 10.1677/joe.0.0400001

Alfonso, S., Gesto, M., and Sadoul, B. (2020). Temperature increase and its effects on fish stress physiology in the context of global warming. *J. Fish. Biol.* 98, 1496–1508.

Aluru, N., and Vijayan, M. M. (2008). Molecular characterization, tissue-specific expression, and regulation of melanocortin 2 receptor in rainbow trout. *Endocrinology* 149, 4577–4588. doi: 10.1210/en.2008-0435

Alves, R. N., Cardoso, J. C. R., Harboe, T., Martins, R. S. T., Manchado, M., Norberg, B., et al. (2017). Duplication of Dio3 genes in teleost fish and their divergent expression in skin during flatfish metamorphosis. *Gen. Comp. Endocrinol.* 246, 279–293. doi: 10.1016/j.ygcen.2017.01.002

Arai, M., Assil, I., and Abou-Samra, A. (2001). Characterization of three corticotropin-releasing factor receptors in catfish: a novel third receptor is predominantly expressed in pituitary and urophysis. *Endocrinology* 142, 446–454. doi: 10.1210/en.142.1.446

Arjona, F. J., Vargas-Chacoff, L., Martin del Rio, M. P., Flik, G., Mancera, J. M., and Klaren, P. H. M. (2011). Effects of cortisol and thyroid hormone on periphearl outer ring deiodination and osmoregulatory parameters in the Senegalese sole (*Solea senegalensis*). *J. Endocrinol.* 208, 323–330.

Bagamasbad, P. D., Bonett, R. M., Sachs, L., Buisine, N., Raj, S., Knoedler, J. R., et al. (2015). Deciphering the regulatory logic of an ancient, ultraconserved nuclear receptor enhancer module. *Mol. Endocrinol.* 29, 856–872. doi: 10.1210/me.2014-1349

Baggerman, B. (1963). The effect of TSH and antithyroid substances on salinity preference and thyroid activity in juvenile pacific salmon. *Can. J. Zool.* 41, 307–319.

Baker, M. E., and Katsu, Y. (2019). Evolution of the mineralocorticoid receptor. *Vitam. Horm.* 109, 17–36. doi: 10.1016/bs.vh.2018.10.009

Balaban, M., and Hill, J. (1971). Effects of thyroxine level and temperature manipulations upon the hatching of chick embryos (*Gallus domesticus*). *Dev. Psychobiol.* 4, 17–35. doi: 10.1002/dev.420040103

Ballard, P. L., Hovey, M. L., and Gonzales, L. K. (1984). Thyroid hormone stimulation of phosphatidylcholine synthesis in cultured fetal rabbit lung lung. since glucocorticoids stimulate surfactant synthesis drome in premature infants, we also examined the choline was determined in organ cultures of rabbit lun. *J. Clin. Invest.* 74, 898–905.

Barnes, R. J., Comline, R. S., and Silver, M. (1977). The effects of bilateral adrenalectomy or hypophysectomy of the foetal lamb in utero. *J. Physiol.* 264, 429–447. doi: 10.1113/jphysiol.1977.sp011676

Barton, B. A., Schreck, C. B., Ewing, R. D., Hemmingsen, A. R., and Patiño, R. (1985). Changes in plasma cortisol during stress and smoltification in Coho Salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 59, 468–471. doi: 10.1016/0016-6480(85)90406-X

Beldade, R., Blandin, A., O'Donnell, R., and Mills, S. (2017). Cascading effects of thermally-induced anemone bleaching on associated anemonefish hormonal stress response and reproduction. *Nat. Commun.* 8:716. doi: 10.1038/s41467-017-00565-w

Bernier, N. J., Flik, G., and Klaren, P. H. M. (2009). Regulation and contribution of corticotropic, melanotropic and thyrotropic axes to the stress response in fishes. *Fish Physiol.* 28, 235–311. doi: 10.1016/S1546-5098(09)28006-X

Berry, X., Banu, X., and Larsen, X. (1991). Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* 349, 438–440.

Besson, M., Feeney, W., Moniz, I., FRançois, L., Brooker, R., Holzer, G., et al. (2020). Anthropogenic stressors impact fish sensory development and survival via thyroid disruption. *Nat. Commun.* 11:3614. doi: 10.1038/s41467-020-17450-8

Bianco, A. C., Salvatore, D., Gereben, B., Berry, M. J., and Larsen, P. R. (2002). Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* 23, 38–89. doi: 10.1210/edrv.23.1.0455

Bidaud, I., Lory, P., Nicolas, P., Bulant, M., and Ladram, A. (2002). Characterization and functional expression of cDNAs encoding thyrotropin-releasing hormone receptor from *Xenopus laevis*: Identification of a novel subtype of thyrotropin-releasing hormone receptor. *Eur. J. Biochem.* 269, 4566–4576. doi: 10.1046/j.1432-1033.2002.03152.x

Bilesimo, P., Jolivet, P., Alfama, G., Buisine, N., Le Mevel, S., Havis, E., et al. (2011). Specific histone lysine 4 methylation patterns define TR-binding capacity and differentiate direct T3 responses. *Mol. Endocrinol.* 25, 225–237. doi: 10.1210/me.2010-0269

Bishop, C. D., Erezyilmaz, D. F., Flatt, T., Georgiou, C. D., Hadfield, M. G., Heyland, A., et al. (2006). What is metamorphosis? *Integr. Comp. Biol.* 46, 655–661. doi: 10.1093/icb/icl004

Björnsson, B. T., Einarsdottir, I. E., and Power, D. (2012). Is salmon smoltification an example of vertebrate metamorphosis? Lessons learnt from work on flatfish larval development. *Aquaculture* 362–363, 264–272. doi: 10.1016/j.aquaculture.2011.03.002

Björnsson, B. T., Stefansson, S. O., and McCormick, S. D. (2011). Environmental endocrinology of salmon smoltification. *Gen. Comp. Endocrinol.* 170, 290–298. doi: 10.1016/j.ygcen.2010.07.003

Boeuf, G. (1993). "Salmonid smolting: a pre-adaptation to the oceanic environment," in *Fish Ecophysiology*, eds J. Rankin and F. Jensen (London: Chapman Press), 105–135.

Bonett, R. M., Hu, F., Bagamasbad, P., and Denver, R. J. (2009). Stressor and glucocorticoid-dependent induction of the immediate early gene krüppel-like factor 9: implications for neural development and plasticity. *Endocrinology* 150, 1757–1765. doi: 10.1210/en.2008-1441

Bonett, R., Hoopfer, E., and Denver, R. (2010). Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. *Gen. Comp. Endocrinol.* 168, 209–219.

Boonstra, R., Bodner, K., Bosson, C., Elehanty, B., Richardson, E., Lunn, N., et al. (2020). The stress of arctic warming on polar bears. *Golb. Chang. Biol.* 26, 4197–4214.

Borges, M., Labourene, J., and Ingbar, S. H. (1980). Changes in hepatic iodothyronine metabolism during ontogeny of the chick embryo. *Endocrinology* 107, 1751–1761. doi: 10.1210/endo-107-6-1751

Bosc, M. (1973). Modification de la durée de gestation de la truie après administration d'ACTH aux foestus. *Comptes Rendus Hebd. Des Seances L Acad. Des Sci. Ser. D* 276, 3183–3186.

Bourgeon, S., Riemer, A. K., Tartu, S., Aars, J., Polder, A., Jenssen, B. M., et al. (2017). Potentiation of ecological factors on the disruption of thyroid hormones by organo-halogenated contaminants in female polar bears (*Ursus maritimus*) from the Barents Sea. *Environ. Res.* 158, 94–104. doi: 10.1016/j.envres.2017.05.034

Brooks, A., Sweeney, G., and Old, R. (1989). Structure and functional expression of a cloned *Xenopus thyroid* hormone receptor. *Nucleic Recept. Res* 17, 9395–9405.

Brown, C., Urbinati, E., Zhang, W., Brown, S., and McComb-Kobza, M. (2014). Maternal thyroid and glucocorticoid hormone interactions in larval fish development, and their applications in aquaculture. *Rev. Fish. Sci. Aquac.* 22, 207–220.

Browne, P., Van Der Wal, L., and Gourmelon, A. (2020). OECD approaches and considerations for regulatory evaluation of endocrine disruptors. *Mol. Cell. Endocrinol.* 504:110675. doi: 10.1016/j.mce.2019.110675

Buchholz, D. R. (2015). More similar than you think: frog metamorphosis as a model of human perinatal endocrinology. *Dev. Biol.* 408, 188–195. doi: 10.1016/j.ydbio.2015.02.018

Buchholz, D. R. (2017). *Xenopus* metamorphosis as a model to study thyroid hormone receptor function during vertebrate developmental transitions. *Mol. Cell. Endocrinol.* 459, 64–70. doi: 10.1016/j.mce.2017.03.020

Buchholz, D. R., and Shi, Y. B. (2018). Dual function model revised by thyroid hormone receptor alpha knockout frogs. *Gen. Comp. Endocrinol.* 265, 214–218. doi: 10.1016/j.ygcen.2018.04.020

Buchholz, D. R., Hsia, S.-C. V., Fu, L., and Shi, Y.-B. (2003). A dominant-negative thyroid hormone receptor blocks amphibian metamorphosis by retaining corepressors at target genes. *Mol. Cell. Biol.* 23, 6750–6758. doi: 10.1128/mcb.23.19.6750-6758.2003

Buchholz, D. R., Tomita, A., Fu, L., Paul, B. D., and Shi, Y.-B. (2004). Transgenic analysis reveals that thyroid hormone receptor is sufficient to mediate the thyroid hormone signal in frog metamorphosis. *Mol. Cell. Biol.* 24, 9026–9037. doi: 10.1128/mcb.24.20.9026-9037.2004

Bury, N. R. (2017). The evolution, structure and function of the ray finned fish (*Actinopterygii*) glucocorticoid receptors. *Gen. Comp. Endocrinol.* 251, 4–11. doi: 10.1016/j.ygcen.2016.06.030

Campinho, M. A., Galay-Burgos, M., Sweeney, G. E., and Power, D. M. (2010). Coordination of deiodinase and thyroid hormone receptor expression during the larval to juvenile transition in sea bream (*Sparus aurata*, Linnaeus). *Gen. Comp. Endocrinol.* 165, 181–194. doi: 10.1016/j.ygcen.2009.06.020

Campinho, M. A., Silva, N., Martins, G. G., Anjos, L., Florindo, C., Roman-Padilla, J., et al. (2018). A thyroid hormone regulated asymmetric responsive centre is correlated with eye migration during flatfish metamorphosis. *Sci. Rep.* 8, 1–12. doi: 10.1038/s41598-018-29957-8

Cardoso, J. C. R., Bergqvist, C. A., Félix, R. C., and Larhammar, D. (2016). Corticotropin-releasing hormone family evolution: five ancestral genes remain in some lineages. *J. Mol. Endocrinol.* 57, 73–86. doi: 10.1530/JME-16-0051

Cardoso, J. C. R., Laiz-Carrion, R., Louro, B., Silva, N., Canario, A. V. M., Mancera, J. M., et al. (2011). Divergence of duplicate POMC genes in gilthead sea bream *Sparus auratus*. *Gen. Comp. Endocrinol.* 173, 396–404. doi: 10.1016/j.ygcen.2010.12.001

Carsia, R. V., Morin, M. E., Rosen, H., and Weber, H. (1987). Ontogenic corticosteroidogenesis of the domestic fowl: response of isolated adrenocortical cells. *Proc. Soc. Exp. Biol. Med.* 184, 436–445.

Casey, B. M., Dashe, J. S., Wells, C. E., McIntire, D. D., Byrd, W., Leveno, K. J., et al. (2005). Subclinical hypothyroidism and pregnancy outcomes. *Obstet. Gynecol.* 105, 239–245. doi: 10.1097/01.AOG.0000152345.99421.22

Castañeda-Cortés, D. C., Arias Padilla, L. F., Langlois, V. S., Somoza, G. M., and Fernandino, J. I. (2019). The central nervous system acts as a transducer of stress-induced masculinization through corticotropin-releasing hormone B. *Development (Cambridge)* 146:dev172866.

Castañeda-Cortés, D. C., Zhang, J., Boan, A. F., Langlois, V. S., and Fernandino, J. I. (2020). High temperature stress response is not sexually dimorphic at the whole-body level and is dependent on androgens to induce sex reversal. *Gen. Comp. Endocrinol.* 299:113605.

Challis, J. R. G., and Hooper, S. (1989). Birth: outcome of a positive cascade. *Baillieres Clin. Endocrinol. Metab.* 3, 781–793. doi: 10.1016/S0950-351X(89)80053-9

Chan, E., Falconer, J., Madsen, G., Rice, K., Webster, E., Chrousos, G., et al. (1998). A corticotropin-releasing hromone type I receptor antagonist delays parturition in sheep. *Endocrinology* 139, 3357–3360.

Chen, X., Ren, C., Teng, Y., Shen, Y., Wu, M., Xiao, H., et al. (2021). Effects of temperature on growth, development and the leptin signaling pathway of *Bufo gargarizans*. *J. Therm. Biol.* 96:102822. doi: 10.1016/j.jtherbio.2020.102822

Chester-Jones, I. (1987). "Structure of the adrenal and interrenal glands," in *Fundamentals of Comparative Vertebrate Endocrinology*, eds I. Chester-Jones, P. M. Ingleton, and J. G. Phillips (Boston, MA: Springer), 95–121. doi: 10.1007/978-1-4899-3617-2_3

Christensen, V. L. (1985). Supplemental thyroid hormones and hatchability of turkey eggs. *Poult. Sci.* 64, 2202–2210. doi: 10.3382/ps.0642202

Cone, R. D. (2006). Studies on the physiological functions of the melanocortin system. *Endocr. Rev.* 27, 736–749. doi: 10.1210/er.2006-0034

Cortés, R., Navarro, S., Agulleiro, M. J., Guillot, R., García-Herranz, V., Sánchez, E., et al. (2014). Evolution of the melanocortin system. *Gen. Comp. Endocrinol.* 209, 3–10. doi: 10.1016/j.ygcen.2014.04.005

Couderq, S., Leemans, M., and Fini, J. B. (2020). Testing for thyroid hormone disruptors, a review of non-mammalian *in vivo* models. *Mol. Cell. Endocrinol.* 508:110779. doi: 10.1016/j.mce.2020.110779

Coughlin, D. J., Forry, J. A., McGlinchey, S. M., Mitchell, J., Saporetti, K. A., and Stauffer, K. A. (2001). Thyroxine induces transitions in red muscle kinetics and steady swimming kinematics in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Zool.* 290, 115–124. doi: 10.1002/jez.1041

Dardente, H., Wood, S., Ebling, F., and Sáenz de Miera, C. (2019). An integrative view of mammalian seasonal neuroendocrinology. *J. Neuroendocrinol.* 31, 1–17. doi: 10.1111/jne.12729

Darras, V. M., and Van Herck, S. L. J. (2012). Iodothyronine deiodinase structure and function: from ascidians to humans. *J. Endocrinol.* 215, 189–206. doi: 10.1530/JOE-12-0204

Darras, V. M., Kotanen, S. P., Geris, K. L., and Kühn, E. R. (1996). Plasma thyroid hormone levels and iodothyronine deiodinase activity following an acute glucocorticoid challenge in embryonic compared with posthatch chickens. *Gen. Comp. Endocrinol.* 104, 203–212. doi: 10.1006/gcen.1996.0163

Darras, V. M., Visser, T. J., Berghman, L. R., and Kühn, E. R. (1992). Ontogeny of type I and type III deiodinase activities in embryonic and posthatch chicks: relationship with changes in plasma triiodothyronine and growth hormone levels. *Comp. Biochem. Physiol. Part A Physiol.* 103, 131–136. doi: 10.1016/0300-9629(92)90252-L

Das, D., Ayromlooi, J., Bandyopadhyay, D., Bandyopadhyay, S., Neogi, A., and Steinberg, H. (1984). Potentiation of surfactant release in fetal lung by thyroid hormone action. *J. Appl. Physiol.* 56, 1621–1626.

Davey, J. C., Becker, K. B., Schneider, M. J., St. Germain, D. L., and Galton, V. A. (1995). Cloning of a cDNA for the type II iodothyronine deiodinase. *J. Biol. Chem.* 270, 26786–26789. doi: 10.1074/jbc.270.45.26786

de Bruijn, R., and Romero, L. M. (2018). The role of glucocorticoids in the vertebrate response to weather. *Gen. Comp. Endocrinol.* 269, 11–32. doi: 10.1016/j.ygcen.2018.07.007

De Groef, B., Grommen, S. V. H., and Darras, V. M. (2013). Hatching the cleidoic egg: the role of thyroid hormones. *Front. Endocrinol. (Lausanne)* 4:63. doi: 10.3389/fendo.2013.00063

De Groef, B., Van Der Geyten, S., Darras, V. M., and Kühn, E. R. (2006). Role of corticotropin-releasing hormone as a thyrotropin-releasing factor in non-mammalian vertebrates. *Gen. Comp. Endocrinol.* 146, 62–68. doi: 10.1016/j.ygcen.2005.10.014

de Jesus, E. G. T., Toledo, J. D., and Simpas, M. S. (1998). Thyroid hormones promote early metamorphosis in grouper (*Epinephelus coioides*) larvae. *Gen. Comp. Endocrinol.* 112, 10–16. doi: 10.1006/gcen.1998.7103

de Jesus, E. G., Inui, Y., and Hirano, T. (1990). Cortisol enhances the stimulating action of thyroid hormones on dorsal fin-ray resorption of flounder larvae *in vitro*. *Gen. Comp. Endocrinol.* 79, 167–173. doi: 10.1016/0016-6480(90)90101-Q

de Jesus, E., Hirano, T., and Inui, Y. (1991). Changes in cortisol and thyroid hormone concentrations during early development and metamorphosis in the Japanese flounder, *Paralichthys olivaceus*. *Gen. Comp. Endocrinol.* 82, 369–376.

De Souza, F. S. J., Bumaschny, V. F., Low, M. J., and Rubinstein, M. (2005). Subfunctionalization of expression and peptide domains following the ancient duplication of the proopiomelanocortin gene in teleost fishes. *Mol. Biol. Evol.* 22, 2417–2427. doi: 10.1093/molbev/msi236

Decherf, S., Seugnet, I., Fini, J. B., Clerget-Froidevaux, M. S., and Demeneix, B. A. (2010). Disruption of thyroid hormone-dependent hypothalamic set-points by environmental contaminants. *Mol. Cell. Endocrinol.* 323, 172–182. doi: 10.1016/j.mce.2010.04.010

Decuypere, E., Scanes, C., and Kühn, E. (1983). Effects of glucocorticoids on circulating concentrations of thyroxine (t4) and triiodothyronine (t3) and on peripheral monodeiodination in pre- and post-hatching chicken. *Horm. Metab. Res.* 15, 233–236.

Defo, M. A., Douville, M., Giraudo, M., Brodeur, P., Boily, M., and Houde, M. (2018). RNA-sequencing to assess the health of wild yellow perch (*Perca flavescens*) populations from the St. Lawrence River, Canada. *Environ. Pollut.* 243, 1657–1668. doi: 10.1016/j.envpol.2018.09.133

Dehal, P., and Boore, J. L. (2005). Two rounds of whole genome duplication in the ancestral vertebrate. *PLoS Biol.* 3:e314. doi: 10.1371/journal.pbio.0030314

Denver, R. (2009). "Endocrinology of complex life cycles: amphibians," in *Hormones, Brain and Behavior*, eds D. Pfaff, A. Arnold, A. Etgen, R. Rubin, and S. Fahrbach (Amsterdam: Elsevier), 707–744.

Denver, R. (2017). "Endocrinology of complex life cycles: amphibians," in *Hormones, Brain and Behavior*, eds D. Pfaff and J. Marian (Oxford: Academic Press), 145–148.

Denver, R. J. (1993). Acceleration of anuran amphibian metamorphosis by corticotropin-releasing hormone-like peptides. *Gen. Comp. Endocrinol.* 91, 38–51. doi: 10.1006/gcen.1993.1102

Denver, R. J. (1997). Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. *Horm. Behav.* 31, 169–179. doi: 10.1006/hbeh.1997.1400

Denver, R. J. (2021). Stress hormones mediate developmental plasticity in vertebrates with complex life cycles. *Neurobiol. Stress* 14:100301. doi: 10.1016/j.ynstr.2021.100301

Denver, R. J., Hu, F., Scanlan, T. S., and Furlow, J. D. (2009). Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in *Xenopus laevis*. *Dev. Biol.* 326, 155–168. doi: 10.1016/j.ydbio.2008.11.005

Dimond, M.-T. (1954). The reactions of developing snapping turtles, *Chelydra serpentina* serpentina (Linné), to thiourea. *J. Exp. Zool.* 127, 93–117.

Dores, R. M., and Garcia, Y. (2015). Views on the co-evolution of the melanocortin-2 receptor, MRAPs, and the hypothalamus/pituitary/adrenal-interrenal axis. *Mol. Cell. Endocrinol.* 408, 12–22. doi: 10.1016/j.mce.2014.12.022

Dores, R. M., Londraville, R. L., Prokop, J., Davis, P., Dewey, N., and Lesinski, N. (2014). Molecular evolution of GPCRs: melanocortin/melanocortin receptors. *J. Mol. Endocrinol.* 52, T29–T42. doi: 10.1530/JME-14-0050

Dores, R., Lecaudé, S., Bauer, D., and Danielson, P. (2002). Analyzing the evolution of the opioid/orphanin gene family. *Mass Spectrom. Rev.* 21, 220–243. doi: 10.1002/mas.10029

Dubois, J., and Dussault, J. (1977). Ontogenesis of thyroid function in the neonatal rat. Thyroxine and triiodothyronine production rates. *Endocrinology* 101, 435–441.

Dupoué, A., Rutschmann, A., Le Galliard, J. F., Clobert, J., Blaimont, P., Sinervo, B., et al. (2018). Reduction in baseline corticosterone secretion correlates with climate warming and drying across wild lizard populations. *J. Anim. Ecol.* 87, 1331–1341. doi: 10.1111/1365-2656.12843

Eales, J., Morin, P., Tsang, P., and Hara, T. (1993). Thyroid hormone deiodination in brain, liver, gill, heart and msucle of Atlantic salmon (*Salmo salar*) during the photoperiodically-induced parr-smolt transformation. II. Outer- and inner-ring 3,5,3'-triiodo-L-thyronine and 3,3',5'-triiodothyronine-L-thyro. *Gen. Comp. Endocrinol.* 90, 157–167.

Ebbesson, L. O. E., Nilsen, T. O., Helvik, J. V., Tronci, V., and Stefansson, S. O. (2011). Corticotropin-releasing factor neurogenesis during midlife development in salmon: genetic, environmental and thyroid hormone regulation. *J. Neuroendocrinol.* 23, 733–741. doi: 10.1111/j.1365-2826.2011.02164.x

Einarsdóttir, I. E., Silva, N., Power, D. M., Smáradóttir, H., and Björnsson, B. T. (2006). Thyroid and pituitary gland development from hatching through metamorphosis of a teleost flatfish, the Atlantic halibut. *Anat. Embryol. (Berl)* 211, 47–60. doi: 10.1007/s00429-005-0055-z

Enriquez-Urzelai, U., San Sebastián, O., Garriga, N., and Llorente, G. A. (2013). Food availability determines the response to pond desiccation in anuran tadpoles. *Oecologia* 173, 117–127. doi: 10.1007/s00442-013-2596-9

Faught, E., Aluru, N., and Vijayan, M. M. (2016). "The molecular stress response," in *Biology of Stress in Fish – Fish Physiology*, eds C. B. Schreck, L. Tort, A. P. Farrell, and C. J. Brauner (Amsterdam: Elsevier Inc), 113–166. doi: 10.1016/B978-0-12-802728-8.00004-7

Fernie, K. J., Chabot, D., Champoux, L., Brimble, S., Alaee, M., Marteinson, S., et al. (2017). Spatiotemporal patterns and relationships among the diet, biochemistry, and exposure to flame retardants in an apex avian predator, the peregrine falcon. *Environ. Res.* 158, 43–53. doi: 10.1016/j.envres.2017.05.035

Filipsson, K., Bergman, E., Greenberg, L., Österling, M., Watz, J., and Erlandsson, A. (2020). Temperature and predator-mediated regulation of plasma cortisol and brain gene expression in juvenile brown trout (*Salmo trutta*). *Front. Zool.* 17:25. doi: 10.1186/s12983-020-00372-y

Fini, J. B., Mughal, B. B., Le Mével, S., Leemans, M., Lettmann, M., Spirhanzlova, P., et al. (2017). Human amniotic fluid contaminants alter thyroid hormone signalling and early brain development in *Xenopus embryos*. *Sci. Rep.* 7, 1–12. doi: 10.1038/srep43786

Fini, J., Le Mevel, S., Turque, N., Palmier, K., Zalko, D., Cravedi, J., et al. (2007). An *in vivo* multiwell-based fluorescent screen for monitoring vertebrate thyroid hormone disruption. *Env. Sci. Technol.* 41, 5908–5914. doi: 10.1021/es0704129

Fleming, M. S., Maugars, G., Lafont, A.-G., Rancon, J., Fontaine, R., Nourizadeh-Lillabadi, R., et al. (2019). Functional divergence of thyrotropin beta-subunit paralogs gives new insights into salmon smoltification metamorphosis. *Sci. Rep.* 9:4561. doi: 10.1038/s41598-019-40019-5

Forhead, A. J., and Fowden, A. L. (2014). Thyroid hormones in fetal growth and prepartum maturation. *J. Endocrinol.* 221, R87–R103. doi: 10.1530/JOE-14-0025

Forhead, A. J., Curtis, K., Kaptein, E., Visser, T. J., and Fowden, A. L. (2006). Developmental control of iodothyronine deiodinases by cortisol in the ovine fetus and placenta near term. *Endocrinology* 147, 5988–5994. doi: 10.1210/en.2006-0712

Forrest, D., Sjoberg, M., and Vennstrom, B. (1990). Contrasting developmental and tissue-specific expression of α and β thyroid hormone receptor genes. *EMBO J.* 9, 1519–1528. doi: 10.1002/j.1460-2075.1990.tb08270.x

Fowden, A. L., and Forhead, A. J. (2009). Hormones as epigenetic signals in developmental programming. *Exp. Physiol.* 94, 607–625. doi: 10.1113/expphysiol.2008.046359

Fowden, A. L., and Forhead, A. J. (2015). Glucocorticoids as regulatory signals during intrauterine development. *Exp. Physiol.* 100, 1477–1487. doi: 10.1113/EP085212

Fowden, A. L., Valenzuela, O. A., Vaughan, O. R., Jellyman, J. K., and Forhead, A. J. (2016). Glucocorticoid programming of intrauterine development. *Domest. Anim. Endocrinol.* 56, S121–S132. doi: 10.1016/j.domaniend.2016.02.014

Freire, R., Van Dort, S., and Rogers, L. J. (2006). Pre- and post-hatching effects of corticosterone treatment on behavior of the domestic chick. *Horm. Behav.* 49, 157–165. doi: 10.1016/j.yhbeh.2005.05.015

Frigerio, D., Moestl, E., and Kotrschal, K. (2001). Excreted metabolites of gonadal steroid hormones and corticosterone in greylag geese (*Anser anser*) from hatching to fledging. *Gen. Comp. Endocrinol.* 124, 246–255. doi: 10.1006/gcen.2001.7706

Furlow, J. D., and Neff, E. S. (2006). A developmental switch induced by thyroid hormone: Xenopus laevis metamorphosis. *Trends Endocrinol. Metab.* 17, 40–47. doi: 10.1016/j.tem.2006.01.007

Galas, L., Raoult, E., Tonon, M. C., Okada, R., Jenks, B. G., Castaño, J. P., et al. (2009). TRH acts as a multifunctional hypophysiotropic factor in vertebrates. *Gen. Comp. Endocrinol.* 164, 40–50. doi: 10.1016/j.ygcen.2009.05.003

Galay-Burgos, M., Power, D. M., Llewellyn, L., and Sweeney, G. E. (2008). Thyroid hormone receptor expression during metamorphosis of Atlantic halibut (*Hippoglossus hippoglossus*). *Mol. Cell. Endocrinol.* 281, 56–63. doi: 10.1016/j.mce.2007.10.009

Galton, V. (1989). The role of 3,5,3'-triiodothyronine in the physiological action of thyroxine in the premetamorphic tadpole. *Endocrinol. Endocrinol.* 124, 2427–2433.

Galton, V. (1990). Mechanisms underlying the acceleration of thyroid hormone-induced tadpole metamorphosis by corticosterone. *Endocrinology* 127, 2997–3002. doi: 10.1210/endo-127-6-2997

Galton, V. A., and Hiebert, A. (1987). The ontogeny of the enzyme systems for the 5- and 5-deiodination of thyroid hormones in chick embryo liver. *Endocrinology* 120, 2604–2610. doi: 10.1210/endo-120-6-2604

Gilbert, M. E., O'Shaughnessy, K. L., and Axelstad, M. (2020). Regulation of thyroid-disrupting chemicals to protect the developing brain. *Endocrinology (United States)* 161, 1–17. doi: 10.1210/endocr/bqaa106

Gilbert, W. M., Eby-Wilkens, E., Plopper, C., Whitsett, J. A., and Tarantal, A. F. (2001). Fetal monkey surfactants after intra-amniotic or maternal administration of betamethasone and thyroid hormone. *Obstet. Gynecol.* 98, 466–470. doi: 10.1016/S0029-7844(01)01417-X

Gilmour, K. M. (2005). Mineralocorticoid receptors and hormones: fishing for answers. *Endocrinology* 146, 44–46. doi: 10.1210/en.2004-1390

Giroux, M., and Schlenk, D. (2021). The effects of temperature and salinity on the endocrinology in two life stages of juvenile rainbow/steelhead trout (*Oncorhynchus mykiss*). *J. Fish. Biol.* 99, 513–523. doi: 10.1111/jfb.14741

Glennemeier, K. A., and Denver, R. J. (2002). Developmental changes in interrenal responsiveness in anuran amphibians. *Integr. Comp. Biol.* 42, 565–573. doi: 10.1093/icb/42.3.565

Godart, M., Frau, C., Farhat, D., Giolito, M., Jamard, C., Le Nevé, C., et al. (2021). Murine intestinal stem cells are highly sensitive to modulation of the T3/TRα1-dependent pathway. *Development* 148:dev194357.

Gomez-Mestre, I., Kulkarni, S., and Buchholz, D. R. (2013). Mechanisms and consequences of developmental acceleration in tadpoles responding to pond drying. *PLoS One* 8:e84266. doi: 10.1371/journal.pone.0084266

Gonzalez, L., Ballard, P., Ertzey, R., and Williams, M. (1986). Glucocorticoids and thyroid hormones stimulate biochemical and morphological differentiation of human fetal lung in organ culture. *J. Clin. Endocrinol. Metab.* 62, 678–691.

Gore, A., Chappell, V., Fenton, S., Flaws, J., Nadal, A., Prins, G., et al. (2015). Executive summary to EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. *Endocr. Rev.* 36, 593–602. doi: 10.1210/er.2015-1093

Gorissen, M., and Flik, G. (2016). "The endocrinology of the stress response in fish: an adaptation-physiological view," in *Biology of Stress In Fish: Fish Physiology*, Vol. 35, eds C. Schreck, L. Tort, A. Farrell, and C. Brauner (London: Elsevier), 75–111.

Grimaldi, A., Buisine, N., Miller, T., Shi, Y. B., and Sachs, L. M. (2013). Mechanisms of thyroid hormone receptor action during development: lessons from amphibian studies. *Biochim. Biophys. Acta Gen. Subj.* 1830, 3882–3892. doi: 10.1016/j.bbagen.2012.04.020

Grone, B. P., and Maruska, K. P. (2015). Divergent evolution of two corticotropin-releasing hormone (CRH) genes in teleost fishes. *Front. Neurosci.* 9:365. doi: 10.3389/fnins.2015.00365

Grossowicz, N. (1946). Influence of thiourea on development of the chick embryo. *Proc. Soc. Exp. Biol. Med.* 63, 151–152.

Gudernatch, J. (1912). Feeding experiments on tadpoles. I. The influence of specific organs given as food on growth and differentiation. A contribution to the knowledge of organs with internal secretion. *Arch. Entwicklungsmech. Org.* 35, 457–483.

Guillemin, R., and Rosenberg, B. (1955). Humoral hypothalamic control of anterior pituitary: a study with combined tissue cultures. *Endocrinology* 57, 599–607.

Guillemin, R., Yamazaki, E., Gard, D. A., Jutisz, M., and Sakiz, E. (1963). *In vitro* secretion of thyrotropin (TSH): stimulation by a hypothalamic peptide (TRF). *Endocrinology* 73, 564–572. doi: 10.1210/endo-73-5-564

Guillemin, R., Yamazaki, E., Jutosz, M., and Sakiz, E. (1962). Présence dans un extrait de tissus hypothalamiques d'une substance stimulant la sécrétion de l'hormone hypophysaire thyréotrope (TSH). Première purification par filtration sur gel Sephadex. *C. R. Acad. Sci. Paris* 255, 1018–1020.

Haba, G., Nishigori, H., Tezuka, Y., Kagami, K., Sugiyama, T., and Nishigori, H. (2011). Effect of antithyroid drug on chick embryos during the last week of development: delayed hatching and decreased cerebellar acetylcholinesterase activity. *J. Obstet. Gynaecol. Res.* 37, 1549–1556. doi: 10.1111/j.1447-0756.2011.01573.x

Haitina, T., Klovins, J., Andersson, J., Fredriksson, R., Lagerström, M. C., Larhammar, D., et al. (2004). Cloning, tissue distribution, pharmacology and three-dimensional modelling of melanocortin receptors 4 and 5 in rainbow trout suggest close evolutionary relationship of these subtypes. *Biochem. J.* 380, 475–486. doi: 10.1042/BJ20031934

Halliday, R., and Buttle, H. R. (1968). The effects of cortisone acetate on the length of the gestation period and the survival of foetal Scottish Blackface lambs. *J. Endocrinol.* 41, 447–448. doi: 10.1677/joe.0.0410447

Harada, M., Yoshinaga, T., Ojima, D., and Iwata, M. (2008). cDNA cloning and expression analysis of thyroid hormone receptor in the coho salmon *Oncorhynchus kisutch* during smoltification. *Gen. Comp. Endocrinol.* 155, 658–667. doi: 10.1016/j.ygcen.2007.09.004

Harris, A., Fang, S., Prosky, J., Braverman, L., and Vagenakis, A. (1978). Decreased outer ring monodeiodination of thyroxine and reverse triiodothyronine in fetal and neonatal rats. *Endocrinology* 103, 2216–2222.

Harris, R. M., Dijkstra, P. D., and Hofmann, H. A. (2014). Complex structural and regulatory evolution of the pro-opiomelanocortin gene family. *Gen. Comp. Endocrinol.* 195, 107–115. doi: 10.1016/j.ygcen.2013.10.007

Harvey, P. (2016). Adrenocortical endocrine disruption. *J. Steroid Biochem. Mol. Biol.* 155, 199–206. doi: 10.1016/j.jsbmb.2014.10.009

Hayes, T., Chan, R., and Licht, P. (1993). Interactions of temperature and steroids on larval growth, development, and metamorphosis in a toad (*Bufo boreas*). *J. Exp. Zool.* 266, 206–215. doi: 10.1002/jez.1402660306

Hayward, L. S., Richardson, J. B., Grogan, M. N., and Wingfield, J. C. (2006). Sex differences in the organizational effects of corticosterone in the egg yolk of quail. *Gen. Comp. Endocrinol.* 146, 144–148. doi: 10.1016/j.ygcen.2005.10.016

Heijlen, M., Houbrechts, A. M., Bagci, E., Van Herck, S. L. J., Kersseboom, S., Esquerra, C. V., et al. (2014). Knockdown of type 3 iodothyronine deiodinase severely perturbs both embryonic and early larval development in zebrafish. *Endocrinology* 155, 1547–1559. doi: 10.1210/en.2013-1660

Helbing, C. C., Crump, K., Bailey, C. M., Kohno, S., Veldhoen, N., Bryan, T., et al. (2006). Isolation of the alligator (*Alligator mississippiensis*) thyroid hormone receptor α and β transcripts and their responsiveness to thyroid stimulating hormone. *Gen. Comp. Endocrinol.* 149, 141–150. doi: 10.1016/j.ygcen.2006.05.019

Henriksen, R., Rettenbacher, S., and Groothuis, T. G. G. (2013). Maternal corticosterone elevation during egg formation in chickens (*Gallus gallus* domesticus) influences offspring traits, partly via prenatal undernutrition. *Gen. Comp. Endocrinol.* 191, 83–91. doi: 10.1016/j.ygcen.2013.05.028

Hinson, J. P., and Raven, P. W. (2006). Effects of endocrine-disrupting chemicals on adrenal function. *Best Pract. Res. Clin. Endocrinol. Metab.* 20, 111–120. doi: 10.1016/j.beem.2005.09.006

Hoar, W. (1976). Smolt transformation: evolution, behaviour, and physiology. *J. Fish. Res. Board Can.* 33, 1234–1252.

Hoar, W. S. (1988). The physiology of smolting salmonids. *Fish Physiol.* 11, 275–343. doi: 10.1016/S1546-5098(08)60216-2

Holbech, H., Matthiessen, P., Hansen, M., Schüürmann, G., Knapen, D., Reuver, M., et al. (2020). ERGO: breaking down the wall between human health and environmental testing of endocrine disrupters. *Int. J. Mol. Sci.* 21:2954. doi: 10.3390/ijms21082954

Holzer, G., Roux, N., and Laudet, V. (2017). Evolution of ligands, receptors and metabolizing enzymes of thyroid signaling. *Mol. Cell. Endocrinol.* 459, 5–13. doi: 10.1016/j.mce.2017.03.021

Hotta, Y., Aritaki, M., Ohta, K., Tagwa, M., and Tanaka, M. (2001a). Development of the digestive system and metamorphosis relating hormones in spotted halibut larvae and early juveniles. *Nippon Suisan Gakkai.* 67, 40–48.

Hotta, Y., Aritaki, M., Tagawa, M., and Tanaka, M. (2001b). Changes in tissue thyroid hormone levels of metamorphosing spotted halibut *Verasper variegatus* reared at different temperatures. *Fish. Sci.* 67, 1119–1124. doi: 10.1046/j.1444-2906.2001.00369.x

Hsu, B. Y., Dijkstra, C., Darras, V. M., de Vries, B., and Groothuis, T. G. G. (2017). Maternal thyroid hormones enhance hatching success but decrease nestling body mass in the rock pigeon (*Columba livia*). *Gen. Comp. Endocrinol.* 240, 174–181. doi: 10.1016/j.ygcen.2016.10.011

Hsu, B.-Y., Sarraude, T., Cossin-Sevrin, N., Crombecque, M., Stier, A., and Ruuskanen, S. (2020). Testing for context-dependent effects of prenatal thyroid hormones on offspring survival and physiology: an experimental temperature manipulation. *Sci. Rep.* 10:14563.

Hu, F., Knoedler, J. R., and Denver, R. J. (2016). A mechanism to enhance cellular responsivity to hormone action: krüppel-like factor 9 promotes thyroid hormone receptor-β autoinduction during postembryonic brain development. *Endocrinology* 157, 1683–1693. doi: 10.1210/en.2015-1980

Hwang, J. I., Moon, M. J., Park, S., Kim, D. K., Cho, E. B., Ha, N., et al. (2013). Expansion of secretin-like G protein-coupled receptors and their peptide ligands via local duplications before and after two rounds of whole-genome duplication. *Mol. Biol. Evol.* 30, 1119–1130. doi: 10.1093/molbev/mst031

Hylka, V. W., and Doneen, B. A. (1983). Ontogeny of embryonic chicken lung: Effects of pituitary gland, corticosterone, and other hormones upon pulmonary growth and synthesis of surfactant phospholipids. *Gen. Comp. Endocrinol.* 52, 108–120. doi: 10.1016/0016-6480(83)90163-6

Ikuta, K., Aida, K., Okumoto, N., and Hanyu, I. (1985). Effects of thyroxine and methyltestosterone on smoltification of masu salmon (*Oncorhynchus masou*). *Aquaculture* 45, 289–303. doi: 10.1016/0044-8486(85)90276-5

Inui, Y., and Miwa, S. (1985). Thyroid hormone induces metamorphosis of flounder larvae. *Gen. Comp. Endocrinol.* 60, 450–454. doi: 10.1016/0016-6480(85)90080-2

Inui, Y., and Miwa, S. (2012). "Metamorphosis of flatfish (pleuronectiformes)," in *Metamorphosis In Fish*, eds S. Dufour, K. Rousseau, and B. Kapoor (Saint Helier: Science Publishers), 107–153.

Irachi, S., Hall, D. J., Fleming, M. S., Maugars, G., Björnsson, B. T., Dufour, S., et al. (2021). Photoperiodic regulation of pituitary thyroid-stimulating hormone and brain deiodinase in Atlantic salmon. *Mol. Cell. Endocrinol.* 519:111056. doi: 10.1016/j.mce.2020.111056

Isorna, E., Obregon, M. J., Calvo, R. M., Vázquez, R., Pendón, C., Falcón, J., et al. (2009). Iodothyronine deiodinases and thyroid hormone receptors regulation during flatfish (*Solea senegalensis*) metamorphosis. *J. Exp. Zool. Part B Mol. Dev. Evol.* 312, 231–246. doi: 10.1002/jez.b.21285

Itoh, K., Watanabe, K., Wu, X., and Suzuki, T. (2010). Three members of the iodothyronine deiodinase family, dio1, dio2 and dio3, are expressed in spatially and temporally specific patterns during metamorphosis of the flounder *Paralichthys olivaceus*. *Zoolog. Sci.* 27, 574–580.

Iwata, M., Yamauchi, K., Nishioka, R., Lin, R., and Bern, H. (1990). Effects of thyroxine, growth hormone and cortisol on salinity preference of juvenile coho salmon (*Oncorhynchus kisutch*). *Mar. Behav. Physiol.* 17, 191–201.

Izaz, A., Pan, T., Wang, L., Zhang, H., Duan, S., Li, E., et al. (2021). Molecular cloning, characterization, and gene expression behavior of glucocorticoid and mineralocorticoid receptors from the Chinese alligator (*Alligator sinensis*). *J. Exp. Zool. B Mol. Dev. Evol.* 336, 50–72.

Izumo, S., and Mahdavi, V. (1988). Thyroid hormone receptor α isoforms generated by alternative splicing differentially activate myosin HC gene transcription. *Nature* 334, 539–542. doi: 10.1038/334539a0

Janczak, A. M., Braastad, B. O., and Bakken, M. (2006). Behavioural effects of embryonic exposure to corticosterone in chickens. *Appl. Anim. Behav. Sci.* 96, 69–82. doi: 10.1016/j.applanim.2005.04.020

Jara, F., Thurman, L., Montiglio, P., Sih, A., and Garcia, T. (2019). Warming-induced shifts in amphibian phenology and behavior lead to altered predator-prey dynamics. *Oecologia* 189, 803–813. doi: 10.1007/s00442-019-04360-w

Jaudet, G. J., and Hatey, J. L. (1984). Variations in aldosterone and corticosterone plasma levels during metamorphosis in *Xenopus laevis* tadpoles. *Gen. Comp. Endocrinol.* 56, 59–65. doi: 10.1016/0016-6480(84)90061-3

Jellyman, J. K., Fletcher, A. J. W., Fowden, A. L., and Giussani, D. A. (2020). Glucocorticoid maturation of fetal cardiovascular function. *Trends Mol. Med.* 26, 170–184. doi: 10.1016/j.molmed.2019.09.005

Jennings, D. H., Moore, M. C., Knapp, R., Matthews, L., and Orchinik, M. (2000). Plasma steroid-binding globulin mediation of differences in stress reactivity in alternative male phenotypes in tree lizards, *Urosaurus ornatus*. *Gen. Comp. Endocrinol.* 120, 289–299. doi: 10.1006/gcen.2000.7564

Jenssen, B. (2006). Endocrine-disrupting chemicals and climate change: A worst-case combination for arctic marine mammals and seabirds? *Env. Heal. Perspect.* 114 Suppl, 76–80. doi: 10.1289/ehp.8057

Jolivet-Jaudet, G., and Leloup-Hâtey, J. (1986). Corticosteroid binding in plasma of *Xenopus laevis*. Modifications during metamorphosis and growth. *J. Steroid Biochem.* 25, 343–350. doi: 10.1016/0022-4731(86)90245-1

Jones, I., Rogers, S. A., Kille, P., and Sweeney, G. E. (2002). Molecular cloning and expression of thyroid hormone receptor alpha during salmonid development. *Gen. Comp. Endocrinol.* 125, 226–235. doi: 10.1006/gcen.2001.7745

Kalliecharan, R., and Hall, B. K. (1974). A developmental study of the levels of progesterone, corticosterone, cortisol, and cortisone circulating in plasma of chick embryos. *Gen. Comp. Endocrinol.* 24, 364–372. doi: 10.1016/0016-6480(74)90149-X

Kanaho, Y. I., Endo, D., and Park, M. K. (2006). Molecular characterization of thyroid hormone receptors from the leopard gecko, and their differential expression in the skin. *Zool. Sci.* 23, 549–556. doi: 10.2108/zsj.23.549

Kiilerich, P., Kristiansen, K., and Madsen, S. S. (2007). Hormone receptors in gills of smolting Atlantic salmon, *Salmo salar*: expression of growth hormone, prolactin, mineralocorticoid and glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 2. *Gen. Comp. Endocrinol.* 152, 295–303. doi: 10.1016/j.ygcen.2006.12.018

Kikuyama, S., Niki, K., and Mayumi, M. (1982). Retardation of thyroxine-induced metamorphosis by Amphenone B in toad tadpoles. *Endocrinol Jpn.* 29, 659–662.

Kingsbury, J., Emery, S., and Adams, A. (1955). Effects of thiourea on the adrenal glands of chick embryos. *Endocrinology* 56, 299–304.

Kloas, W., Reinecke, M., and Hanke, W. (1997). Stage-dependent changes in adrenal steroids and catecholamines during development in *Xenopus laevis*. *Gen. Comp. Endocrinol.* 108, 416–426. doi: 10.1006/gcen.1997.6998

Kobayashi, H. (1958). Effect of desoxycorticosterone acetate on metamorphosis induced by throxine in anuran tadpoles. *Endocrinology* 62, 371–377. doi: 10.1210/endo-62-4-371

Kobayashi, Y., Chiba, H., Yamanome, T., Schiöth, H. B., and Takahashi, A. (2011). Melanocortin receptor subtypes in interrenal cells and corticotropic activity of α-melanocyte-stimulating hormones in barfin flounder, *Verasper moseri*. *Gen. Comp. Endocrinol.* 170, 558–568. doi: 10.1016/j.ygcen.2010.11.019

Kortenkamp, A., Axelstad, M., Baig, A. H., Bergman, Å, Bornehag, C. G., Cenijn, P., et al. (2020). Removing critical gaps in chemical test methods by developing new assays for the identification of thyroid hormone system-disrupting chemicals—the athena project. *Int. J. Mol. Sci.* 21:3123. doi: 10.3390/ijms21093123

Krain, L. P., and Denver, R. J. (2004). Developmental expression and hormonal regulation of glucocorticoid and thyroid hormone receptors during metamorphosis in *Xenopus laevis*. *J. Endocrinol.* 181, 91–104. doi: 10.1677/joe.0.1810091

Krief, S., Berny, P., Gumisiriza, F., Gross, R., Demeneix, B., Fini, J. B., et al. (2017). Agricultural expansion as risk to endangered wildlife: pesticide exposure in wild chimpanzees and baboons displaying facial dysplasia. *Sci. Total Environ.* 598, 647–656. doi: 10.1016/j.scitotenv.2017.04.113

Krief, S., Inglesias-Gonzalez, A., Appenzeller, B., Okimat, J., Fini, J.-B., Demeneix, B., et al. (2020). Road impact in a protected area with rich biodiversity: the case of the Sebitoli road in Kibale National Park, Uganda. *Env. Sci. Pollut. Res. Int.* 27, 27914–27925. doi: 10.1007/s11356-020-09098-0

Kudo, H., Tsuneyoshi, Y., Nagae, M., Adachi, S., Yamauchi, K., Ueda, H., et al. (1994). Detection of thyroid hormone receptors in the olfactory system and brain of wild masu salmon, Oncorhynchus masou (Brevoort), during smolting by *in vitro* autoradiography. *Aquac. Res.* 25, 171–181. doi: 10.1111/are.1994.25.s2.171

Kulkarni, S. S., and Buchholz, D. R. (2012). Beyond synergy: Corticosterone and thyroid hormone have numerous interaction effects on gene regulation in *Xenopus tropicalis* tadpoles. *Endocrinology* 153, 5309–5324. doi: 10.1210/en.2012-1432

Kulkarni, S. S., and Buchholz, D. R. (2014). Corticosteroid signaling in frog metamorphosis. *Gen. Comp. Endocrinol.* 203, 225–231. doi: 10.1016/j.ygcen.2014.03.036

Kulkarni, S. S., Denver, R. J., Gomez-Mestre, I., and Buchholz, D. R. (2017). Genetic accommodation via modified endocrine signalling explains phenotypic divergence among spadefoot toad species. *Nat. Commun.* 8, 1–6. doi: 10.1038/s41467-017-00996-5

Kulkarni, S., and Buchholz, D. (2013). Developmental programs and endocrine disruption in frog metamorphosis: the perspective from microarray analysis. *Curr. Top. Dev. Biol.* 103, 329–364. doi: 10.1016/B978-0-12-385979-2.00012-5

Kwok, A. H. Y., Wang, Y., Wang, C. Y., and Leung, F. C. (2007). Cloning of chicken glucocorticoid receptor (GR) and characterization of its expression in pituitary and extrapituitary tissues. *Poult. Sci.* 86, 423–430. doi: 10.1093/ps/86.2.423

Lafont, A., Hardman, L., Dirks, R., von den Thillart, G., Tomkiewicz, J., and Dufour, S. (2014). "Characterization and regulation during reproduction of four nuclear corticosteroid receptors in the European eel, *Anguilla anguilla*," in *Proceedings of the 10th International Symposium on Reproductive Physiology of Fish*, olhão.

Lamers, W. H., Mooren, P. G., Griep, H., Endert, E., Degenhart, H. J., and Charles, R. (1986). Hormones in perinatal rat and spiny mouse: relation to altricial and precocial timing of birth. *Am. J. Physiol. Endocrinol. Metab.* 251, E78–E85. doi: 10.1152/ajpendo.1986.251.1.e78

Langdon, J. S., Thorpe, J. E., and Roberts, R. J. (1984). Effects of cortisol and acth on gill Na+/K+-ATPase, SDH and chloride cells in juvenile atlantic salmon *Salmo salar* L. *Comp. Biochem. Physiol. Part A Physiol.* 77, 9–12. doi: 10.1016/0300-9629(84)90004-5

Langhorne, P., and Simpson, T. (1981). "Natural changes in serum cortisol in *Atlantic salmon* (*Salmo salar* L.) during parr-smolt transformation," in *Stress and Fish*, ed. A. Pickering (Cambridge, MA: Academic Press), 349–350.

Langhorne, P., and Simpson, T. H. (1986). The interrelationship of cortisol, Gill (Na + K) ATPase, and homeostasis during the Parr-Smolt transformation of atlantic salmon (*Salmo salar* L.). *Gen. Comp. Endocrinol.* 61, 203–213. doi: 10.1016/0016-6480(86)90198-X

Lazcano, I., and Orozco, A. (2018). Revisiting available knowledge on teleostean thyroid hormone receptors. *Gen. Comp. Endocrinol.* 265, 128–132. doi: 10.1016/j.ygcen.2018.03.022

Le Roy, A., and Seebacher, F. (2020). Mismatched light and temperature cues disrupt locomotion and energetics via thyroid-dependent mechanisms. *Conserv. Physiol.* 8, 1–11. doi: 10.1093/conphys/coaa051

Lee, S., Ji, K., and Choi, K. (2014). Effects of water temperature on perchlorate toxicity to the thyroid and reproductive system of *Oryzias latipes*. *Ecotoxicol. Environ. Saf.* 108, 311–317. doi: 10.1016/j.ecoenv.2014.07.016

Leemans, M., Couderq, S., Demeneix, B., and Fini, J. B. (2019). Pesticides with potential thyroid hormone-disrupting effects: a review of recent data. *Front. Endocrinol. (Lausanne)* 10:743. doi: 10.3389/fendo.2019.00743

Leloup, I., Buscaglia, M., and de Luze, A. (1981). [Modulation of T4 to T3 conversion. Comparative aspects (author's transl)]. *Ann. Endocrinol. (Paris)* 42, 454–460.

Leloup, J., and Buscaglia, M. (1977). Triiodothyronine, hormone of amphibian metamorphosis. *Comptes Rendus Hebd. Des Seances L Acad. Des Sci. Ser. D* 284, 2261–2263.

Lema, S. C., Chow, M. I., Resner, E. J., Westman, A. A., May, D., Dittman, A. H., et al. (2016). Endocrine and metabolic impacts of warming aquatic habitats: Differential responses between recently isolated populations of a eurythermal desert pupfish. *Conserv. Physiol.* 4, 1–17. doi: 10.1093/conphys/cow047

Li, X., Li, Z., Deng, Y., Zhang, J., Li, J., and Wang, Y. (2020). Characterization of a novel thyrotropin-releasing hormone receptor, TRHR3, in chickens. *Poult. Sci.* 99, 1643–1654. doi: 10.1016/j.psj.2019.10.062

Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., Nome, T., et al. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature* 533, 200–205. doi: 10.1038/nature17164

Liggins, G. C. (1968). Premature parturition after infusion of corticotrophin or cortisol into foetal lambs. *J. Endocrinol.* 42, 323–329. doi: 10.1677/joe.0.0420323

Liggins, G. C. (1969). Premature delivery of foetal lambs infused with glucocorticoids. *J. Endocrinol.* 45, 515–523. doi: 10.1677/joe.0.0450515

Liggins, G., Schellenberg, J.-C., Manzal, M., Kitterman, J. A., and Lee, C.-C. (1988). Synergism of cortisol and thyrotropin-releasing hormone in lung maturation in fetal sheep. *J. Appl. Physiol.* 65, 1880–1884.

Lima, A., Ferreira, L., Silva, D., Gomes, F., and Titon, S. (2020). Thermal sensitivity of Bullfrog's immune response kept at different temperatures. *J. Exp. Zool. A Ecol. Integr. Physiol.* 333, 767–778. doi: 10.1002/jez.2436

Little, A. G. (2021). Thyroid hormone regulation of thermal acclimation in ectotherms: physiological mechanisms and ecoevolutionary implications. *Mol. Cell. Endocrinol.* 530:111285. doi: 10.1016/j.mce.2021.111285

Little, A. G., Kunisue, T., Kannan, K., and Seebacher, F. (2013). Thyroid hormone actions are temperature-specific and regulate thermal acclimation in zebrafish (*Danio rerio*). *BMC Biol.* 11:26. doi: 10.1186/1741-7007-11-26

Little, A. G., Loughland, I., and Seebacher, F. (2020). What do warming waters mean for fish physiology and fisheries? *J. Fish Biol.* 97, 328–340. doi: 10.1111/jfb.14402

Little, A., and Seebacher, F. (2014). Thyroid hormone regulates cardiac performance during cold acclimation in zebrafish (*Danio rerio*). *J. Exp. Biol.* 217, 718–725. doi: 10.1242/jeb.096602

Logan, D. W., Bryson-Richardson, R. J., Pagán, K. E., Taylor, M. S., Currie, P. D., and Jackson, I. J. (2003). The structure and evolution of the melanocortin and MCH receptors in fish and mammals. *Genomics* 81, 184–191. doi: 10.1016/S0888-7543(02)00037-X

Lorgen, M., Casadei, E., Król, E., Douglas, A., Birnie, M. J., Ebbesson, L. O. E., et al. (2015). Functional divergence of type 2 deiodinase paralogs in the Atlantic salmon. *Curr. Biol.* 25, 936–941. doi: 10.1016/j.cub.2015.01.074

Lovejoy, D. A., and Balment, R. J. (1999). Evolution and physiology of corticotropin-releasing factor (CRF) family of neuropeptides in vertebrates. *Gen. Comp.Endocrinol.* 115, 1–22.

Lovejoy, D. A., Chang, B. S. W., Lovejoy, N. R., and del Castillo, J. (2014). Molecular evolution of GPCRs: CRH/CRH receptors. *J. Mol. Endocrinol.* 52, T46–T60. doi: 10.1530/JME-13-0238

Luzeena Raja, G., Divya Subhashree, K., Lite, C., Santosh, W., and Barathi, S. (2019). Transient exposure of methylparaben to zebrafish (*Danio rerio*) embryos altered cortisol level, acetylcholinesterase activity and induced anxiety-like behaviour. *Gen. Comp. Endocrinol.* 279, 53–59. doi: 10.1016/j.ygcen.2018.11.001

Madsen, S. S. (1990). The role of cortisol and growth hormone in seawater adaptation and development of hypoosmoregulatory mechanisms in sea trout parr (*Salmo trutta* trutta). *Gen. Comp. Endocrinol.* 79, 1–11. doi: 10.1016/0016-6480(90)90082-W

Manchado, M., Infante, C., Rebordinos, L., and Cañavate, J. P. (2009). Molecular characterization, gene expression and transcriptional regulation of thyroid hormone receptors in *Senegalese sole*. *Gen. Comp. Endocrinol.* 160, 139–147. doi: 10.1016/j.ygcen.2008.11.001

Männistö, T., Mendola, P., Reddy, U., and Laughon, S. K. (2013). Neonatal outcomes and birth weight in pregnancies complicated by maternal thyroid disease. *Am. J. Epidemiol.* 178, 731–740. doi: 10.1093/aje/kwt031

Marchand, O., Duffraisse, M., Triqueneaux, G., Safi, R., and Laudet, V. (2004). Molecular cloning and developmental expression patterns of thyroid hormone receptors and T3 target genes in the turbot (*Scophtalmus maximus*) during post-embryonic development. *Gen. Comp. Endocrinol.* 135, 345–357. doi: 10.1016/j.ygcen.2003.10.012

Marchand, O., Safi, R., Escriva, H., Van Rompaey, E., Prunet, P., and Laudet, V. (2001). Molecular cloning and characterization of thyroid hormone receptors in teleost fish. *J. Mol. Endocrinol.* 26, 51–65. doi: 10.1677/jme.0.0260051

Marie, C. (1981). Ontogenesis of the adrenal glucocorticoids and of the target function of the enzymatic tyrosine transaminase activity in the chick embryo. *J. Endocrinol.* 90, 193–200.

Mashaly, M. M. (1991). Effect of exogenous corticosterone on chicken embryonic development. *Poult. Sci.* 70, 371–374. doi: 10.3382/ps.0700371

Maugars, G., Dufour, S., Cohen-Tannoudji, J. L., and Quérat, B. (2014). Multiple thyrotropin b-subunit and thyrotropin receptor-related genes arose during vertebrate evolution. *PLoS One* 9:e111361. doi: 10.1371/journal.pone.0111361

Maugars, G., Mauvois, X., Rousseau, K., and Dufour, S. (2016). "Evolution of the corticotropin-releasing hormone paralogs in teleosts," in *Proceedings of the 8th International Symposium on Fish Endocrinology*, Gothenburg.

Mazurais, D., Ducouret, B., Tujague, M., Valotaire, Y., D'Cotta, H., Gallais, C., et al. (1998). Regulation of the glucocorticoid receptor mRNA levels in the gills of Atlantic salmon (*Salmo salar*) during smoltification. *Bull. Fr. Pêche Piscic.* 350–351, 499–510.

McBride, J., Higgs, D., Fagerlund, U., and Buckley, J. (1982). Thyroid hormones and steroid hormones: potential for control of growth and smoltification of salmonids. *Aquaculture* 28, 201–210.

McCormick, S. D. (2001). Endocrine control of osmoregulation in teleost fish. *Am. Zool.* 41, 781–794. doi: 10.1093/icb/41.4.781

McCormick, S. D. (2012). Smolt physiology and endocrinology. *Fish Physiol.* 32, 199–251. doi: 10.1016/B978-0-12-396951-4.00005-0

McCormick, S. D., Dickhoff, W. W., Duston, J., Nishioka, R. S., and Bern, H. A. (1991). Developmental differences in the responsiveness of gill Na+, K+-ATPase to cortisol in salmonids. *Gen. Comp. Endocrinol.* 84, 308–317. doi: 10.1016/0016-6480(91)90054-A

McCormick, S. D., Lerner, D. T., Monette, M. Y., Nieves-Puigdoller, K., Kelly, J. T., and Björnsson, B. T. (2009). Taking it with you when you go: how perturbations to the freshwater environment, including temperature, dams, and contaminants, affect marine survival of salmon. *Am. Fish. Soc. Symp.* 69, 195–214.

McCormick, S. D., Regish, A., O'Dea, M. F., and Shrimpton, J. M. (2008). Are we missing a mineralocorticoid in teleost fish? Effects of cortisol, deoxycorticosterone and aldosterone on osmoregulation, gill Na+,K+-ATPase activity and isoform mRNA levels in Atlantic salmon. *Gen. Comp. Endocrinol.* 157, 35–40. doi: 10.1016/j.ygcen.2008.03.024

McGlashan, J. K., Thompson, M. B., Van Dyke, J. U., and Spencer, R. J. (2017). Thyroid hormones reduce incubation period without developmental or metabolic costs in murray river short-necked turtles (*Emydura macquarii*). *Physiol. Biochem. Zool.* 90, 34–46. doi: 10.1086/689744

McLean, M., Bisits, A., Davies, J., Woods, R., Lowry, P., and Smith, R. (1995). A placental clock controlling the length of human pregnancy. *Nat. Med.* 1, 460–463. doi: 10.1038/nm0595-460

McLeese, J. M., Johnsson, J., Huntley, T. F. M., Clarke, W. C., and Weisbart, M. (1994). Seasonal changes in osmoregulation, cortisol, and cortisol receptor activity in the gills of parr/smolt of steelhead trout and steelhead-rainbow trout hybrids, *Oncorhynchus mykiss*. *Gen. Comp. Endocrinol.* 93, 103–113. doi: 10.1006/gcen.1994.1012

McNabb, F. M. A. (2006). Avian thyroid development and adaptive plasticity. *Gen. Comp. Endocrinol.* 147, 93–101. doi: 10.1016/j.ygcen.2005.12.011

McNabb, F. M. A., Stanton, F. W., and Dicken, S. G. (1984). Post-hatching thyroid development and body growth in precocial vs altricial birds. *Comp. Biochem. Physiol. Part A Physiol.* 78, 629–635. doi: 10.1016/0300-9629(84)90609-1

Medler, K. F., and Lance, V. A. (1998). Sex differences in plasma corticosterone levels in alligator (*Alligator mississippiensis*) embryos. *J. Exp. Zool.* 280, 238–244.

Mekuchi, M., Saito, Y., Aoki, Y., Masuda, T., Iigo, M., and Yanagisawa, T. (2011). Molecular cloning, gene structure, molecular evolution and expression analyses of thyrotropin-releasing hormone receptors from medaka (*Oryzias latipes*). *Gen. Comp. Endocrinol.* 170, 374–380. doi: 10.1016/j.ygcen.2010.10.013

Metz, J. R., Geven, E. J. W., Van Den Burg, E. H., and Flik, G. (2005). ACTH, α-MSH, and control of cortisol release: cloning, sequencing, and functional expression of the melanocortin-2 and melanocortin-5 receptor in *Cyprinus carpio*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, 814–826. doi: 10.1152/ajpregu.00826.2004

Meyer, A., and Schartl, M. (1999). Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions. *Curr. Opin. Cell Biol.* 11, 699–704. doi: 10.1016/S0955-0674(99)00039-3

Miranda, L. A., Affanni, J. M., and Paz, D. A. (2000). Corticotropin-releasing factor accelerates metamorphosis in *Bufo arenarum*: effect on pituitary ACTH and TSH cells. *J. Exp. Zool.* 286, 473–480.

Miwa, S., and Inui, Y. (1983). Effects of thyroxine and thiourea on the parr-smolt transformation of amago salmon (*Oncorhynchus rhodurus*). *Bull. Natl. Res. Inst. Aquac.* 4, 41–52.

Miwa, S., and Inui, Y. (1985). Effects of l-thyroxine and ovine growth hormone on smoltification of amago salmon (*Oncorhynchus rhodurus*). *Gen. Comp. Endocrinol.* 58, 436–442. doi: 10.1016/0016-6480(85)90116-9

Miwa, S., and Inui, Y. (1987). Effects of various doses of thyroxine and triiodothyronine on the metamorphosis of flounder (*Paralichthys olivaceus*). *Gen. Comp. Endocrinol.* 67, 356–363. doi: 10.1016/0016-6480(87)90190-0

Miwa, S., Tagawa, M., Inui, Y., and Hirano, T. (1988). Thyroxine surge in metamorphosing flounder larvae. *Gen. Comp. Endocrinol.* 70, 158–163. doi: 10.1016/0016-6480(88)90105-0

Mizuno, S., Ura, K., Onodera, Y., Fukada, H., Misaka, N., Hara, A., et al. (2001). Changes in transcript levels of gill cortisol receptor during smoltification in Wild Masu Salmon, *Oncorhynchus masou*. *Zool. Sci.* 18, 853–860. doi: 10.2108/zsj.18.853

Mommsen, T. P., Vijayan, M. M., and Moon, T. W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev. Fish Biol. Fish.* 9, 211–268. doi: 10.1023/A:1008924418720

Moore, C. S., Cristol, D. A., Maddux, S. L., Varian-Ramos, C. W., and Bradley, E. L. (2014). Lifelong exposure to methylmercury disrupts stress-induced corticosterone response in zebra finches (*Taeniopygia guttata*). *Environ. Toxicol. Chem.* 33, 1072–1076. doi: 10.1002/etc.2521

Moreira, L., Diamante, G., Giroux, M., Coffin, S., Xu, E., Moledo de Souza Abessa, D., et al. (2018). Impacts of salinity and temperature on the thyroidogenic effects of the biocide diuron in M*enidia beryllina*. *Env. Sci. Technol.* 52, 3146–3155. doi: 10.1021/acs.est.7b04970

Mulay, S., Giannopoulos, G., and Solomon, S. (1973). Corticosteroid levels in mother an dfetus of the rabbit during gestation. *Endocrinology* 93, 1342–1348.

Nagae, M., Fuda, H., Hara, A., Saneyoshi, M., and Yamauchi, K. (1994). Changes in serum concentrations of immunoglobulin M (IgM), cortisol and thyroxine (T4) during Smoltification in the Masu Salmon *Oncorhynchus masou*. *Fish. Sci.* 60, 241–242. doi: 10.2331/fishsci.60.241

Nakane, Y., and Yoshimura, T. (2019). Photoperiodic regulation of reproduction in vertebrates. *Annu. Rev. Anim. Biosci.* 7, 173–194.

Nakano, M., Hasunuma, I., Minagawa, A., Iwamuro, S., Yamamoto, K., Kikuyama, S., et al. (2018). Possible involvement of thyrotropin-releasing hormone receptor 3 in the release of prolactin in the metamorphosing bullfrog larvae. *Gen. Comp. Endocrinol.* 267, 36–44. doi: 10.1016/j.ygcen.2018.05.029

Nisenbaum, L., Martin, P., Fuentes, M., Besseau, L., Magnanou, E., McCormick, S., et al. (2020). Effects of a temperature rise on melatonin and thyroid hormones during smoltification of Atlantic salmon, *Salmo salar*. *J. Comp. Physiol. B* 190, 731–748. doi: 10.1007/s00360-020-01304-2

O'Shaughnessy, K. L., and Gilbert, M. E. (2020). Thyroid disrupting chemicals and developmental neurotoxicity – new tools and approaches to evaluate hormone action. *Mol. Cell. Endocrinol.* 518:110663. doi: 10.1016/j.mce.2019.110663

Olivereau, M. (1975). Histophysiologie de l'axe hypophyso-corticosurrenalien chez le saumon de l'Atlantique (Cycle en eau douce, vie thalassique et reproduction). *Gen. Comp. Endocrinol.* 27, 9–27.

Olson, J. M., McNabb, F. M. A., Jablonski, M. S., and Ferris, D. V. (1999). Thyroid development in relation to the development of endothermy in the red-winged blackbird (*Agelaius phoeniceus*). *Gen. Comp. Endocrinol.* 116, 204–212. doi: 10.1006/gcen.1999.7363

On, J. S. W., Arokiaraj, A. W. R., and Chow, B. K. C. (2019). Molecular evolution of CRH and CRHR subfamily before the evolutionary origin of vertebrate. *Peptides* 120:170087. doi: 10.1016/j.peptides.2019.04.014

Orozco, A., and Valverde-R, C. (2005). Thyroid hormone deiodination in fish. *Thyroid* 15, 799–813. doi: 10.1089/thy.2005.15.799

Orozco, A., Valverde, R. C., Olvera, A., and García, G. C. (2012). Iodothyronine deiodinases: a functional and evolutionary perspective. *J. Endocrinol.* 215, 207–219. doi: 10.1530/JOE-12-0258

Ortiz-Santaliestra, M., and Marco, A. (2015). Influence of dissolved oxygen conditions on toxicity of ammonium nitrate to larval natterjack toads. *Arch. Env. Contam. Toxicol.* 69, 95–103. doi: 10.1007/s00244-014-0126-3

Orton, F., and Tyler, C. R. (2015). Do hormone-modulating chemicals impact on reproduction and development of wild amphibians? *Biol. Rev.* 90, 1100–1117. doi: 10.1111/brv.12147

Owens, D., and Morris, Y. (1985). The comparative endocrinology of sea turtles. *Copeia* 3, 723–735.

Paris, M., and Laudet, V. (2008). The history of a developmental stage: metamorphosis in chordates. *Genesis* 46, 657–672. doi: 10.1002/dvg.20443

Parisi, C., and Guerriero, G. (2019). Antioxidative defense and fertility rate in the assessment of reprotoxicity risk posed by global warming. *Antioxidants* 8:622. doi: 10.3390/antiox8120622

Park, D., Freel, K. L., Daniels, K. D., and Propper, C. R. (2016). Interactions between a small chronic increase in diel water temperature and exposure to a common environmental contaminant on development of Arizona tiger salamander larvae. *Gen. Comp. Endocrinol.* 238, 69–77. doi: 10.1016/j.ygcen.2016.06.021

Patel, J., Landers, K., Li, H., Mortimer, R. H., and Richard, K. (2011). Thyroid hormones and fetal neurological development. *J. Endocrinol.* 209, 1–8. doi: 10.1530/JOE-10-0444

Petrović, T. G., Kijanović, A., Tomašević, N. K., Gavrić, J. P., Despotović, S. G., Gavrilović, B. R., et al. (2021). Effects of desiccation on metamorphic climax in *Bombina variegata*: changes in levels and patterns of oxidative stress parameters. *Animals* 11:953. doi: 10.3390/ani11040953

Pinsky, M., Eikeset, A., McCauley, D., Payne, J., and Sunday, J. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569, 108–111. doi: 10.1038/s41586-019-1132-4

Porter, T. E., Ghavam, S., Muchow, M., Bossis, I., and Ellestad, L. (2007). Cloning of partial cDNAs for the chicken glucocorticoid and mineralocorticoid receptors and characterization of mRNA levels in the anterior pituitary gland during chick embryonic development. *Domest. Anim. Endocrinol.* 33, 226–239. doi: 10.1016/j.domaniend.2006.05.006

Pottinger, T. G. (2017). Modulation of the stress response in wild fish is associated with variation in dissolved nitrate and nitrite. *Environ. Pollut.* 225, 550–558. doi: 10.1016/j.envpol.2017.03.021

Préau, L., Fini, J. B., Morvan-Dubois, G., and Demeneix, B. (2015). Thyroid hormone signaling during early neurogenesis and its significance as a vulnerable window for endocrine disruption. *Biochim. Biophys. Acta Gene Regul. Mech.* 1849, 112–121. doi: 10.1016/j.bbagrm.2014.06.015

Premdas, F., and Eales, J. (1976). The influence of TSH and ACTH on purine and pteridine deposition in the skin of rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* 54, 576–581.

Quan, J., Kang, Y., Luo, Z., Zhao, G., Ma, F., Li, L., et al. (2020). Identification and characterization of long noncoding RNAs provide insight into the regulation of gene expression in response to heat stress in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. Part D Genomics Proteomics* 36:100707. doi: 10.1016/j.cbd.2020.100707

Racic, A., Tylan, C., and Langkilde, T. (2020). Effects of temperature on plasma corticosterone in a native lizard. *Sci. Rep.* 10, 1–8. doi: 10.1038/s41598-020-73354-z

Radchuk, V., Reed, T., Teplitsky, C., van de Pol, M., Charmantier, A., Hassall, C., et al. (2019). Adaptive responses of animals to climate change are most likely insufficient. *Nat. Commun.* 10, 1–14. doi: 10.1038/s41467-019-10924-4

Raine, J. C., Cameron, C., Vijayan, M. M., MacKenzie, D. S., and Leatherland, J. F. (2005). Effect of fasting on thyroid hormone levels, and TRα and TRβ mRNA accumulation in late-stage embryo and juvenile rainbow trout, *Oncorhynchus mykiss*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 140, 452–459. doi: 10.1016/j.cbpb.2005.02.007

Ranjan, M., Wong, J., and Shi, Y. B. (1994). Transcriptional repression of *Xenopus* TRβ gene is mediated by a thyroid hormone response element located near the start site. *J. Biol. Chem.* 269, 24699–24705. doi: 10.1016/s0021-9258(17)31447-3

Redding, J. M., Patiño, R., and Schreck, C. B. (1991). Cortisol effects on plasma electrolytes and thyroid hormones during smoltification in coho salmon *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 81, 373–382. doi: 10.1016/0016-6480(91)90164-2

Reddy, P. K., and Lam, T. J. (1991). Effect of thyroid hormones on hatching in the tilapia, *Oreochromis mossambicus*. *Gen. Comp. Endocrinol.* 81, 484–491.

Reyns, G. E., Venken, K., Morreale De Escobar, G., Kühn, E. R., and Darras, V. M. (2003). Dynamics and regulation of intracellular thyroid hormone concentrations in embryonic chicken liver, kidney, brain, and blood. *Gen. Comp. Endocrinol.* 134, 80–87. doi: 10.1016/S0016-6480(03)00220-X

Richman, N. H., Tai de Diaz, S., Nishioka, R. S., Prunet, P., and Bern, H. A. (1987). Osmoregulatory and endocrine relationships with chloride cell morphology and density during smoltification in coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 60, 265–285. doi: 10.1016/0044-8486(87)90293-6

Robertson, O. (1949). Production of silvery smolt stage in rainbow trout by intramuscular injection of mammalian thyroid extract and thyrotropic hormone. *J. Exp. Zool.* 110, 337–355.

Romanoff, A., and Laufer, H. (1956). The effect of injected thiourea on the development of some organs of the chick embryo. *Endocrinology* 59, 611–619. doi: 10.1210/endo-59-6-611

Rondeau, E. B., Minkley, D. R., Leong, J. S., Messmer, A. M., Jantzen, J. R., Von Schalburg, K. R., et al. (2014). The genome and linkage map of the northern pike (*Esox lucius*): conserved synteny revealed between the salmonid sister group and the neoteleostei. *PLoS One* 9:e102089. doi: 10.1371/journal.pone.0102089

Rousseau, J. P., Buteau-Poulin, A., and Kinkead, R. (2019). Maternal thyroid hormone deficiency and cardiorespiratory disorder in rat pups. *Exp. Neurol.* 320:112960. doi: 10.1016/j.expneurol.2019.112960

Rousseau, K., Martin, P., Boeuf, G., and Dufour, S. (2012). "Salmonid secondary metamorphosis: smoltification," in *Metamorphosis in Fish*, eds S. Dufour, K. Rousseau, and B. Kappor (St Helier: Science Publishers), 167–215.

Routti, H., Atwood, T. C., Bechshoft, T., Boltunov, A., Ciesielski, T. M., Desforges, J. P., et al. (2019). State of knowledge on current exposure, fate and potential health effects of contaminants in polar bears from the circumpolar Arctic. *Sci. Total Environ.* 664, 1063–1083. doi: 10.1016/j.scitotenv.2019.02.030

Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R. P., Martinelli, R., Galeotti, P., et al. (2005). Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Horm. Behav.* 47, 592–605. doi: 10.1016/j.yhbeh.2005.01.006

Rupik, W. (2011). Structural and ultrastructural differentiation of the thyroid gland during embryogenesis in the grass snake *Natrix natrix* L. (Lepidosauria, Serpentes). *Zoology* 114, 284–297. doi: 10.1016/j.zool.2011.05.002

Ruthsatz, K., Dausmann, K. H., Drees, C., Becker, L. I., Hartmann, L., Reese, J., et al. (2020). Altered thyroid hormone levels affect the capacity for temperature-induced developmental plasticity in larvae of *Rana temporaria* and *Xenopus laevis*. *J. Therm. Biol.* 90:102599. doi: 10.1016/j.jtherbio.2020.102599

Ruuskanen, S., Groothuis, T. G. G., Schaper, S. V., Darras, V. M., de Vries, B., and Visser, M. E. (2016). Temperature-induced variation in yolk androgen and thyroid hormone levels in avian eggs. *Gen. Comp. Endocrinol.* 235, 29–37. doi: 10.1016/j.ygcen.2016.05.026

Ruuskanen, S., Hsu, B. Y., and Nord, A. (2021). Endocrinology of thermoregulation in birds in a changing climate. *Mol. Cell. Endocrinol.* 519:111088. doi: 10.1016/j.mce.2020.111088

Sachs, L. M., and Buchholz, D. R. (2017). Frogs model man: *in vivo* thyroid hormone signaling during development. *Genesis* 55, e23000. doi: 10.1002/dvg.23000

Sachs, L. M., and Buchholz, D. R. (2019). Insufficiency of thyroid hormone in frog metamorphosis and the role of glucocorticoids. *Front. Endocrinol. (Lausanne)* 10:287. doi: 10.3389/fendo.2019.00287

Sachs, L., Damjanovski, S., Jones, P., Li, Q., Amano, T., Ueda, S., et al. (2000). Dual functions of thyroid hormone receptors during *Xenopus* development. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 126, 199–211.

Saffran, M., Schally, A., and Benfey, B. (1955). Stimulation of the release of corticotropin from the adenohypophysis by a neurohypophysial factor. *Endocrinology* 57, 439–444.

Saino, N., Romano, M., Ferrari, R. P., Martinelli, R., and Møller, A. P. (2005). Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *J. Exp. Zool. Part A Comp. Exp. Biol.* 303, 998–1006. doi: 10.1002/jez.a.224

Saito, Y., Mekuchi, M., Kobayashi, N., Kimura, M., Aoki, Y., Masuda, T., et al. (2011). Molecular cloning, molecular evolution and gene expression of cDNAs encoding thyrotropin-releasing hormone receptor subtypes in a teleost, the sockeye salmon (*Oncorhynchus nerka*). *Gen. Comp. Endocrinol.* 174, 80–88. doi: 10.1016/j.ygcen.2011.07.011

Salis, P., Roux, N., Huang, D., Marcionetti, A., Mouginot, P., Reynaud, M., et al. (2021). Thyroid hormones regulate the formation and environmental plasticity of white bars in clownfishes. *Proc. Natl. Acad. Sci. U.S.A.* 118, 1–9. doi: 10.1073/pnas.2101634118

Sangild, P. T., Fowden, A. L., and Trahair, J. F. (2000). How does the foetal gastrointestinal tract develop in preparation for enteral nutrition after birth? *Livest. Prod. Sci.* 66, 141–150. doi: 10.1016/S0301-6226(00)00221-9

Sap, J., Muñoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., et al. (1986). The c-erb-A protein is a high-affinity receptor for thyroid hormone. *Nature* 324, 635–640. doi: 10.1038/324635a0

Sarraude, T., Hsu, B. Y., Groothuis, T., and Ruuskanen, S. (2020). Testing the short- and long-term effects of elevated prenatal exposure to different forms of thyroid hormones. *PeerJ* 8, 1–25. doi: 10.7717/peerj.10175

Saunders, R. L., McCormick, S. D., Henderson, E. B., Eales, J. G., and Johnston, C. E. (1985). The effect of orally administered 3,5,3′-triiodo-L-thyronine on growth and salinity tolerance of Atlantic salmon (*Salmo salar* L.). *Aquaculture* 45, 143–156. doi: 10.1016/0044-8486(85)90265-0

Schaaf, M. J. M., Champagne, D., Van Laanen, I. H. C., Van Wijk, D. C. W. A., Meijer, A. H., Meijer, O. C., et al. (2008). Discovery of a functional glucocorticoid receptor β-isoform in zebrafish. *Endocrinology* 149, 1591–1598. doi: 10.1210/en.2007-1364

Schellenberg, J., Liggins, G., Manzai, M., Kitterman, J., and Lee, C. (1988). Synergistic hormonal effects on lung maturation in fetal sheep. *J. Appl. Physiol.* 65, 94–100.

Schmidt, J., Andree, R., Davis, K., Treese, S., and Satterlee, D. (2009). Influence of maternal corticosterone treatment on incubation length of eggs laid by Japanese quail hens selected for divergent adrenocortical stress responsiveness. *Br. Poult. Sci.* 50, 739–747. doi: 10.1080/00071660903317571

Schreiber, A. M., and Specker, J. L. (1998). Metamorphosis in the summer flounder, Paralichthys dentatus: stage-specific developmental response to altered thyroid status. *Gen. Comp. Endocrinol.* 111, 156–166.

Schreiber, V., Eckertova, A., Franc, Z., Koci, J., Rybak, M., and Kmentova, V. (1961). Effect of a fraction of bovine hypothalamic extract on the release of TSH by rat adenohypophyses *in vitro*. *Experientia* 17, 264–265.

Schriks, M., van der Linden, S. C., Stoks, P. G. M., van der Burg, B., Puijker, L., de Voogt, P., et al. (2013). Occurrence of glucocorticogenic activity in various surface waters in The Netherlands. *Chemosphere* 93, 450–454. doi: 10.1016/j.chemosphere.2013.04.091

Scott, T. R., Johnson, W. A., Satterlee, D. G., and Gildersleeve, R. P. (1981). Circulating levels of corticosterone in the serum of developing chick embryos and newly hatched chicks. *Poult. Sci.* 60, 1314–1320. doi: 10.3382/ps.0601314

Shepherdley, C. A., Daniels, C. B., Orgeig, S., Richardson, S. J., Evans, B. K., and Darras, V. M. (2002). Glucocorticoids, thyroid hormones, and iodothyronine deiodinases in embryonic saltwater crocodiles. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283, 1155–1163. doi: 10.1152/ajpregu.00015.2002

Shi, Y. B. (2021). Life without thyroid hormone receptor. *Endocrinology (United States)* 162, 1–12. doi: 10.1210/endocr/bqab028

Shi, Y. B., Liang, V. C. T., Parkison, C., and Cheng, S. Y. (1994). Tissue-dependent developmental expression of a cytosolic thyroid hormone protein gene in *Xenopus*: its role in the regulation of amphibian metamorphosis. *FEBS Lett.* 355, 61–64. doi: 10.1016/0014-5793(94)01173-7

Shibata, Y., Tanizaki, Y., Zhang, H., Lee, H., Dasso, M., and Shi, Y. B. (2021). Thyroid hormone receptor is essential for larval epithelial apoptosis and adult epithelial stem cell development but not adult intestinal morphogenesis during *Xenopus* tropicalis metamorphosis. *Cells* 10:536. doi: 10.3390/cells10030536

Shibata, Y., Wen, L., Okada, M., and Shi, Y. (2020). Organ-specific requirements for thyroid hormone receptor ensure temporal coordination of tissue-specific transformations and completion of *Xenopus* Metamorphosis. *Thyroid* 30, 300–313. doi: 10.1089/thy.2019.0366

Shibusawa, K., Saito, S., Fukuda, M., Kawai, T., and Yoshimura, F. (1955). On the role of the hypothalamic-neurohypophyseal neurosecretion in the liberation of the adenohypophyseal hormines. *Endocrinol. Jpn.* 2, 47–56.

Shin, H. S., Choi, Y. J., Kim, N. N., Lee, J., Ueda, H., and Choi, C. Y. (2014). Effects of exogenous cortisol and seawater adaptation on thyroid hormone receptors in the smolt stage of the sockeye salmon, *Oncorhynchus nerka*. *Ichthyol. Res.* 61, 9–16. doi: 10.1007/s10228-013-0365-8

Shrimpton, J. M. (1996). Relationship between size, gill corticosteroid receptors, Na+-K+ ATPase activity and smolting in juvenile coho salmon (*Oncorhynchus kisutch*) in autumn and spring. *Aquaculture* 147, 127–140. doi: 10.1016/S0044-8486(96)01390-7

Shrimpton, J. M., and McCormick, S. D. (1998). Seasonal differences in plasma cortisol and gill corticosteroid receptors in upper and lower mode juvenile Atlantic salmon. *Aquaculture* 168, 205–219. doi: 10.1016/S0044-8486(98)00350-0

Shrimpton, J. M., and McCormick, S. D. (1999). Responsiveness of gill Na+/K+-ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. *J. Exp. Biol.* 202, 987–995.

Shrimpton, J. M., and McCormick, S. D. (2003). Environmental and endocrine control of gill corticosteroid receptor number and affinity in Atlantic salmon (*Salmo salar*) during smolting. *Aquaculture* 222, 83–99. doi: 10.1016/S0044-8486(03)00104-2

Shrimpton, J., and Randall, D. (1994). Downregulation of corticosteroid receptors in gills of coho salmon due to stress and cortisol treatment. *Am. J. Physiol. Integr. Comp. Physiol.* 267, R432–R438.

Siegel, H. S., and Gould, N. R. (1976). Chick embryonic plasma proteins and binding capacity for corticosterone. *Dev. Biol.* 50, 510–516. doi: 10.1016/0012-1606(76)90169-X

Silver, M., and Fowden, A. L. (1989). Pituitary-adrenocortical activity in the fetal pig in the last third of gestation. *Q. J. Exp. Physiol.* 74, 197–206. doi: 10.1113/expphysiol.1989.sp003255

Silverin, B., and Rudas, P. (1996). Thyroid hormones in nestling great tits (*Parus major*). *Gen. Comp. Endocrinol.* 103, 138–141. doi: 10.1006/gcen.1996.0104

Sinha, M. D., Ringer, R. K., Coleman, T. H., and Zindel, H. C. (1959). The effect of injected thiouracil on body weight and hatchability of the chick embryo. *Poult. Sci.* 38, 1405–1409. doi: 10.3382/ps.0381405

Sirakov, M., and Plateroti, M. (2011). The thyroid hormones and their nuclear receptors in the gut: from developmental biology to cancer. *Biochim. Biophys. Acta Mol. Basis Dis.* 1812, 938–946. doi: 10.1016/j.bbadis.2010.12.020

Sirakov, M., Kress, E., Nadjar, J., and Plateroti, M. (2014). Thyroid hormones and their nuclear receptors: new players in intestinal epithelium stem cell biology? *Cell. Mol. Life Sci.* 71, 2897–2907. doi: 10.1007/s00018-014-1586-3

Sirsat, T. S., and Dzialowski, E. M. (2020). Manipulating plasma thyroid hormone levels at hatching alters development of endothermy and ventilation in Pekin duck (*Anas platyrhynchos* domestica). *J. Exp. Biol.* 223(Pt 22):jeb237701. doi: 10.1242/jeb.237701

Sjöberg, M., Vennström, B., and Forrest, D. (1992). Thyroid hormone receptors in chick retinal development: differential expression of mRNAs for α and N-terminal variant β receptors. *Development* 114, 39–47.

Solbakken, J. S., Norberg, B., Watanabe, K., and Pittman, K. (1999). Thyroxine as a mediator of metamorphosis of Atlantic halibut, *Hippoglossus hippoglossus*. *Environ. Biol. Fishes* 56, 53–65. doi: 10.1023/a:1007542526040

Spaan, K., Haigis, A. C., Weiss, J., and Legradi, J. (2019). Effects of 25 thyroid hormone disruptors on zebrafish embryos: a literature review of potential biomarkers. *Sci. Total Environ.* 656, 1238–1249. doi: 10.1016/j.scitotenv.2018.11.071

Specker, J. L. (1982). Interrenal function and smoltification. *Aquaculture* 28, 59–66. doi: 10.1016/0044-8486(82)90008-4

Specker, J. L., Eales, J. G., Tagawa, M., and Tyler, W. A. (2000). Parr-smolt transformation in Atlantic salmon: thyroid hormone deiodination in liver and brain and endocrine correlates of change in rheotactic behavior. *Can. J. Zool.* 78, 696–705. doi: 10.1139/z99-258

Specker, J., and Schreck, C. (1982). Changes in plasma corticosteroids during smoltification of coho salmon *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 46, 53–58.

Spirhanzlova, P., Fini, J. B., Demeneix, B., Lardy-Fontan, S., Vaslin-Reimann, S., Lalere, B., et al. (2019). Composition and endocrine effects of water collected in the Kibale national park in Uganda. *Environ. Pollut.* 251, 460–468. doi: 10.1016/j.envpol.2019.05.006

St. Germain, D. L., Schwartzman, R. A., Croteau, W., Kanamori, A., Wang, Z., Brown, D. D., et al. (1994). A thyroid hormone-regulated gene in *Xenopus laevis* encodes a type III iodothyronine 5-deiodinase. *Proc. Natl. Acad. Sci. U.S.A* 91, 7767–7771. doi: 10.1073/pnas.91.16.7767

Starck, J., and Ricklefs, R. (1998). "Patterns of development: the altricial-precocial spectrum," in *Avian Growth And Development*, eds J. Starck and R. Ricklefs (Oxford: Oxford Univ. Press), 3–30.

Steegborn, C., and Schweizer, U. (2020). Structure and mechanism of iodothyronine deiodinases - what we know, what we don't know, and what would be nice to know. *Exp. Clin. Endocrinol. Diabetes* 128, 375–378. doi: 10.1055/a-1022-9916

Stenzel, D., and Huttner, W. B. (2013). Role of maternal thyroid hormones in the developing neocortex and during human evolution. *Front. Neuroanat.* 7:19. doi: 10.3389/fnana.2013.00019

Sterner, Z. R., Shewade, L. H., Mertz, K. M., Sturgeon, S. M., and Buchholz, D. R. (2020). Glucocorticoid receptor is required for survival through metamorphosis in the frog *Xenopus tropicalis*. *Gen. Comp. Endocrinol.* 291:113419. doi: 10.1016/j.ygcen.2020.113419

Sullivan, L. C., Orgeig, S., and Daniels, C. B. (2002a). Control of the development of the pulmonary surfactant system in the saltwater crocodile, *Crocodylus porosus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283, 1164–1176. doi: 10.1152/ajpregu.00009.2002

Sullivan, L. C., Orgeig, S., and Daniels, C. B. (2002b). Regulation of pulmonary surfactant secretion in the developing lizard, *Pogona vitticeps*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 133, 539–546. doi: 10.1016/S1095-6433(02)00187-3

Sullivan, L. C., Orgeig, S., Wood, P. G., and Daniels, C. B. (2001). The ontogeny of pulmonary surfactant secretion in the embryonic green sea turtle (*Chelonia mydas*). *Physiol. Biochem. Zool.* 74, 493–501. doi: 10.1086/322158

Sullivan, L., Orgeig, S., and Daniels, C. (2003). The role of extrinsic and intrinsic factors in the evolution of the control of pulmonary surfactant maturation during development in the amniotes. *Physiol. Biochem. Zool.* 76, 281–295.

Sun, B. J., Li, T., Mu, Y., McGlashan, J. K., Georges, A., Shine, R., et al. (2016). Thyroid hormone modulates offspring sex ratio in a turtle with temperature-dependent sex determination. *Proc. R. Soc. B Biol. Sci.* 283:20161206. doi: 10.1098/rspb.2016.1206

Sundell, H., Gray, M., Relier, J.-P., Kovar, I., Catterton, W., Switt, L., et al. (1979). The effects of ACTH on lung maturation in fetal lambs. *Am. J. Pathol.* 97, 393–410.

Sundell, K., Jutfelt, F., Ágústsson, T., Olsen, R. E., Sandblom, E., Hansen, T., et al. (2003). Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr-smolt transformation of Atlantic salmon *Salmo salar*. *Aquaculture* 222, 265–285. doi: 10.1016/S0044-8486(03)00127-3

Sundström, G., Dreborg, S., and Larhammar, D. (2010). Concomitant duplications of opioid peptide and receptor genes before the origin of jawed vertebrates. *PLoS One* 5:e10512. doi: 10.1371/journal.pone.0010512

Suzuki, M. R., and Kikuyama, S. (1983). Corticoids augment nuclear binding capacity for triiodothyronine in bullfrog tadpole tail fins. *Gen. Comp. Endocrinol.* 52, 272–278. doi: 10.1016/0016-6480(83)90122-3

Sweeting, R. M., Alexander, G. P., Eales, J. G., and McKeown, B. A. (1994). Hepatic and branchial thyroid hormone deiodinase activities associated with the parr-smolt transformation of coho salmon (*Oncorhynchus kisutch*). *Gen. Comp. Endocrinol.* 94, 171–181. doi: 10.1006/gcen.1994.1073

Székely, D., Cogălniceanu, D., Székely, P., Armijos-Ojeda, D., Espinosa-Mogrovejo, V., and Denoël, M. (2020). How to recover from a bad start: size at metamorphosis affects growth and survival in a tropical amphibian. *BMC Ecol.* 20:24. doi: 10.1186/s12898-020-00291-w

Tagawa, M., Miwa, S., Inuii, Y., de Jesus, E., and Hirano, T. (1990). Changes in thyroid hormone concentrations during early development and metamorphosis of the flounder, *Paralichthys olivaceus*. *Zool. Sci.* 7, 93–96.

Taillebois, L., Keith, P., Valade, P., Torres, P., Baloche, S., Dufour, S., et al. (2011). Involvement of thyroid hormones in the control of larval metamorphosis in (Teleostei: Gobioidei) at the time of river recruitment. *Gen. Comp. Endocrinol.* 173, 281–288. doi: 10.1016/j.ygcen.2011.06.008

Takeuchi, S., and Takahashi, S. (1998). Melanocortin receptor genes in the chicken–tissue distributions. *Gen. Comp. Endocrinol.* 112, 220–231. doi: 10.1006/gcen.1998.7167

Tanabe, Y. (1982). Ontogenetic aspect of steroidogenesis by gonads and adrenals of ducks and its role on sex differentiation. *J. Yamashina Inst. Ornithol.* 14, 151–156. doi: 10.3312/jyio1952.14.151

Tanabe, Y., Saito, N., and Nakamura, T. (1986). Ontogenetic steroidogenesis by testes, ovary, and adrenals of embryonic and postembryonic chickens (*Gallus domesticus*). *Gen. Comp. Endocrinol.* 63, 456–463. doi: 10.1016/0016-6480(86)90146-2

Tanabe, Y., Yano, T., and Nakamura, T. (1983). Steroid hormone synthesis and secretion by testes, ovary, and adrenals of embryonic and postembryonic ducks. *Gen. Comp. Endocrinol.* 49, 144–153. doi: 10.1016/0016-6480(83)90018-7

Tanangonan, J. B., Tagawa, M., Tanaka, M., and Hirano, T. (1989). Changes in tissue thyroxine level of metamorphosing japanese flounder *Paralichthys olivaceus* reared at different temperatures. *Nippon Suisan Gakkai.* 55, 485–490. doi: 10.2331/suisan.55.485

Telemeco, R. S., and Addis, E. A. (2014). Temperature has species-specific effects on corticosterone in alligator lizards. *Gen. Comp. Endocrinol.* 206, 184–192. doi: 10.1016/j.ygcen.2014.07.004

Thambirajah, A. A., Koide, E. M., Imbery, J. J., and Helbing, C. C. (2019). Contaminant and environmental influences on thyroid hormone action in amphibian metamorphosis. *Front. Endocrinol. (Lausanne)* 10:276. doi: 10.3389/fendo.2019.00276

Thompson, C., Weinberger, C., Lebo, R., and Evans, R. (1987). Identification of a novel thyroid hormone receptor expressed in the mammalian central nervous system. *Science* 237, 1610–1614. doi: 10.1126/science.3629259

Tipsmark, C. K., Jørgensen, C., Brande-Lavridsen, N., Engelund, M., Olesen, J. H., and Madsen, S. S. (2009). Effects of cortisol, growth hormone and prolactin on gill claudin expression in Atlantic salmon. *Gen. Comp. Endocrinol.* 163, 270–277. doi: 10.1016/j.ygcen.2009.04.020

Tona, K., Onagbesan, O., Bruggeman, V., De Smit, L., Figueiredo, D., and Decuypere, E. (2007). Non-ventilation during early incubation in combination with dexamethasone administration during late incubation: 1. Effects on physiological hormone levels, incubation duration and hatching events. *Domest. Anim. Endocrinol.* 33, 32–46. doi: 10.1016/j.domaniend.2006.04.002

Trahair, J. F., Perry, R. A., and Robinson, P. M. (1987a). Studies on the maturation of the small intestine in the fetal sheep. I. The effects of bilateral adrenalectomy. *Q. J. Exp. Physiol.* 72, 61–69.

Trahair, J. F., Perry, R. A., Silver, M., and Robinson, P. (1987b). Studies on the maturation of the small intestine in the fetal sheep. II. The effects of exogenous cortisol. *Q. J. Exp. Physiol.* 72, 71–79. doi: 10.1113/expphysiol.1987.sp003056

Trahair, J., and Sangild, P. (1997). Systemic and luminal influences on the perinatal development of the gut. *Equine Vet. J.* 29, 40–50.

Trudeau, V. L., Thomson, P., Zhang, W. S., Reynaud, S., Navarro-Martin, L., and Langlois, V. S. (2020). Agrochemicals disrupt multiple endocrine axes in amphibians. *Mol. Cell. Endocrinol.* 513:110861. doi: 10.1016/j.mce.2020.110861

Tseng, C., and Johnson, L. (1986). Role of thyroxine in functional gastric development. *Am. J. Physiol.* 251, G111–G116.

Ura, K., Hara, A., and Yamauchi, K. (1994). Serum thyroid hormone, guanine and protein profiles during smoltification and after thyroxine treatment in the masu salmon, *Oncorhynchus masou*. *Comp. Biochem. Physiol. Part A Physiol.* 107, 607–612. doi: 10.1016/0300-9629(94)90359-X

Usal, M., Veyrenc, S., Darracq–Ghitalla-Ciock, M., Regnault, C., Sroda, S., Fini, J. B., et al. (2021). Transgenerational metabolic disorders and reproduction defects induced by benzo[a]pyrene in *Xenopus tropicalis*. *Environ. Pollut.* 269:116109. doi: 10.1016/j.envpol.2020.116109

Vale, W., Spiess, J., Rivier, C., and Rivier, J. (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science* 213, 1394–1397. doi: 10.1097/00006254-198205000-00013

Van Herck, S. L. J., Delbaere, J., Bourgeois, N. M. A., McAllan, B. M., Richardson, S. J., and Darras, V. M. (2015). Expression of thyroid hormone transporters and deiodinases at the brain barriers in the embryonic chicken: insights into the regulation of thyroid hormone availability during neurodevelopment. *Gen. Comp. Endocrinol.* 214, 30–39. doi: 10.1016/j.ygcen.2015.02.021

Van Rensburg, S. J. (1967). Gestation in sheep after foetal adrenalectomy and cortisol acetate administration. *J. Endocrinol.* 38, 83–84.

Van Tuyl, M., Blommaart, P. E., De Boer, P. A. J., Wert, S. E., Ruijter, J. M., Islam, S., et al. (2004). Prenatal exposure to thyroid hormone is necessary for normal postnatal development of murine heart and lungs. *Dev. Biol.* 272, 104–117. doi: 10.1016/j.ydbio.2004.03.042

Vargas-Chacoff, L., Dann, F., Paschke, K., Oyarzún-Salazar, R., Nualart, D., Martínez, D., et al. (2021). Freshening effect on the osmotic response of the Antarctic spiny plunderfish *Harpagifer antarcticus*. *J. Fish. Biol.* 98, 1558–1571. doi: 10.1111/jfb.14676

Västermark, Å, and Schiöth, H. B. (2011). The early origin of melanocortin receptors, agouti-related peptide, agouti signalling peptide, and melanocortin receptor-accessory proteins, with emphasis on pufferfishes, elephant shark, lampreys, and amphioxus. *Eur. J. Pharmacol* 660, 61–69. doi: 10.1016/j.ejphar.2010.10.106

Veillette, P. A., and Young, G. (2005). Tissue culture of sockeye salmon intestine: functional response of Na +-K+-ATPase to cortisol. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, 1598–1605. doi: 10.1152/ajpregu.00741.2004

Verma, G., Khan, M. F., Akhtar, W., Alam, M. M., Akhter, M., and Shaquiquzzaman, M. (2018). Molecular interactions of bisphenols and analogs with glucocorticoid biosynthetic pathway enzymes: an in silico approach. *Toxicol. Mech. Methods* 28, 45–54. doi: 10.1080/15376516.2017.1356415

Vijayan, M., and Leatherland, J. (1989). Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon (*Oncorhynchus kisutch* Walbaum). *Can. J. Zool.* 67, 2746–2750.

Virtanen, E., and Soivio, A. (1985). The patterns of T3, T4, cortisol and Na+K+-ATPase during smoltification of hatchery-reared *Salmo salar* and comparison with wild smolts. *Aquaculture* 45, 97–109. doi: 10.1016/0044-8486(85)90261-3

Výboh, P., Zeman, M., Juráni, M., Buyse, J., and Decuypere, E. (1996). Plasma thyroid hormone and growth hormone patterns in precocial Japanese quail and altricial European starlings during postnatal development. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 114, 23–27. doi: 10.1016/0742-8413(95)02106-X

Wada, H. (2008). Glucocorticoids: Mediators of vertebrate ontogenetic transitions. *Gen. Comp. Endocrinol.* 156, 441–453. doi: 10.1016/j.ygcen.2008.02.004

Walpita, C. N., Van der Geyten, S., Rurangwa, E., and Darras, V. M. (2007). The effect of 3,5,3'-triiodothyronine supplementation on zebrafish (*Danio rerio*) embryonic development and expression of iodothyronine deiodinases and thyroid hormone receptors. *Gen. Comp. Endocrinol.* 152, 206–214.

Wang, J., Li, J., Zhang, X., and Sun, X. (2012). Transcriptome analysis reveals the time of the fourth round of genome duplication in common carp (*Cyprinus carpio*). *BMC Genomics* 13:96. doi: 10.1186/1471-2164-13-96

Warburton, D., Parton, L., Buckley, S., Cosico, L., Enns, G., and Saluna, T. (1988). Combined effects of corticosteroid, thyroid hormones, and (β-agonist on surfactant, pulmonary mechanics, and β-receptor binding in fetal lamb lung. *Pediatr. Res.* 24, 166–170. doi: 10.1203/00006450-198808000-00005

Weber, B. M., Bowers, E. K., Terrell, K. A., Falcone, J. F., Thompson, C. F., and Sakaluk, S. K. (2018). Pre- and postnatal effects of experimentally manipulated maternal corticosterone on growth, stress reactivity and survival of nestling house wrens. *Funct. Ecol.* 32, 1995–2007. doi: 10.1111/1365-2435.13126

Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J., and Evans, R. M. (1986). The c-erb-A gene encodes a thyroid hormone receptor. *Nature* 324, 641–646. doi: 10.1038/324641a0

Weiss, S. L., Johnston, G., and Moore, M. C. (2007). Corticosterone stimulates hatching of late-term tree lizard embryos. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 146, 360–365. doi: 10.1016/j.cbpa.2006.11.009

Weitzel, J. M., Viergutz, T., Albrecht, D., Bruckmaier, R., Schmicke, M., Tuchscherer, A., et al. (2017). Hepatic thyroid signaling of heat-stressed late pregnant and early lactating cows. *J. Endocrinol.* 234, 129–141. doi: 10.1530/JOE-17-0066

Wen, L., He, C., Sifuentes, C. J., and Denver, R. J. (2019). Thyroid hormone receptor alpha is required for thyroid hormone-dependent neural cell proliferation during tadpole metamorphosis. *Front. Endocrinol. (Lausanne)* 10:396. doi: 10.3389/fendo.2019.00396

Wentworth, B. C., and Hussein, M. O. (1985). Serum corticosterone levels in embryos, newly hatched, and young turkey poults. *Poult. Sci.* 64, 2195–2201. doi: 10.3382/ps.0642195

Williams, G. (2011). Extrathyroidal expression of TSH receptor. *Ann. Endocrinol.* 72, 68–73.

Wilson, J., Berntsen, H. F., Zimmer, K. E., Verhaegen, S., Frizzell, C., Ropstad, E., et al. (2016). Do persistent organic pollutants interact with the stress response? Individual compounds, and their mixtures, interaction with the glucocorticoid receptor. *Toxicol. Lett.* 241, 121–132. doi: 10.1016/j.toxlet.2015.11.014

Wise, P. M., and Frye, B. E. (1973). Functional development of the hypothalamo-hypophyseal-adrenal cortex axis in the chick embryo, *Gallus domesticus*. *J. Exp. Zool.* 185, 277–291. doi: 10.1002/jez.1401850302

Yamada, T., Donai, H., Okauchi, M., Tagawa, M., and Araki, K. (2011). Induction of ambicoloration by exogenous cortisol during metamorphosis of spotted halibut Verasper variegatus. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 160, 174–180. doi: 10.1016/j.cbpb.2011.08.004

Yamano, K., and Inui, Y. (1995). cDNA cloning of thyroid hormone receptor beta for the Japanese flounder. *Gen. Comp. Endocrinol.* 99, 197–203.

Yamano, K., and Miwa, S. (1998). Differential gene expression of thyroid hormone receptor α and β in fish development. *Gen. Comp. Endocrinol.* 109, 75–85. doi: 10.1006/gcen.1997.7011

Yamano, K., Arakai, K., Sekikawa, K., and Inui, Y. (1994). Cloning of thyroid hormone receptor genes expressed in metamorphosing flounder. *Dev. Genet.* 15, 378–382.

Yaoita, Y., and Brown, D. D. (1990). A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. *Genes Dev.* 4, 1917–1924. doi: 10.1101/gad.4.11.1917

Yaoita, Y., Shi, Y.-B., and Brown, D. D. (1990). *Xenopus laevis* α and β thyroid hormone receptors. *Proc. Natl. Acad. Sci. U.S.A.* 87, 7090–7094.

Yoon, B. H., Romero, R., Jun, J. K., Maymon, E., Mazor, M., and Park, J. S. (1998). An increase in fetal plasma cortisol but not dehydroepiandrosterone sulfate is followed by the onset of preterm labor in patients with preterm premature rupture of the membranes. *Am. J. Obstet. Gynecol.* 179, 1107–1114.

Yoshimura, T. (2013). Thyroid hormone and seasonal regulation of reproduction. *Front. Neuroendocrinol.* 34:157–166. doi: 10.1016/j.yfrne.2013.04.002

Young, G. (1986). Cortisol secretion *in vitro* by the interrenal of coho salmon (*Oncorhynchus kisutch*) during smoltification relationship with plasma thyroxine and plasma cortisol. *Gen. Comp. Endocrinol.* 63, 191–200. doi: 10.1016/0016-6480(86)90156-5

Young, G., Björnsson, B., Prunet, P., Lin, R., and Bern, H. (1989). Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones, and cortisol. *Gen. Comp. Endocrinol.* 74, 335–345.

Zena, L. A., Dillon, D., Hunt, K. E., Navas, C. A., Buck, C. L., and Bícego, K. C. (2020). Hormonal correlates of the annual cycle of activity and body temperature in the South-American tegu lizard (*Salvator merianae*). *Gen. Comp. Endocrinol.* 285:113295. doi: 10.1016/j.ygcen.2019.113295

Zimmer, C., Boogert, N. J., and Spencer, K. A. (2013). Developmental programming: cumulative effects of increased pre-hatching corticosterone levels and post-hatching unpredictable food availability on physiology and behaviour in adulthood. *Horm. Behav.* 64, 494–500. doi: 10.1016/j.yhbeh.2013.07.002

Zoeller, R. T., Tan, S. W., and Tyl, R. W. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Crit. Rev. Toxicol.* 37, 11–53. doi: 10.1080/10408440601123446

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

REVIEW
published: 07 October 2021
doi: 10.3389/fevo.2021.734031

Constraints and Opportunities for the Evolution of Metamorphic Organisms in a Changing Climate

Yuichiro Suzuki and Lyanna Toh*

Department of Biological Sciences, Wellesley College, Wellesley, MA, United States

We argue that developmental hormones facilitate the evolution of novel phenotypic innovations and timing of life history events by genetic accommodation. Within an individual's life cycle, metamorphic hormones respond readily to environmental conditions and alter adult phenotypes. Across generations, the many effects of hormones can bias and at times constrain the evolution of traits during metamorphosis; yet, hormonal systems can overcome constraints through shifts in timing of, and acquisition of tissue specific responses to, endocrine regulation. Because of these actions of hormones, metamorphic hormones can shape the evolution of metamorphic organisms. We present a model called a developmental goblet, which provides a visual representation of how metamorphic organisms might evolve. In addition, because developmental hormones often respond to environmental changes, we discuss how endocrine regulation of postembryonic development may impact how organisms evolve in response to climate change. Thus, we propose that developmental hormones may provide a mechanistic link between climate change and organismal adaptation.

Keywords: metamorphosis, hormones, genetic accommodation, climate change, plasticity

OPEN ACCESS

Edited by:
Fedor N. Shkil, Severtsov Institute of Ecology and Evolution (RAS), Russia

Reviewed by:
Xavier Belles, Instituto de Biología Evolutiva (IBE), Spain
Marco António Campinho, University of Algarve, Portugal

****Correspondence:***
Yuichiro Suzuki
ysuzuki@wellesley.edu

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *30 June 2021*
Accepted: *16 September 2021*
Published: *07 October 2021*

Citation:
Suzuki Y and Toh L (2021) Constraints and Opportunities for the Evolution of Metamorphic Organisms in a Changing Climate. Front. Ecol. Evol. 9:734031. doi: 10.3389/fevo.2021.734031

THE ROLE OF HORMONES IN METAMORPHOSIS

Approximately 80% of animals undergo metamorphosis—the transition from a larval to an adult stage (**Figure 1**; Werner, 1988). One key tenant of metamorphosis is that the pre-metamorphic or larva stage and its subsequent adult stage often occupy different habitats (Bishop et al., 2006). The change in habitat (such as from aquatic to terrestrial, or terrestrial to aerial) may be accompanied by a shift in nutrition and feeding behavior or different means of locomotion which necessitates distinct morphological, physiological and/or behavioral adaptations. In many metamorphic species, such as frogs and insects, the larvae devote much of their resources to growth, whereas the adults divert much of their energy toward reproduction and dispersal. In other species, especially marine invertebrates, the larval stage is dedicated toward dispersal and much of their growth commences once they settle. Because of their distinct roles, the larvae and adults often look nothing like each other. Metamorphosis then serves as a transitional period during which tissue remodeling and adult development can occur. Moreover, metamorphosis allows larval and adult life stages to evolve independently although certain aspects of the adult stage may depend on the larval development and experiences (Moran, 1994; Lee et al., 2013; Collet and Fellous, 2019; Moore and Martin, 2019).

Hormones play salient roles during metamorphosis. In response to either internal or environmental signals, dynamics of endocrine regulators begin to change toward the end of the larval life. These endocrine regulators are secreted into the circulatory system and orchestrate

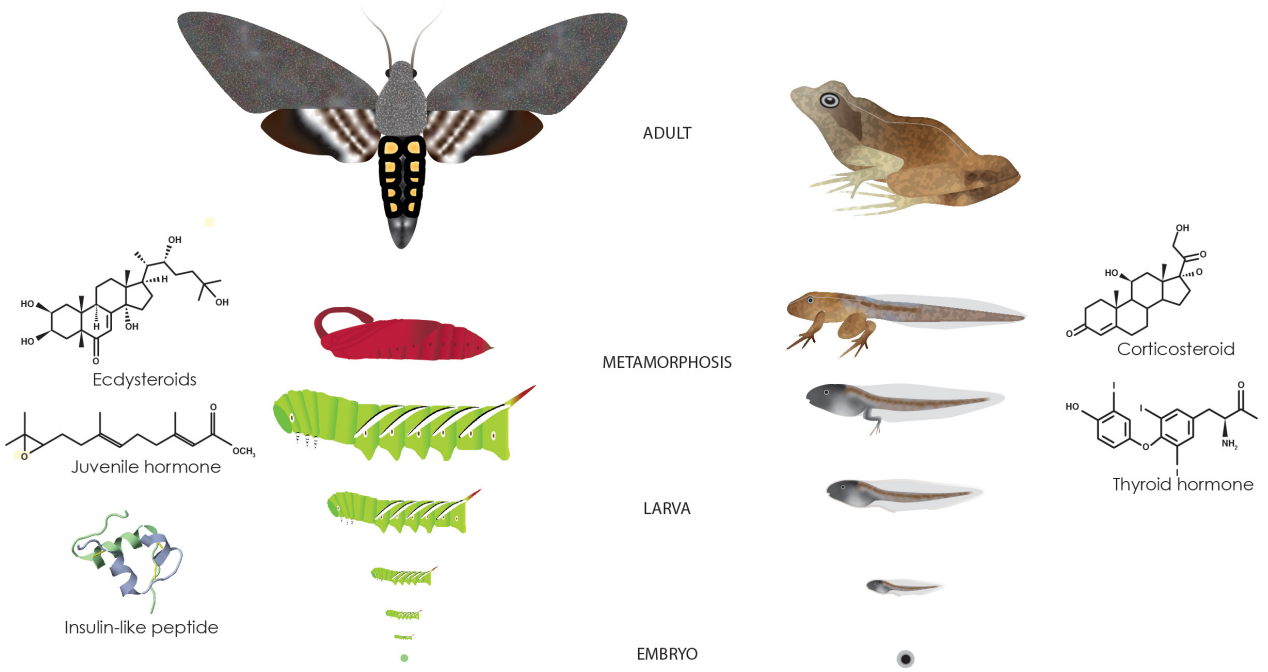


FIGURE 1 | Metamorphosis in insects and vertebrates. Holometabolous insects and anurans grow as a larva and undergoes metamorphosis before developing into an adult. Major hormones involved in this process are depicted next to the drawings. Insulin-like peptide is Dilp5 from *Drosophila* (generated using FirstGlance in Jmol at http://first-glance.jmol.org).

complex metabolic and/or morphogenetic processes in target tissues. In organisms that have adult body plans that differ radically from larval body plans, key body plan regulators that were involved in embryonic development, such as Hox genes, play major roles in shaping the adult body (Gaur et al., 2001; Lombardo and Slack, 2001; Tomoyasu et al., 2005; Chesebro et al., 2009; Hrycaj et al., 2010; Chou et al., 2019). Although these endocrine regulators act during other developmental stages, metamorphosis is a time when they coordinate drastic changes in gene expression and morphogenesis in multiple tissues (White et al., 1999; Arbeitman et al., 2002; Helbing et al., 2003; Li and White, 2003; Alves et al., 2016; Zhao et al., 2016; Wang et al., 2019). In addition, hormones play an important role in determining body size by impacting both how fast and how long an animal grows (Lorenz et al., 2009; Nijhout et al., 2014).

Below, we discuss how these endocrine processes might influence organismal evolution in the face of climate change. We will first discuss how hormones orchestrate the dramatic morphological changes that occur during metamorphosis. We then discuss how hormones respond to environmental conditions. Next, we will explore how hormones may bias evolution and how organisms might overcome constraints imposed by hormones. Furthermore, we will introduce the concept of "developmental goblet" to offer a visual representation of how hormones might impact the evolution of metamorphic organisms. Finally, we will explain how hormones can facilitate the evolution of novel traits by a process called genetic accommodation and discuss how climate change might impact the evolution of organisms by impacting their endocrine system.

Despite the prevalence of metamorphosis across the animal kingdom, metamorphosis likely evolved several times independently (Wolpert, 1999) although the molecular machinery used for metamorphosis was likely present in the common ancestor of all bilaterians (Fuchs et al., 2014). Therefore, the specific developmental events during metamorphosis differ between taxa. Our review focuses on vertebrates and insects where endocrine regulation of development has been best studied. Amphibians are one of the models for understanding the impacts of ecological changes as they are particularly susceptible to ecological disturbances (Hopkins, 2007). Insects are the most diverse group of organisms. In particular, those that undergo complete metamorphosis (the Holometabola, which have distinct larval, pupal and adults stages), have enjoyed extraordinary success (Yang, 2001). Ecological services of insects provide major economic contributions (Losey and Vaughan, 2006). With global climate change leading to mismatches in the timing of metamorphosis and flowering time, both insect and plant communities face dire consequences (Hegland et al., 2009; Høye et al., 2013; Kudo and Ida, 2013; Forrest, 2016).

Metamorphic Hormones in Vertebrates and Non-insect Invertebrates

Within a particular phylum, the specific endocrine regulators involved in metamorphosis appear to be similar. In most vertebrates, thyroid hormone signaling is a key endocrine pathway that regulates growth, development/morphogenesis and metabolism (Rabah et al., 2019). Thyroid hormone is produced

and secreted from the thyroid gland and plays a chief role in metamorphosis in amphibians and fish (Gudernatsch, 1912). The main form of thyroid hormone secreted from the thyroid gland is thyroxine (T4), which is biologically inactive and is subsequently converted to the biologically active triiodothyronine (T3), which coordinates metamorphosis (Denver et al., 2002). This conversion is mediated by the enzyme type II iodothyronine deiodinase (Davey et al., 1995). In target tissues, thyroid hormone enters the cell and regulates the expression of target genes in several different ways. In vertebrates, T3 typically binds to the nuclear Thyroid hormone receptor (TR) (Sap et al., 1986; Weinberger et al., 1986), which together with the co-receptor retinoid co-receptor (RXR), bind to DNA and regulate transcription (Zhang and Kahl, 1993; Zhang and Lazar, 2000). The peak in thyroid hormone titers coincides with the beginning of metamorphosis and coordinates myriad morphological and physiological changes from resorption of the tail to growth of limbs and remodeling of the gut (Shi, 2000; Brown and Cai, 2007). Different tissues of a tadpole undergo metamorphic changes at distinct time points. For example, a metamorphosizing tadpole grows its limbs before losing its tail so that it can continue to swim while the limbs grow out. This tissue specific timing of metamorphosis is regulated by the distinct timing of appearance of mRNAs encoding TR, RXR and type II iodothyronine deiodinase (Yaoita and Brown, 1990; Kawahara et al., 1991; Wong and Shi, 1995; Shi et al., 1996; Cai and Brown, 2004). Thyroid hormone is both necessary and sufficient for metamorphosis in teleost fishes. For example, when flounder larvae are exposed to T4, they can accelerate metamorphosis, leading to small juveniles, whereas disruption of thyroid hormone production by thiourea leads to retention of larval traits (Inui and Miwa, 1985). Exogenous thyroid hormone is also sufficient to induce early metamorphosis in larvae of the grouper, *Epinephelus coioides* (de Jesus et al., 1998).

Thyroid hormone is part of the hypothalamic–pituitary–thyroid (HPT) axis (**Figure 2A**). As in mammals, thyroid stimulating hormone (TSH), which is secreted from the pituitary gland, stimulates the production of thyroid hormone. In amphibians, TSH release is in turn regulated by corticotropin releasing hormone (CRH) from the hypothalamus rather than the thyrotropin-releasing hormone as is the case in mammals (Denver, 1999). CRH is a potent regulator of metamorphosis and appears to overcome the negative feedback of thyroid hormone on TSH release (Manzon and Denver, 2004). In teleost fishes, the role of CRH in regulating thyroid production appears to be limited to some species (Larsen et al., 1998; Campinho et al., 2015).

The HPT axis interacts with the hypothalamic–pituitary–interrenal (HPI) axis, which responds to stress. The HPT axis begins with the hypothalamus releasing CRH, which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) (**Figure 2A**). ACTH acts on the interrenal glands to release corticosteroids, the key mediator of stress responses.

Corticosteroids also interact with the thyroid hormone pathway and regulate the developmental changes induced by thyroid hormone. The application of hydrocortisone accelerates T3- and T4-induced metamorphosis in *Bufo bufo*, *Rana hechsheri* and *Rana pipiens* (Frieden and Naile, 1955). Corticosterone was also found to stimulate T3-induced metamorphosis in *Xenopus laevis* (Gray and Janssens, 1990). Corticosteroids act on tissues by enhancing tissue sensitivity to thyroid hormone: Aldosterone and corticosterone increase T3 binding in tadpole tails (Niki et al., 1981; Suzuki and Kikuyama, 1983), and cultured tadpole tails exposed to corticosteroids express higher transcript levels of *type II deiodinase* and *TR* (Krain and Denver, 2004; Bonett et al., 2010). It is thus possible that the production of corticosteroids due to environmental stressor can accelerate metamorphosis by enhancing tissue sensitivity to thyroid hormone (Wada, 2008; Denver, 2021; **Figure 2**). The evidence for teleost fishes is more ambiguous: Although cortisol can enhance the impacts of T3 on fin-ray resorption of the Japanese flounder, *Paralichthys olivaceus*, *in vitro*, the timing of metamorphosis is not impacted by cortisol *in vivo* (de Jesus et al., 1990). The lack of *in vivo* effects may be because sufficient amount of cortisol is produced endogenously (de Jesus et al., 1990).

Thyroid hormone can play an essential role during metamorphosis of other Deuterostomes (**Box 1**), including several Echinoderm species (Chino et al., 1994; Heyland and Hodin, 2004; Heyland et al., 2006) and possibly also ascidians (Patricolo et al., 1981, 2001). Whether thyroid hormone acts *via* TR is not as well-established in these non-vertebrate Deuterostomes although TR is present in all Deuterostomes studied to date (Taylor and Heyland, 2017). Intriguingly, recent studies have also suggested the involvement of thyroid hormone signaling in accelerating molluscan metamorphosis (Fukazawa et al., 2001; Taylor and Heyland, 2017). Although regulators of corticosteroid action have been identified outside vertebrates (Baker, 2010), the role of corticosteroids during metamorphosis in these species remains unknown.

Metamorphic Hormones in Insects

Before undergoing metamorphosis, most insects undergo several larval molts—the process involving the shedding of the exoskeleton to allow for growth. Within insects, the main developmental hormones are juvenile hormone (JH) and ecdysteroids (Nijhout, 1998; Truman, 2019; **Figure 1**). Generally, periodic surges of the 20-hydroxyecdysone (20E) trigger larval-larval molting as well as the initiation of metamorphosis. During the larval stage, JH prevents a larva from undergoing metamorphosis and therefore came to known as the "*status quo* hormone" (Riddiford, 1996). JH alters the effects of 20E action and inhibits metamorphic genes from being activated (Nijhout, 1998; Liu et al., 2009; Jindra et al., 2013, 2015). When bound to the Ecdysone receptor (EcR), 20E activates a transcriptional cascade of genes which induces molting (Riddiford et al., 2000) and adult tissue morphogenesis by activating a transcription factor called Ecdysone-induced protein 93 (E93) (Belles and Santos, 2014; Jindra, 2019; Truman and Riddiford, 2019). Conversely, JH binds to its receptor Methoprene-tolerant (Met) and induces the expression of Krüppel homolog 1 (Kr-h1), which represses E93 (Belles and Santos, 2014). Together, these regulators comprise the MEKRE93 pathway, which appears to be highly conserved across most insects studied to date (Belles, 2019, 2020). During metamorphosis, these regulators play

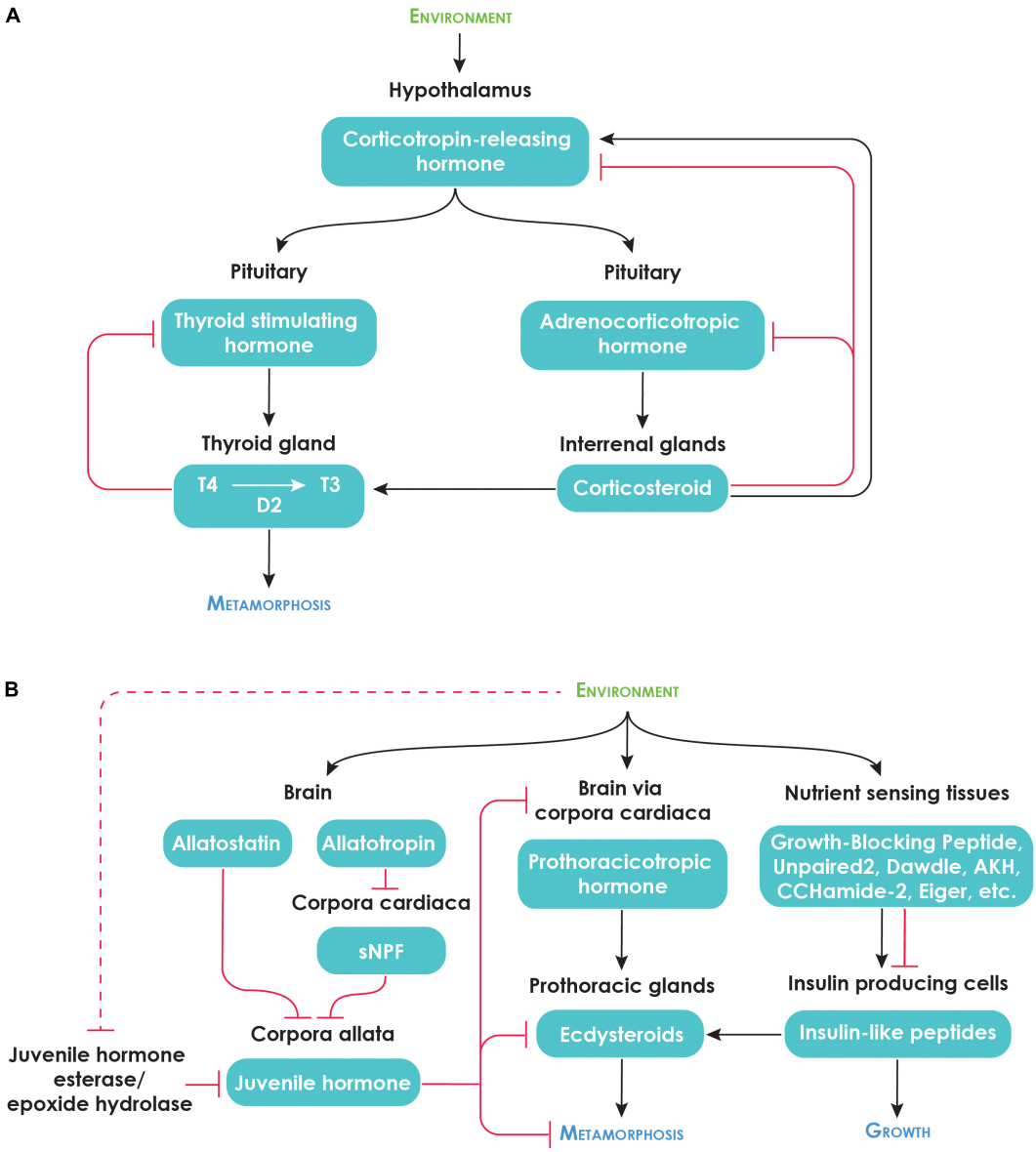


FIGURE 2 | Neuroendocrine regulation of metamorphosis. **(A)** Metamorphic regulation of amphibians [Modified from Denver (2013)]. **(B)** Hormonal regulation of insect growth and metamorphosis. The details of JH regulation are based on lepidopteran studies. Many of the regulators secreted by the nutrient-sensing tissues were identified in *Drosophila melanogaster*. We do not yet know how conserved these factors are across all insects. Dotted red line indicates effect of stress on JH esterase activity.

critical roles in regulating metamorphic timing (Rountree and Bollenbacher, 1986; Mirth et al., 2005; Yamanaka et al., 2013; Hatem et al., 2015; Nijhout, 2015) and hence the final body size (Nijhout and Williams, 1974; Caldwell et al., 2005; Callier and Nijhout, 2013; Nijhout et al., 2014). We will discuss how hormones impact body size in Section "ENVIRONMENTAL IMPACTS ON METAMORPHIC HORMONES." In addition, tissue proliferation and morphogenesis are also regulated by these hormones through their action on many target genes (Champlin and Truman, 1998a,b; Truman and Riddiford, 2002, 2007; Mirth et al., 2009; Herboso et al., 2015).

Just as the major metamorphic hormones of vertebrates are regulated by the brain, the production of metamorphic hormones in insects is also regulated by the brain, which can integrate various environmental cues (**Figure 2B**). Ecdysteroids production and release is regulated by prothoracicotropic hormone (PTTH), which is synthesized in the brain and released by the corpora cardiaca. JH synthesis and release is stimulated and inhibited by neuroendocrine factors called allatotropins and allatostatins, respectively, although the roles of these factors in the regulation of metamorphosis remain poorly understood (Goodman and Granger, 2005; Nijhout et al., 2014). Based on

BOX 1 | Terms used in this review.

Terms used in this review	Definition
Cryptic genetic variation	Hidden genetic variation of a trait that is revealed under environmental stress. This genetic variation contributes to genetic accommodation of a phenotype in a population of organisms
Deuterostomes	The animals include chordates and echinoderms and are characterized by the development of the anus before the mouth during embryogenesis. Its sister group is called the protostomes, which develop the mouth before the anus.
Developmental bias	A bias on the production of certain phenotypes due to the underlying developmental system
Developmental constraint	A limitation on the production of certain phenotypes due to the underlying developmental system
Developmental drive	A positive drive that leads to the production of certain phenotypes due to the underlying developmental system
Developmental goblet	A model for metamorphic organisms depicting that the phylotypic stage and metamorphosis represent the times when development is most conserved.
Developmental hourglass	A model for embryogenesis that shows that the mid-embryonic stage called the phylotypic stage is the time of highest developmental conservation.
Genetic accommodation	An evolutionary process by which an environmentally or mutationally induced novel phenotype either becomes fixed or becomes readily induced by small environmental fluctuations in a population. It is characterized by either an increase or decrease in phenotypic plasticity
Genetic assimilation	A special case of genetic accommodation whereby an environmentally induced novel phenotype becomes fixed in a population even without the initial environmental input. In this case, phenotypic plasticity of the trait disappears and becomes robust (or canalized)
Hormonal pleiotropy or hormonal integration	Hormonal pleiotropy or hormonal integration occurs when a hormonal system influences more than one distinct trait.
Modularity	The degree to which a trait can develop and evolve independently of another. A module in a biological system can be defined at the molecular, cellular or tissue level.
Phenotypic plasticity	The ability of an organism with the same genotype to give rise to different phenotypes depending on the environment
Phylotypic stage	A developmentally conserved stage that occurs during mid-embryogenesis. Each phylum is thought to have a characteristic phylotypic stage
Physiological homeostasis	The ability of the endocrine system to respond to the environment so that developmental and metabolic processes can proceed normally. We propose that physiological homeostasis is key to an organism's ability to cope with climate change and suggest that genetic variation in physiological homeostasis might dive the process of genetic accommodation
Polyphenisms	A special case of phenotypic plasticity where two or more distinct phenotypes arise as a consequence of a change in the environment

studies done in the silkworm, *Bombyx mori*, the allatostatins appear to act directly on the corpora allata whereas allatotropins appear to act indirectly by inhibiting Short neuropeptide F (sNPF), an inhibitor of JH biosynthesis that is produced in the corpora cardiaca (Kaneko and Hiruma, 2014; **Figure 2B**). JH activity is also modulated by JH degradation enzymes, JH esterase (JHE) and JH epoxide hydrolase (JHEH).

In addition to these two metamorphic hormones, Insulin-like peptides act on the Insulin/Target of rapamycin (TOR) signaling pathway and impact growth of insects (Koyama et al., 2020). This pathway plays an important role in regulating growth rate and determining the overall body size of the adult (Brogiolo et al., 2001; Geminard et al., 2009). Nutritional availability influences growth in almost all animals, and Insulin/TOR signaling pathway links growth of organisms to nutrient availability (Masumura et al., 2000; Ikeya et al., 2002; Geminard et al., 2009). In addition, this pathway plays a major role during metamorphosis to control tissue specific growth (Shingleton et al., 2005; Tang et al., 2011). Insulin-like peptides are often released in response to nutrients (Park et al., 2014) although in many cases, the interaction is indirect. For example, in fruitfly larvae, different tissues sense amino acids and sugars and release factors that then travel to cells that release insulin-like peptides (**Figure 2B**; Colombani et al., 2003; Geminard et al., 2009; Kim and Neufeld, 2015; Sano et al., 2015; Agrawal et al., 2016; Koyama and Mirth, 2016; Nässel and Broeck, 2016). Once released, insulin-like peptides travel to other parts of the body where they bind to the Insulin receptor, which activates a signal transduction cascade that ultimately leads to the phosphorylation of the forkhead transcription factor, Forkhead box O (FoxO), which regulates many developmentally and physiologically relevant genes (Koyama et al., 2020). The Insulin/TOR signaling pathway interacts with the ecdysteroid signaling pathway in a complex manner: Insulin/TOR signaling regulates the production of ecdysone, thus impacting the timing of metamorphosis (Mirth et al., 2005), while ecdysteroids also act to suppress Insulin signaling (Colombani et al., 2005; Mirth et al., 2014).

ENVIRONMENTAL IMPACTS ON METAMORPHIC HORMONES

Although the production of the hormones mentioned above are regulated by gene products, they also respond readily to environmental conditions. In this section, we address how the environment can impact hormonal systems. Where possible,

we also review how metamorphic hormones respond to these environmental cues and impact phenotypes.

Environmental Impacts of Vertebrate Metamorphic Hormones

In vertebrates, various environmental cues have been shown to influence hormone titers. For example, T4, T3 and corticosteroid levels all increase rapidly when tadpoles of the Western spadefoot toad, *Scapiopus hammondii* encounter decreasing water levels (Denver, 1998). These environmental changes are sensed by the brain neurons, which trigger an increase in CRH release from the hypothalamus, activating the HPT axis (Denver, 1998; Boorse and Denver, 2003). These changes are correlated with an earlier onset of and small body size at metamorphosis (Denver et al., 1998).

Temperature also impacts T3 and corticosteroid levels. In leopard frog tadpoles, *Lithobates pipiens*, corticosteroid levels peak earlier and T3 levels are elevated at higher temperatures (Freitas et al., 2017). Similarly, and tadpoles of the American bullfrog, *Lithobates catesbeianus*, also have elevated T3 levels at higher temperatures (Freitas et al., 2016). Higher temperatures are associated with faster growth and earlier onset of metamorphosis and smaller sizes at metamorphosis (Smith-Gill and Berven, 1979; Leips and Travis, 1994; Álvarez and Nicieza, 2002). Although hormonal changes could explain some of these changes, it is also possible that the phenotypic effects could also result from increased rates of intrinsic biochemical reactions and an overall reduction in cell size (Atkinson and Sibly, 1997).

Nutrition also impacts the timing of metamorphosis of anurans. There is a critical size above which food deprivation accelerates metamorphosis (Leips and Travis, 1994) and leads to smaller body sizes at the time of metamorphosis (Denver et al., 1998; Nicieza, 2000). These impacts appear to be regulated by hormones. T3 and corticotropin-releasing hormone levels are increased in food restricted mid-prometamorphic *S. hammondii* tadpoles (Boorse and Denver, 2003), and thyroid glands from starved late pre- to early prometamorphic *Rana catesbeiana* tadpoles also produce significantly higher amounts of T4 (Wright et al., 1999).

Environmental Regulation of Insect Metamorphic Hormones

In insects, a complex interaction between various endocrine regulators determines the timing of metamorphosis (Koyama et al., 2020). Within a particular species, the timing of metamorphosis can shift depending on environmental conditions, such as temperature and nutrient availability (Davidowitz et al., 2003). Both heritable differences in developmental time and plastic responses to the environment may involve alterations in endocrine regulators. In the lab, higher temperatures almost always lead to small adult body sizes by shortening the growth period (Davidowitz et al., 2003, 2004; Klok and Harrison, 2013). Observations in the field are much more complex and appear to depend on several factors including the number of generations, temperature, survival, and photoperiod (e.g., Roff, 1980; Atkinson, 1994; Imasheva et al., 1994; James et al., 1997; Horne et al., 2015).

Although studies have explored the cellular basis of temperature-dependent differences in body size (Partridge et al., 1994; Atkinson and Sibly, 1997; Zwaan et al., 2000), we still do not have a clear understanding of how temperature during the growth period impacts endocrine events that regulate life history transitions. However, the environment can impact hormones that regulate growth. A recent study on the cricket *Modicogryllus siamensis* demonstrated that higher rearing temperatures lead to enhanced Insulin/TOR signaling, leading to faster growth rate (Miki et al., 2020). Insulin/TOR signaling, however, does not impact the number of instars in *M. siamensis*; instead, the timing of JH decline impacts the duration of the juvenile growth period in a photoperiod-dependent manner (Miki et al., 2020). Thus, body size determination appears to rely on a complex interaction of endocrine regulators that respond differently to distinct environmental cues. Furthermore, we still do not understand how temperature influences the duration of larval stage, and more studies are needed to address this issue.

In addition, the environment can impact the timing of diapause and adult eclosion. Diapause is a dormant stage in insects that is equivalent to hibernation in vertebrates. Depending on the species, diapause can occur during different life history stages but metamorphic hormones often play prominent roles in regulating both the entry and duration of diapause (Chippendale and Yin, 1975; Zdarek and Denlinger, 1975; Sim and Denlinger, 2008, 2013). Environmental conditions, such as temperatures, can impact metamorphic hormones to influence the timing and duration of diapause (Turnock et al., 1986; Green and Kronforst, 2019; Cambron et al., 2021). We suspect that hormonal responses to environmental conditions are the norm, and that species can utilize these cues to coordinate life history transitions and phenotypic outcomes.

We end this section by discussing how hormones play prominent roles in polyphenisms. Polyphenic organisms can produce two or more distinct phenotypes from one genotype depending on the environment. A classic example of a polyphenism includes the polyphenisms of horned beetles where smaller male beetles have no horns on the head or the thorax, whereas larger male beetles grow horns (Kijimoto et al., 2013). These alternative morphs are both adaptive: Horned males use their horn as weapons to engage in male-male combat and guard the tunnels in which females are found, whereas hornless males "sneak by" the males by creating side-tunnels and gain access to the females (Emlen, 1997). Other examples of polyphenisms include the diet-induced polyphenisms of the caterpillars of *Nemoria arizonaria,* which can either develop into oak twig-resembling larvae or catkin-resembling morphs (Greene, 1989), and butterfly wing polyphenisms, where adult morphs adopt distinct wing color patterns depending on the season (Nijhout, 2003).

In polyphenisms, hormones play a salient role in instructing identical genomes to give rise to distinct adult morphologies that are adapted to particular environments (Nijhout, 1999, 2003). Because of the major effects developmental hormones have on adult tissue morphogenesis, small changes in the

endocrine system can lead to profound changes during metamorphosis that results in distinct, and at times spectacular, adult phenotypes (**Figure 3**). In many polyphenisms, the endocrine centers integrate environmental stimuli encountered by the larva and adjusts the amount and timing of hormone production/release/response. For example, in the squinting bush brown butterfly *Bicyclus anynana*, the adult wing has eyespots that serve as defense against potential predators. Depending on the environment, both the ecdysteroid titers and the amount of ecdysone receptors expressed on the wing discs change (Monteiro et al., 2015) and impact the size of eyespots. In another butterfly, *Precis coenia*, the wings can be red and brown depending on the photoperiod and the temperature and their impacts on ecdysteroid levels during the early pupal stage (Rountree and Nijhout, 1995). Similarly, the alternative morphs of horned beetles are regulated by the titers of JH and ecdysteroids that are modulated by the amount of nutritional consumption (Emlen and Nijhout, 1999, 2001).

THE ROLE OF HORMONES IN BIASING EVOLUTION

Metamorphosis is a time when the same developmental hormone coordinates changes in multiple tissues at once (known as hormonal pleiotropy or hormonal integration) (**Box 1** and **Figure 4A**). Hormonal pleiotropy may influence the evolutionary trajectory of organisms. The effect of hormonal pleiotropy on the evolution of organisms is dependent on the way each tissue responds to hormones (Ketterson et al., 2009). If increases in hormones enhance fitness of all traits, hormonal systems will likely evolve rapidly. In contrast, if increases in hormones leads to fitness enhancing changes in some tissues but not others, antagonistic selection may constrain the evolution of the traits involved (McGlothlin and Ketterson, 2008). For example, a hormone might promote the growth of a body part which might contribute to increased fitness. If the same hormone also promotes growth of another structure which reduces fitness, hormonal pleiotropy may prevent one trait from increasing in size while reducing the size of the other trait. Although tissue responses to hormones can evolve over time, in the short term, hormonal pleiotropy can prevent rapid adaptive changes (Ketterson and Nolan, 1999). In addition, because the same metamorphic hormone can also regulate myriad of other traits beyond metamorphosis (Hayes, 1997; Flatt et al., 2005; Deal and Volkoff, 2020), endocrine regulation that has been shaped by natural selection during another life history stage could also impact endocrine regulation during metamorphosis. For example, in insects, JH plays roles in behavior (Huang et al., 1991; Sullivan et al., 2000; Zhang et al., 2020), reproduction (Bilen et al., 2013; Santos et al., 2019) and aging (Yamamoto et al., 2013). The non-metamorphic roles of thyroid hormone has not been studied as extensively in metamorphic vertebrates, but in fishes, it appears to impact embryonic survival, larval growth (Ayson and Lam, 1993; Alinezhad et al., 2020), and gonadal sex ratios (Sharma and Patino, 2013).

In particular, in insects, many adult tissues (e.g., eyes, legs, wing) arise from the proliferating tissues called imaginal cells that proliferate in response to ecdysteroids (Champlin and Truman, 1998b; Nijhout and Grunert, 2002; Nijhout et al., 2007; Herboso et al., 2015). Programmed death of larval cells in various tissues is also coordinated by ecdysteroids (Nicolson et al., 2015). A change in the production of, or response to, metamorphic hormones can lead to catastrophic changes in the development of larvae, typically resulting in the death right before pupation (Cherbas et al., 2003; Davis et al., 2005; Tan and Palli, 2008; Ohhara et al., 2015). Moreover, tissue growth is coordinated by hormones such that disruption of one tissue can impact metamorphosis of the whole organism (Cherbas et al., 2003; Colombani et al., 2012).

This does not necessarily mean that developmental events regulated by metamorphic hormones always evolve slowly. If changes in the same hormone exert favorable changes across most tissues, selection on the endocrine system can allow for the rapid evolution of coordinated changes in multiple tissues and lead to dramatically altered phenotypes. The evolution of organisms that retain juvenile traits as reproductive adults (for example, the Mexican axolotl, the strepsipteran *Xenos vesparum* or the Japanese mealybug, *Planococcus kraunhiae*) often arise from changes in endocrine-dependent regulators (Rosenkilde and Ussing, 1996; Chafino et al., 2018; Vea et al., 2019). Thus, hormonal pleiotropy, at least in the short term, likely biases the way traits evolve and can acts as a developmental constraint (Smith et al., 1985) or a developmental drive (**Box 1**; Arthur, 2001).

THE ROLE OF HORMONES IN FACILITATING THE EVOLUTION OF ADULT PHENOTYPES

Although the highly pleiotropic developmental physiology might temporarily slow the evolution of metamorphic processes, the same endocrine regulators can also contribute to phenotypic diversification. Two distinct processes can lead to phenotypic diversification of adult morphologies: heterochrony and modularization or co-option of endocrine-dependent processes.

Heterochronic Shifts of Metamorphosis Can Promote Adult Size Diversity

A glance at the organisms living around us highlights the diversity of body sizes across species. Although body sizes and hence the timing of metamorphosis can be impacted by environmental conditions, these differences can be explained by specific-specific differences: No matter how much a fruit fly larva eats, it will never grow as large as a bullfrog. At least some of the diversity of body size can be explained by genetic changes in the timing of metamorphosis (heterochrony).

Heterochronic shifts in the timing of thyroid hormone-mediated metamorphosis can impact adult sizes in Deuterostomes. In amphibians, premature exposure to thyroid hormone can cause the tadpole to initiate metamorphosis at

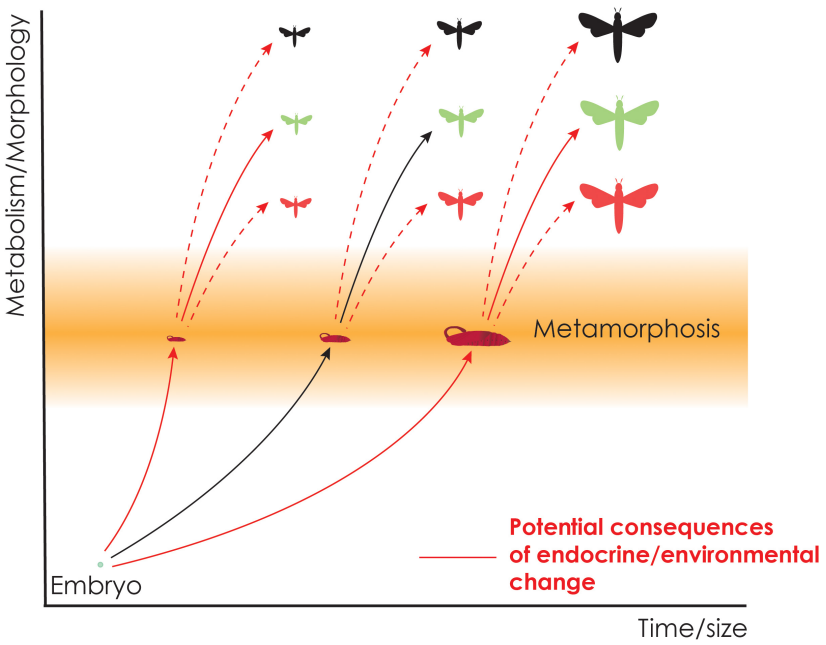


FIGURE 3 | Potential consequences of environmental changes on the timing of metamorphosis and the development of adult phenotypes during metamorphosis. Adult body size can become larger or smaller through changes in the timing of metamorphosis (solid red lines), or distinct morphologies may develop in response to environmental changes (dotted red lines). These events are often regulated by endocrine processes that respond to environmental cues.

a much smaller size than normally observed (Gudernatsch, 1912; Shi et al., 1996). In contrast, experimental ablation of thyroid glands can cause the tadpole to continue feeding and grow to an enormous size (Allen, 1916). An extreme case of heterochronic shifts has been documented in the direct-developing anuran, *Eleutherodactylus coqui* (Callery and Elinson, 2000). In this species, thyroid hormone production is initiated during embryogenesis such that the tadpole state is bypassed and a miniature adult frog hatches from the eggs. Shifting the timing of metamorphosis thus has profound impacts on the size of the adult. Similarly, exposure to thyroid hormone or thyroid hormone inhibitors can accelerate or delay, respectively, the timing metamorphosis in echinoderms (Heyland and Hodin, 2004).

In insects, body size can respond readily to artificial selection with corresponding shifts in the timing of metamorphosis (Grunert et al., 2015). In fact, it is the heritable changes in the endocrine response to the environment that often appears to be under selection and to underlie the divergent life history strategies. For instance, insects have evolved distinct responses to starvation depending on the feeding ecology (Callier and Nijhout, 2013; Hatem et al., 2015; Nijhout, 2015; Nagamine et al., 2016; Helm et al., 2017; Xu et al., 2020). In species that feed on ephemeral food sources, starvation often triggers an immediate switch to metamorphic induction by activating ecdysteroid production, ensuring that the larvae regardless of their size will initiate metamorphosis (Mirth et al., 2005; Helm et al., 2017). In species that have reliable food supply, starvation halts ecdysteroid synthesis, leading to a delay in the timing of metamorphosis (Nijhout, 2015; Xu et al., 2020). Moreover, different species have distinct threshold sizes, which is the size checkpoint that determines when a larva can metamorphose (Nijhout, 1975). Threshold size plays a critical role in the final size of the adult and does so by ultimately determining the timing of JH decline (Chafino et al., 2019; He et al., 2020).

In species with larvae that feed and grow, changes in the timing or rate of metamorphic hormone synthesis, release or sensitivity can influence final adult size (**Figure 3**). Because the endocrine regulators themselves do not change, such changes can occur without disrupting the process of metamorphosis itself. Thus, heterochronic shifts in the timing of metamorphosis, and hence the evolution of final adult size, may occur over just a few generations. We note that heterochronic changes can also occur at the level of individual tissues or behavior. Such heterochronic shifts can occur when traits become modularized and respond to hormones in a trait-specific manner (see next section).

Modularization and Co-option of Hormone Action Promotes Adult Phenotypic Diversification

Although the pleiotropic effects of metamorphic hormones might temporarily constrain evolution of metamorphic events, the sensitivity of target tissues to hormones may not be constrained in the same manner. Adaptive change in the sensitivity of tissues allows individual traits to be regulated independently from the rest of the body. Modularization (**Box 1**), or the evolution of a unique set of responses to hormones, releases the constraints imposed by the pleiotropic effects of endocrine regulators. Endocrine regulators can also be recruited to regulate

new developmental event in a tissue specific manner (a process known as co-option) (True and Carroll, 2002).

The most obvious demonstration of modularization and/or co-option of hormonal pathways in adult development is seen in insect polyphenisms. A recent survey of nymphalid butterflies has demonstrated that 20E titers fluctuate in a thermally sensitive manner regardless of the effect on wing coloration (Bhardwaj et al., 2020). Thus, in polyphenic butterflies, the pigment specification and/or synthesis pathways appear to have co-opted the pre-existing thermally-sensitive ecdysteroid peak of metamorphosis so that the adult wing coloration can be modulated by the larval environment. This example suggests that (1) hormonal levels respond readily to the environment and (2) target tissues can evolve to respond uniquely to the fluctuating hormones.

In other polyphenic traits, hormones that regulate growth of the body can have an exaggerated effect on specific parts of the body. The impressive weapons of rhinoceros beetles grow larger because insulin signaling has an outsized effect on the growth of the head horns (Emlen et al., 2012). Similarly, the disproportionate growth of the horns and mandibles in some beetle species is regulated by localized effects of hormones that arise due to tissue specific sensitivities to metamorphic hormones (Emlen and Nijhout, 1999, 2001; Gotoh et al., 2011, 2014). Thus, when individual tissues acquire the ability to uniquely respond to hormones, phenotypes can overcome hormonal pleiotropy and diversify (**Figure 4B**). Such changes could arise, for example, by the increased production of the hormone receptor or by more efficient conversion of the prohormone to an active hormone in a particular tissue (**Figure 4B**).

Finally, we note that modularity facilitates heterochronic shifts of modules. Hormones can act on individual modularized traits and either speed up or slow down development relative to an ancestral trait. Thus, heterochronic changes and modularization can both facilitate phenotypic diversification. For example, changes in thyroid hormone have been suggested to underlie the diversification of barb species in Lake Tana: Experimental alterations of thyroid hormone levels in Lake Tana barbs *Labeobarbus intermedius*, for example, can accelerate or slow down craniogenesis and produce a bony skull that resembles that of *Labeobarbus brevicephalus* and *Labeobarbus megastoma*, respectively (Smirnov et al., 2012; Shkil and Smirnov, 2016). Thyroid hormone does not uniformly impact craniogenesis. Rather, different skull bones have distinct sensitivities to thyroid hormones, allowing thyroid hormone to heterochronically alter the development of skull bones in a modular fashion (Shkil et al., 2012; Shkil and Smirnov, 2016).

THE DEVELOPMENTAL GOBLET: METAMORPHOSIS AS BOTH A CONSTRAINED AND EVOLVABLE STAGE IN DEVELOPMENT

In embryos, the phylotypic stage (**Box 1**) has been proposed to be a time when development is highly constrained and embryos resemble each other across species (Raff, 1996). This understanding led to the conceptualization of a developmental hourglass (**Box 1**), which has a broad base and broad top that sandwiches a narrow opening, representing the conserved phylotypic stage (Duboule, 1994; Raff, 1996). During the phylotypic stage, complex gene regulatory interactions pattern the major body plans, and any alterations in the interactions are likely to have profound changes in the body plan and the survival of an embryo (Galis and Metz, 2001). Because of these developmental constraints (Smith et al., 1985), gene interactions are predicted to be relatively stable across different species of a phylum, which share similar body plans.

We have discussed how pleiotropy of hormone action can bias development and how release from pleiotropic regulation *via* modularization and/or co-option of endocrine regulation can allow for diversification of traits. Across species, we propose that the amount of constraint could still be larger during metamorphosis than during the larval or adult stage. We, therefore, suggest that the early portion of metamorphosis represents a second developmentally constrained stage, during which the endocrine mechanisms controlling life history transitions are conserved. Drost et al. (2017) have also hypothesized that metamorphosis may be another constrained stage. Conversely, the larval and late metamorphic stages are less constrained and developmentally uncoupled from each other, allowing divergent stage-specific adaptations (Moran, 1994). If we were to graphically depict the amount of phenotypic and/or developmental variability across post-embryonic development of various metamorphic species within a phylum, we expect an hourglass shape to emerge where the constriction corresponds to metamorphosis, and the broad base and the broad top correspond to the larger phenotypic and/or developmental variability of larvae and adults, respectively (**Figure 5**). The width of the constriction would then depend upon the degree to which tissues have become modularized or uniquely sensitive to hormones: the more modularized the tissues, the less constricted the hourglass.

The phenotypic diversity of metamorphic animals can then be depicted as two stacked developmental hourglasses, composed of an embryonic and a post-embryonic hourglass (**Figure 5**). The resulting goblet shape may therefore be more appropriate for metamorphic animals with complex life cycles: the base and the cup representing early embryogenesis and adult development, respectively, and the bulge in the stem of a goblet representing the late embryo/larval stage (**Figure 5**). We call this the developmental goblet (**Box 1**).

We suspect that hormonal pleiotropy will constrain the metamorphic stage. However, unlike the embryonic phylotypic stage, the constraints could be more easily overcome by modularization of hormonally regulated traits, and co-option of endocrine regulation can lead to diversification of particular body parts or specific metabolic process. In animals that undergo drastic changes in body plans, metamorphosis is a post-embryonic developmental stage when the expression and/or activity of conserved developmental genes, such as homeobox genes, are modulated by the action of metamorphic hormones

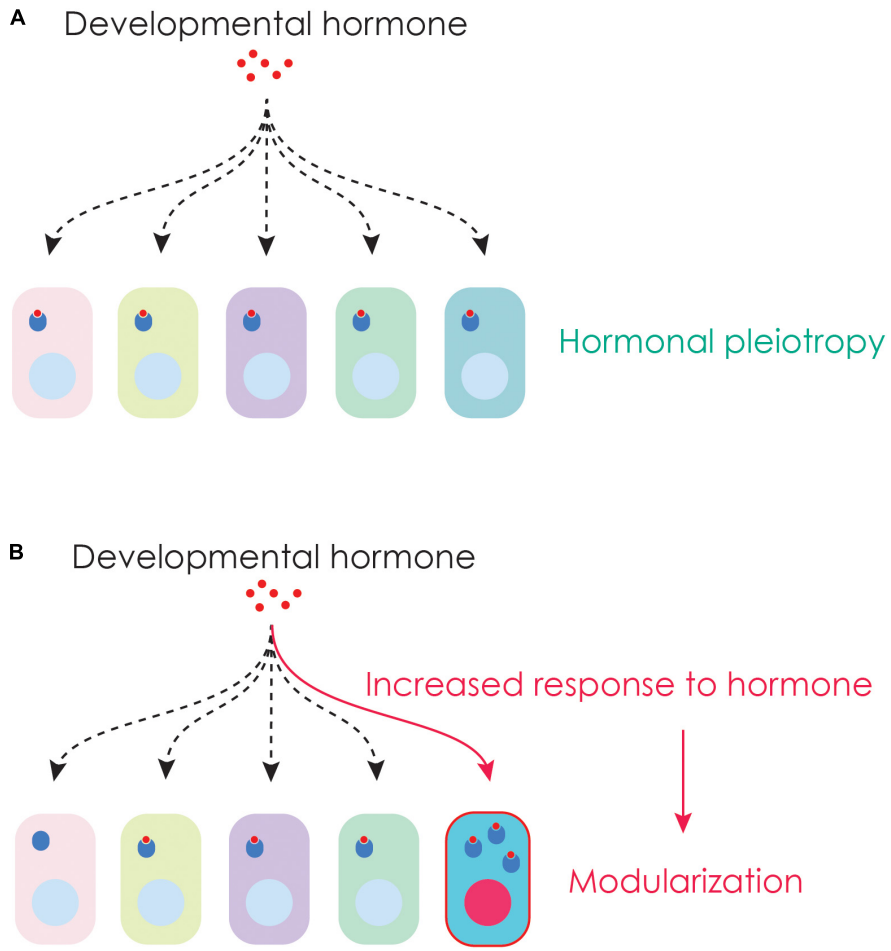


FIGURE 4 | Hormonal pleiotropy and modularization. **(A)** Hormonal pleiotropy occurs when one hormone impacts many tissues at the same time. **(B)** Specific tissues can overcome constraints imposed by pleiotropy by evolving a unique response to hormones. Such changes could arise, for example, by the cells becoming more sensitive to hormones by producing additional hormone receptors.

and their targets (Gaur et al., 2001; Monier et al., 2005; Mou et al., 2012). In insects undergoing metamorphosis, ecdysteroids activate various signaling networks in a tissue-specific manner (Li and White, 2003). In anurans, Hox genes involved in limb development are activated during limb outgrowth (Lombardo and Slack, 2001), and thyroid hormone-induced metamorphosis in axolotls has been shown to activate the expression of Hox5a in the heart (Gaur et al., 2001). In flatfish, developmental genes are also regulated by thyroid hormone in a tissue-specific manner during metamorphosis (Alves et al., 2016). Thus, hormones coordinate metamorphic events across a variety of tissues, but individual tissues can respond at different times and in distinct ways by activating target developmental genes in a tissue-specific manner. Thus, metamorphosis offers opportunities for innovation and phenotypic diversification.

Finally, we note that the shape of the goblet will likely depend on the taxon. In metamorphic organisms that undergo dramatic tissue reprogramming and remodeling (e.g., insects with complete metamorphosis), the constriction during metamorphosis maybe more pronounced than organisms in which adult organs develop from preexisting larval organs changes (e.g., fishes).

DEVELOPMENTAL HOMEOSTASIS AS A DRIVER OF EVOLUTION BY GENETIC ACCOMMODATION UNDER A CHANGING CLIMATE

Phenotypic plasticity is the ability of an organism with the same genotype to give rise to different phenotypes depending on the environment (**Box 1**). Phenotypic plasticity has been recognized as an important of how populations might respond to climate change (Reed et al., 2011; Merila and Hendry, 2014; Rodrigues and Beldade, 2020). Moreover, phenotypic plasticity has been proposed to facilitate phenotypic evolution by allowing organisms to explore novel morphospace under altered environmental or genetic backgrounds (West-Eberhard, 2003; Nijhout et al., 2021). Specifically, when genetic differences

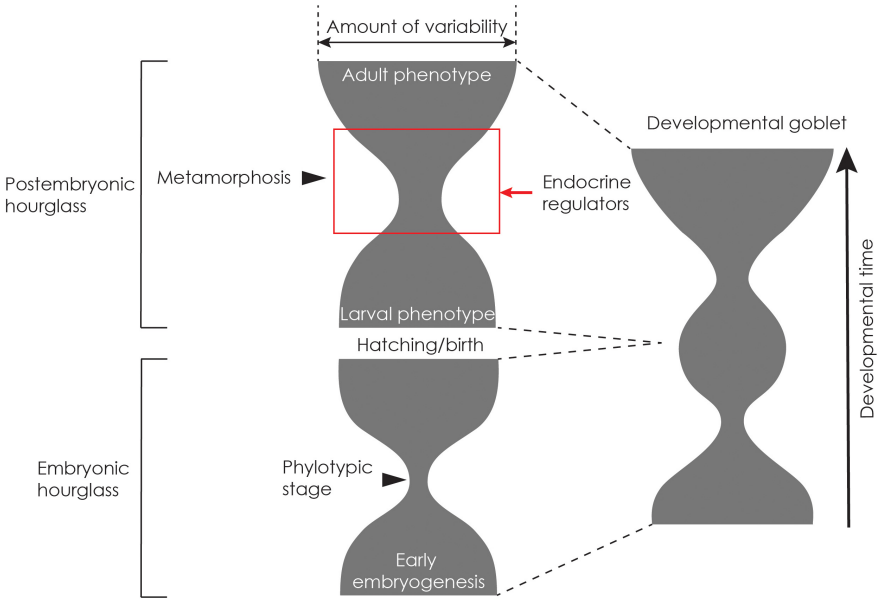


FIGURE 5 | The developmental goblet model for metamorphic organisms. The phenotypic diversity of metamorphic animals results from two stacked developmental hourglasses, the developmental goblet, which is composed of an embryonic hourglass and a postembryonic hourglass. The horizontal width of the hourglass represents phenotypic diversity across a taxon. The vertical axis represents developmental time with the top of the goblet representing the adult stage. Early larval ecologies are diverse and are reflected in the diversity of larval phenotypes. During metamorphosis, a small number of developmental hormones constrain development. Subsequently, developmental trajectories diverge to generate various adult morphologies.

underlie the organisms' variable phenotypic responses to the novel environment, natural selection can act on the induced phenotypes. The genetic variation underlying the phenotypic variation under the novel environment is called cryptic genetic variation (**Box 1**), which is normally hidden but is exposed under stressful or novel environments (Gibson and Dworkin, 2004). Selection on these revealed cryptic genetic variants can lead to evolution of novel phenotypes. Genetic accommodation (**Box 1**) is the name given to such an evolutionary process (West-Eberhard, 2003).

Climate change dependent phenotypic plasticity may lead to adaptive evolution by genetic accommodation (Kelly, 2019). Although several mechanisms have been proposed to explain genetic accommodation, developmental hormones may play a role in this process (**Figure 6**; Lema and Kitano, 2013; Kulkarni et al., 2017; Lafuente and Beldade, 2019; Levis and Pfennig, 2019; Lema, 2020; Suzuki et al., 2020). Developmental hormones often regulate both trait development and homeostasis, thus serving as the nexus between the environment and development (Dufty et al., 2002; Denver, 2009; Xu et al., 2013; **Figure 3**). Hormonal changes can manifest as phenotypic differences and the degree to which a developmental hormone impacts the phenotype and facilitate phenotypic evolution can vary according to the cryptic genetic variation that is revealed under stressful conditions (Suzuki and Nijhout, 2006, 2008; Suzuki et al., 2020).

Altered temperature and precipitation patterns due to climate change (Trenberth, 2011; Intergovernmental Panel on Climate Change, 2014), and resulting changes in food availability, may lead to such stressful environments that disrupt physiological homeostasis and reveal cryptic genetic variation. If the population encounters a directional change in environmental conditions (e.g., warmer and moister) over multiple generations, hormonally mediated traits may evolve by either shifting the timing of life history transitions or by altering the adult phenotypes by co-option or modularization of hormonally mediated traits (**Figure 6**). For example, in amphibians, desiccation stress and nutritional stress have both been shown to lead to changes in stress hormones, which in turn impacts that timing of metamorphosis and life history transitions (Denver, 1997; Denver et al., 2002; Wada, 2008; Ledon-Rettig et al., 2009; Kulkarni and Buchholz, 2014). In spadefoot toads, aridification has been proposed to have led to the evolution of species with shorter larval periods by adjustments in thyroid hormone titers through genetic accommodation (Gomez-Mestre and Buchholz, 2006; Kulkarni et al., 2017).

In insects, JH levels increase or fail to decline in larvae exposed to stressful environments (Cymborowski et al., 1982; Rauschenbach et al., 1987; Jones et al., 1990; Browder et al., 2001; Suzuki and Nijhout, 2006; Xu et al., 2020), possibly due to the inhibition of the JH degradation enzyme, JHE (Hirashima et al., 1995), and/or changes in JH binding proteins, which may alter the bioavailability of JH (Tauchman et al., 2007). Cryptic genetic variation that confers differential sensitivity to heat or nutritional stress could lead to variation in JH levels that selection could act upon. Similarly, 20E levels has been shown to increase in response to thermal stress in adult *Drosophila virilis* (Hirashima et al., 2000), and in the common cutworm, *Spodoptera litura*, mild thermal stress upregulates the expression of *EcR* during

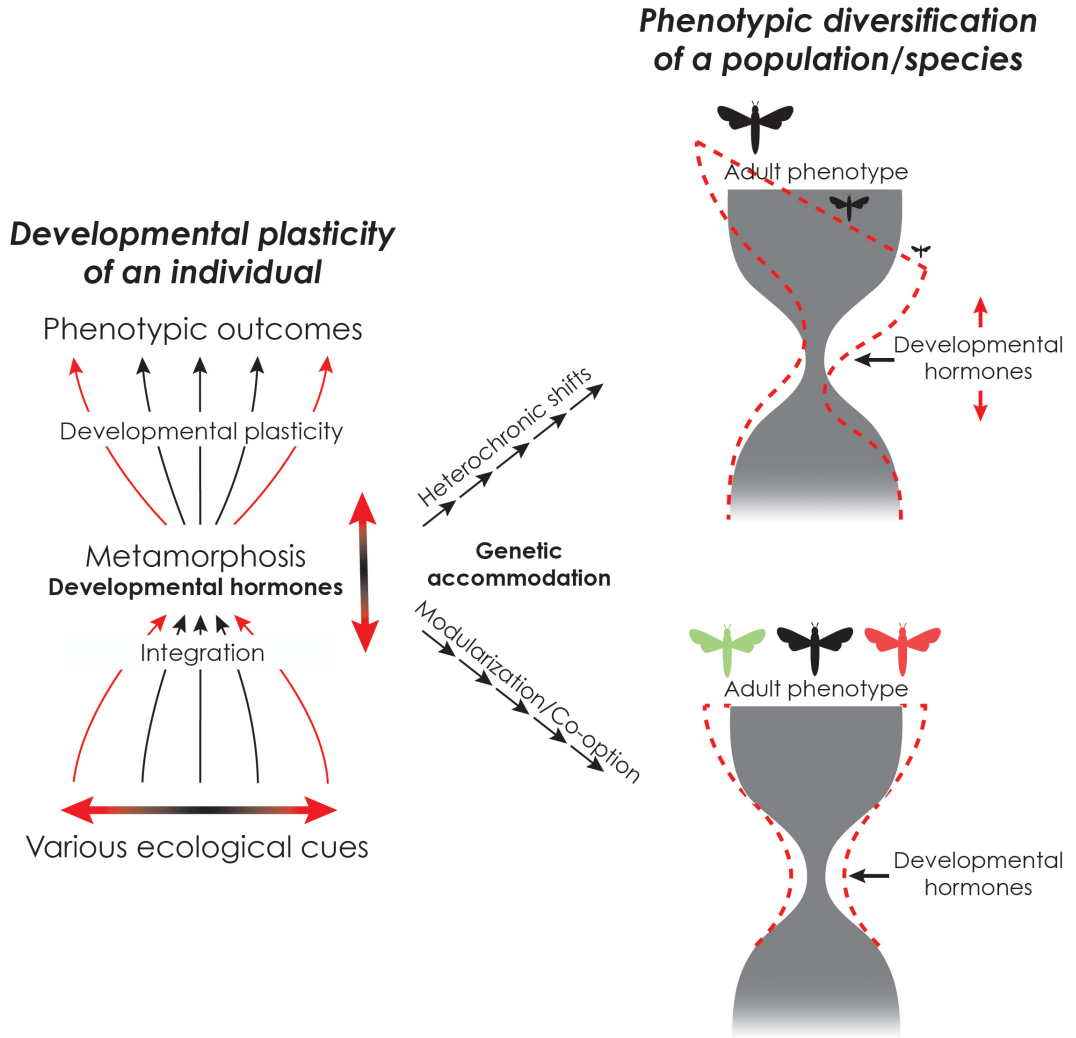


FIGURE 6 | Genetic accommodation *via* endocrine changes leads to changes in body size and morphology.(Left) Developmental plasticity of an individual. Extreme environmental conditions can lead to changes in the timing of metamorphosis or adult morphogenesis through changes in the timing and amount of endocrine action (solid red lines). (Right) Such changes can be selected for and become genetically accommodated in a population over multiple generations, leading to changes in phenotypic diversity (dotted red lines).

metamorphosis (Shen et al., 2014). Thus, environmental stress can impact both JH and ecdysteroid signaling.

Finally, evolution of hormonal systems could also impact insect diapause through genetic accommodation. Emergence of adults is regulated by hormones, and selection for environmentally sensitive alleles of endocrine regulators has been proposed for the evolution of diapause by genetic accommodation (Schiesari and O'Connor, 2013). Moreover, climate change may impact the timing of entry and exit from diapause (Forrest, 2016). Taken together, genetic accommodation of hormonal regulation may play a role in the evolution of the timing of metamorphosis and life history transitions.

Genetic accommodation mediated by physiological homeostasis can change the shape of the developmental goblet in two ways: Either the height can change, or the width of the upper constriction and shape of the "cup" might change (**Figure 7**). For example, parts of the hourglass may lengthen due to changes in diapause, dormancy or the timing of metamorphosis, each of which would increase the height of the hourglass (**Figure 7A**). Alternatively, the width and shape of the upper cup can change by increased modularity and release from hormonal pleiotropy (**Figure 7B**). Of course, different species respond in disparate ways to varying environmental conditions. Thus, the overall effect of genetic accommodation on a group of species will be the total of such changes.

Climate change will impact the length of the growing season, the timing of metamorphosis and the size of adult organisms which can impact fitness (Honěk, 1993; Blanckenhorn, 2000; Blanckenhorn and Demont, 2004; Daufresne et al., 2009; Sheridan and Bickford, 2011). Although these changes certainly have many proximate causes (Atkinson, 1994; Atkinson and Sibly, 1997; Verberk et al., 2021), how a species adapts in response

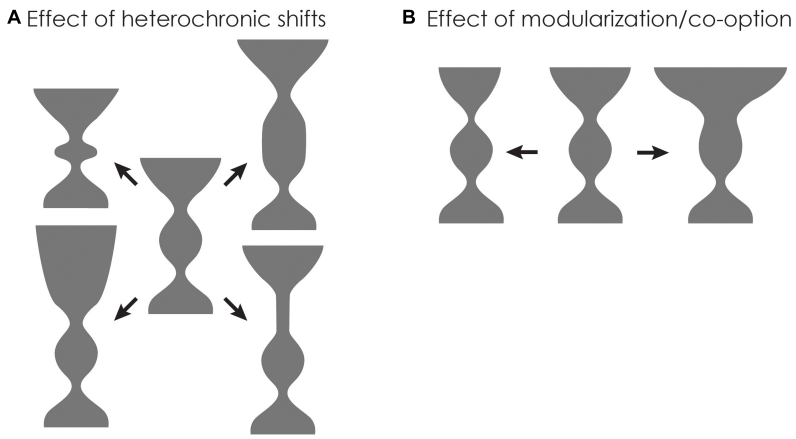


FIGURE 7 | Changes in developmental endocrinology in response to climate change can lead to changes in body size and metabolism/morphology. **(A)** Potential effects of heterochronic shifts on the shape of the developmental goblet. Changes in endocrine system can lead to alteration in duration of the larval or metamorphic stages. **(B)** Potential effects of modularization/co-option on the shape of the developmental goblet. Changes in endocrine system can lead to alteration in phenotypic diversity of the adult stages.

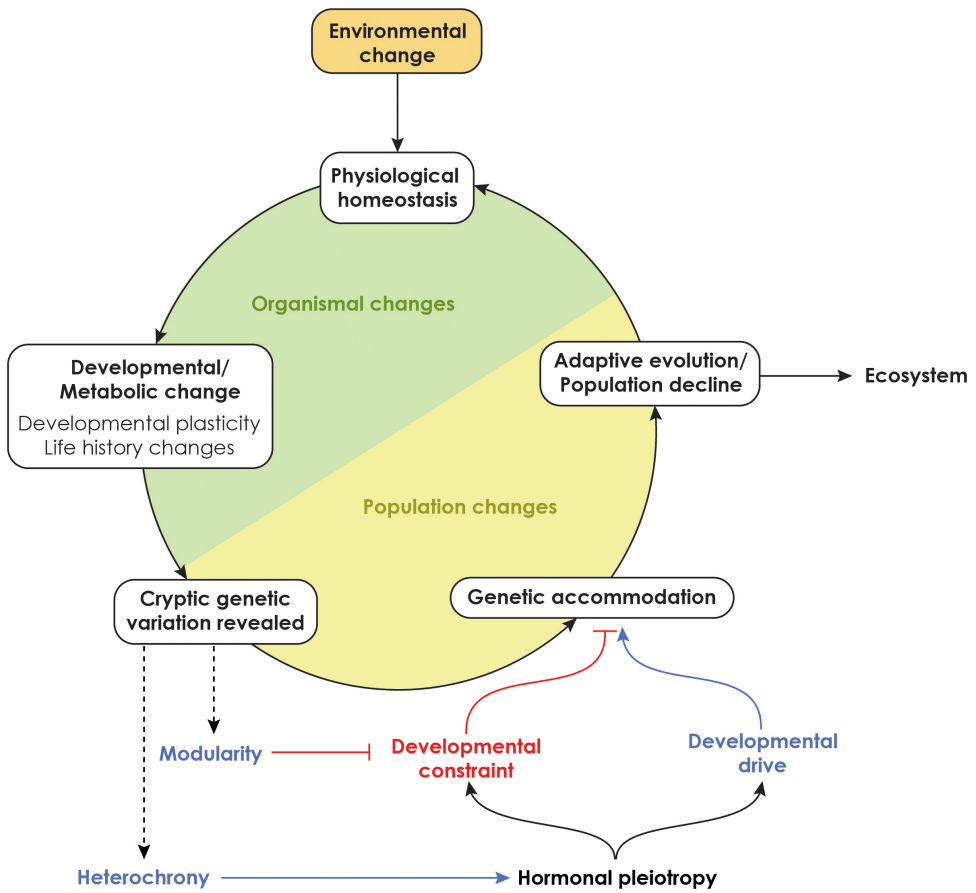


FIGURE 8 | Impact of climate change on developmental hormones and their ultimate impacts on populations and the ecosystem. Climate change can impact developmental physiology of organisms that can influence their development and life history. Cryptic genetic variation that is revealed as a consequence of climate change can fuel genetic accommodation of traits. The degree to which members of a population can adjust their development and physiology determines whether a population thrives or declines.

to climate change may also depend on the developmental system as well as the amount and nature of cryptic genetic variation: stabilization of the newly induced phenotypes can lead to genetic assimilation (or fixation) of the novel phenotypes (**Box 1**); selection on the novel phenotypes could lead to increased phenotypic plasticity; or selection in the altered environment could lead to compensatory genetic changes that restores the original phenotype (i.e., genetic compensation) (Grether, 2005). Because so many traits are regulated by hormones, changes in hormonal response requires uncoupling of tissues and subsequent evolution of appropriate tissues specific adaptations—modularization of adaptation. Whether such changes can happen fast enough to keep up with the rapid pace of climate change remains unclear. Therefore, metamorphic organisms may not be able to evolve in all directions depicted in **Figure 7**. Instead, certain directions of change may occur more rapidly than others.

CONCLUSION

Climate change in the Anthropocene has dramatically accelerated extinction rates (Waters et al., 2016). However, how evo-devo intersects with climate change remains poorly studied (Campbell et al., 2017; Gilbert, 2021). Recent studies have begun to identify alleles that are involved in organismal response to climate change (Franks and Hoffmann, 2012; Merila and Hendry, 2014), but the mechanistic basis of evolution of organisms in response to climate change is still lacking (Chmura et al., 2019). In metamorphic organism, hormones play critical roles in life history transitions. Because of the multitude of roles they play, hormones can bias the way organisms develop and evolve, leading to changes in the shapes of the developmental goblet. We propose that developmental homeostasis may be a contributor for adaptive evolution especially in a changing climate (**Figure 8**). As organisms face climate change, changes in homeostatic mechanisms may allow rapid adaptive responses in metabolism that are followed by phenological and morphological changes that alter the shape of the developmental goblet. In particular, physiological homeostasis can allow the expression of hidden genetic variation that can promote adaptive evolution. The amount of hidden genetic variation present in a population for such adaptive changes may then be a determinant of whether a population thrives or collapses.

AUTHOR CONTRIBUTIONS

YS and LT wrote sections of the manuscript. Both authors contributed to the manuscript revision, read, and approved the submitted version.

FUNDING

This work was funded by the National Science Foundation grant IOS-2002354 and funding from Wellesley College. The publication fees for this article were supported by the Wellesley College Library and Technology Services Open Access Fund.

ACKNOWLEDGMENTS

We would like to thank Drs. Julia Bowsher, Marianne Moore, and Nick Rodenhouse, and members of the Suzuki lab for their constructive feedback on earlier drafts of this work. We would also like to thank the reviewers of this work who have provided helpful and constructive criticisms that helped to improve this work.

REFERENCES

Agrawal, N., Delanoue, R., Mauri, A., Basco, D., Pasco, M., Thorens, B., et al. (2016). The *Drosophila* TNF eiger is an adipokine that acts on insulin-producing cells to mediate nutrient response. *Cell Metab.* 23, 675–684. doi: 10.1016/j.cmet.2016.03.003

Alinezhad, S., Abdollahpour, H., Jafari, N., and Falahatkar, B. (2020). Effects of thyroxine immersion on Sterlet sturgeon (*Acipenser ruthenus*) embryos and larvae: variations in thyroid hormone levels during development. *Aquaculture* 519:734745. doi: 10.1016/j.aquaculture.2019.734745

Allen, B. M. (1916). The results of the extirpation of the anterior lobe of the hypophysis and of the thyroid of *Rana pipiens* larvae. *Science* 44, 755–758. doi: 10.1126/science.44.1143.755

Álvarez, D., and Nicieza, A. G. (2002). Effects of temperature and food quality on anuran larval growth and metamorphosis. *Funct. Ecol.* 16, 640–648. doi: 10.1046/j.1365-2435.2002.00658.x

Alves, R. N., Gomes, A. S., Stueber, K., Tine, M., Thorne, M. A., Smaradottir, H., et al. (2016). The transcriptome of metamorphosing flatfish. *BMC Genomics* 17:413. doi: 10.1186/s12864-016-2699-x

Arbeitman, M. N., Furlong, E. E., Imam, F., Johnson, E., Null, B. H., Baker, B. S., et al. (2002). Gene expression during the life cycle of *Drosophila melanogaster*. *Science* 297, 2270–2275. doi: 10.1126/science.1072152

Arthur, W. (2001). Developmental drive: an important determinant of the direction of phenotypic evolution. *Evol. Dev.* 3, 271–278. doi: 10.1046/j.1525-142x.2001.003004271.x

Atkinson, D. (1994). Temperature and organism size – a biological law for ectotherms? *Adv. Ecol. Res.* 25, 1–58. doi: 10.1016/S0065-2504(08)60212-3

Atkinson, D., and Sibly, R. M. (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends Ecol. Evol.* 12, 235–239. doi: 10.1016/S0169-5347(97)01058-6

Ayson, F. G., and Lam, T. J. (1993). Thyroxine injection of female rabbitfish (*Siganus guttatus*) broodstock: changes in thyroid hormone levels in plasma, eggs, and yolk-sac larvae, and its effect on larval growth and survival. *Aquaculture* 109, 83–93. doi: 10.1016/0044-8486(93)90488-K

Baker, M. E. (2010). Evolution of 11beta-hydroxysteroid dehydrogenase-type 1 and 11beta-hydroxysteroid dehydrogenase-type 3. *FEBS Lett.* 584, 2279–2284. doi: 10.1016/j.febslet.2010.03.036

Belles, X. (2019). The innovation of the final moult and the origin of insect metamorphosis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 374:20180415. doi: 10.1098/rstb.2018.0415

Belles, X. (2020). *Insect Metamorphosis*. Cambridge, MA: Academic Press.

Belles, X., and Santos, C. G. (2014). The MEKRE93 (Methoprene tolerant-Kruppel homolog 1-E93) pathway in the regulation of insect metamorphosis, and the homology of the pupal stage. *Insect Biochem. Mol. Biol.* 52, 60–68. doi: 10.1016/j.ibmb.2014.06.009

Bhardwaj, S., Jolander, L. S., Wenk, M. R., Oliver, J. C., Nijhout, H. F., and Monteiro, A. (2020). Origin of the mechanism of phenotypic plasticity in satyrid butterfly eyespots. *eLife* 9:e49544. doi: 10.7554/eLife.49544

Bilen, J., Atallah, J., Azanchi, R., Levine, J. D., and Riddiford, L. M. (2013). Regulation of onset of female mating and sex pheromone production by juvenile hormone in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 110, 18321–18326. doi: 10.1073/pnas.1318119110

Bishop, C. D., Erezyilmaz, D. F., Flatt, T., Georgiou, C. D., Hadfield, M. G., Heyland, A., et al. (2006). What is metamorphosis? *Integr. Comp. Biol.* 46, 655–661. doi: 10.1093/icb/icl004

Blanckenhorn, W. U. (2000). The evolution of body size: What keeps organisms small? *Q. Rev. Biol.* 75, 385–407. doi: 10.1086/393620

Blanckenhorn, W. U., and Demont, M. (2004). Bergmann and converse bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integr. Comp. Biol.* 44, 413–424. doi: 10.1093/icb/44.6.413

Bonett, R. M., Hoopfer, E. D., and Denver, R. J. (2010). Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. *Gen. Comp. Endocrinol.* 168, 209–219. doi: 10.1016/j.ygcen.2010.03.014

Boorse, G. C., and Denver, R. J. (2003). Endocrine mechanisms underlying plasticity in metamorphic timing in spadefoot toads. *Integr. Comp. Biol.* 43, 646–657. doi: 10.1093/icb/43.5.646

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., and Hafen, E. (2001). An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr. Biol.* 11, 213–221. doi: 10.1016/S0960-9822(01)00068-9

Browder, M. H., D'Amico, L. J., and Nijhout, H. F. (2001). The role of low levels of juvenile hormone esterase in the metamorphosis of *Manduca sexta*. *J. Insect. Sci.* 1:11. doi: 10.1673/031.001.1101

Brown, D. D., and Cai, L. (2007). Amphibian metamorphosis. *Dev. Biol.* 306, 20–33. doi: 10.1016/j.ydbio.2007.03.021

Cai, L., and Brown, D. D. (2004). Expression of type II iodothyronine deiodinase marks the time that a tissue responds to thyroid hormone-induced metamorphosis in *Xenopus laevis*. *Dev. Biol.* 266, 87–95. doi: 10.1016/j.ydbio.2003.10.005

Caldwell, P. E., Walkiewicz, M., and Stern, M. (2005). Ras activity in the *Drosophila* prothoracic gland regulates body size and developmental rate via ecdysone release. *Curr. Biol.* 15, 1785–1795. doi: 10.1016/j.cub.2005.09.011

Callery, E. M., and Elinson, R. P. (2000). Thyroid hormone-dependent metamorphosis in a direct developing frog. *Proc. Natl. Acad. Sci. U.S.A.* 97, 2615–2620. doi: 10.1073/pnas.050501097

Callier, V., and Nijhout, H. F. (2013). Body size determination in insects: a review and synthesis of size- and brain-dependent and independent mechanisms. *Biol. Rev. Camb. Philos. Soc.* 88, 944–954. doi: 10.1111/brv.12033

Cambron, L. D., Yocum, G. D., Yeater, K. M., and Greenlee, K. J. (2021). Overwintering conditions impact insulin pathway gene expression in diapausing *Megachile rotundata*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 256:110937. doi: 10.1016/j.cbpa.2021.110937

Campbell, C. S., Adams, C. E., Bean, C. W., and Parsons, K. J. (2017). Conservation evo-devo: preserving biodiversity by understanding its origins. *Trends Ecol. Evol.* 32, 746–759. doi: 10.1016/j.tree.2017.07.002

Campinho, M. A., Silva, N., Roman-Padilla, J., Ponce, M., Manchado, M., and Power, D. M. (2015). Flatfish metamorphosis: A hypothalamic independent process? *Mol. Cell. Endocrinol.* 404, 16–25. doi: 10.1016/j.mce.2014.12.025

Chafino, S., Lopez-Escardo, D., Benelli, G., Kovac, H., Casacuberta, E., Franch-Marro, X., et al. (2018). Differential expression of the adult specifier E93 in the strepsipteran *Xenos vesparum* Rossi suggests a role in female neoteny. *Sci. Rep.* 8:14176. doi: 10.1038/s41598-018-32611-y

Chafino, S., Urena, E., Casanova, J., Casacuberta, E., Franch-Marro, X., and Martin, D. (2019). Upregulation of E93 gene expression acts as the trigger for metamorphosis independently of the threshold size in the beetle *Tribolium castaneum*. *Cell Rep.* 27, 1039–1049.e2. doi: 10.1016/j.celrep.2019.03.094

Champlin, D. T., and Truman, J. W. (1998a). Ecdysteroid control of cell proliferation during optic lobe neurogenesis in the moth *Manduca sexta*. *Development* 125, 269–277. doi: 10.1242/dev.125.2.269

Champlin, D. T., and Truman, J. W. (1998b). Ecdysteroids govern two phases of eye development during metamorphosis of the moth, *Manduca sexta*. *Development* 125, 2009–2018. doi: 10.1242/dev.125.11.2009

Cherbas, L., Hu, X., Zhimulev, I., Belyaeva, E., and Cherbas, P. (2003). EcR isoforms in *Drosophila*: testing tissue-specific requirements by targeted blockade and rescue. *Development* 130, 271–284. doi: 10.1242/dev.00205

Chesebro, J., Hrycaj, S., Mahfooz, N., and Popadic, A. (2009). Diverging functions of Scr between embryonic and post-embryonic development in a hemimetabolous insect, *Oncopeltus fasciatus*. *Dev. Biol.* 329, 142–151. doi: 10.1016/j.ydbio.2009.01.032

Chino, Y., Saito, M., Yamasu, K., Suyemitsu, T., and Ishihara, K. (1994). Formation of the adult rudiment of sea urchins is influenced by thyroid hormones. *Dev. Biol.* 161, 1–11. doi: 10.1006/dbio.1994.1001

Chippendale, G. M., and Yin, C. M. (1975). Reappraisal of proctodone involvement in the hormonal regulation of larval diapause. *Biol. Bull.* 149, 151–164. doi: 10.2307/1540486

Chmura, H. E., Kharouba, H. M., Ashander, J., Ehlman, S. M., Rivest, E. B., and Yang, L. H. (2019). The mechanisms of phenology: the patterns and processes of phenological shifts. *Ecol. Monogr.* 89:e01337. doi: 10.1002/ecm.1337

Chou, J., Ferris, A. C., Chen, T., Seok, R., Yoon, D., and Suzuki, Y. (2019). Roles of Polycomb group proteins Enhancer of zeste (E(z)) and Polycomb (Pc) during metamorphosis and larval leg regeneration in the flour beetle *Tribolium castaneum*. *Dev. Biol.* 450, 34–46. doi: 10.1016/j.ydbio.2019.03.002

Collet, J., and Fellous, S. (2019). Do traits separated by metamorphosis evolve independently? Concepts and methods. *Proc. R. Soc. B* 286:20190445. doi: 10.1098/rspb.2019.0445

Colombani, J., Andersen, D. S., and Leopold, P. (2012). Secreted peptide Dilp8 coordinates *Drosophila* tissue growth with developmental timing. *Science* 336, 582–585. doi: 10.1126/science.1216689

Colombani, J., Bianchini, L., Layalle, S., Pondeville, E., Dauphin-Villemant, C., Antoniewski, C., et al. (2005). Antagonistic actions of ecdysone and insulins determine final size in *Drosophila*. *Science* 310, 667–670. doi: 10.1126/science.1119432

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., and Leopold, P. (2003). A nutrient sensor mechanism controls *Drosophila* growth. *Cell* 114, 739–749. doi: 10.1016/S0092-8674(03)00713-X

Cymborowski, B., Bogus, M., Beckage, N. E., Williams, C. M., and Riddiford, L. M. (1982). Juvenile hormone titres and metabolism during starvation-induced supernumerary larval moulting of the tobacco hornworm, *Manduca sexta* L. *J. Insect Physiol.* 28, 129–135. doi: 10.1016/0022-1910(82)90120-2

Daufresne, M., Lengfellner, K., and Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12788–12793. doi: 10.1073/pnas.0902080106

Davey, J. C., Becker, K. B., Schneider, M. J., St Germain, D. L., and Galton, V. A. (1995). Cloning of a cDNA for the type II iodothyronine deiodinase. *J. Biol. Chem.* 270, 26786–26789. doi: 10.1074/jbc.270.45.26786

Davidowitz, G., D'Amico, L. J., and Nijhout, H. F. (2003). Critical weight in the development of insect body size. *Evol. Dev.* 5, 188–197. doi: 10.1046/j.1525-142X.2003.03026.x

Davidowitz, G., D'Amico, L. J., and Nijhout, H. F. (2004). The effects of environmental variation on a mechanism that controls insect body size. *Evol. Ecol. Res.* 6, 49–62.

Davis, M. B., Carney, G. E., Robertson, A. E., and Bender, M. (2005). Phenotypic analysis of EcR-A mutants suggests that EcR isoforms have unique functions during *Drosophila* development. *Dev. Biol.* 282, 385–396. doi: 10.1016/j.ydbio.2005.03.019

de Jesus, E. G., Inui, Y., and Hirano, T. (1990). Cortisol enhances the stimulating action of thyroid hormones on dorsal fin-ray resorption of flounder larvae *in vitro*. *Gen. Comp. Endocrinol.* 79, 167–173. doi: 10.1016/0016-6480(90)90101-Q

de Jesus, E. G., Toledo, J. D., and Simpas, M. S. (1998). Thyroid hormones promote early metamorphosis in grouper (*Epinephelus coioides*) larvae. *Gen. Comp. Endocr.* 112, 10–16. doi: 10.1006/gcen.1998.7103

Deal, C. K., and Volkoff, H. (2020). The role of the thyroid axis in fish. *Front. Endocrinol.* 11:596585. doi: 10.3389/fendo.2020.596585

Denver, R. J. (1997). Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. *Horm. Behav.* 31, 169–179. doi: 10.1006/hbeh.1997.1383

Denver, R. J. (1998). Hormonal correlates of environmentally induced metamorphosis in the Western spadefoot toad, *Scaphiopus hammondii*. *Gen. Comp. Endocrinol.* 110, 326–336. doi: 10.1006/gcen.1998.7082

Denver, R. J. (1999). Evolution of the corticotropin-releasing hormone signaling system and its role in stress-induced phenotypic plasticity. *Ann. N. Y. Acad. Sci.* 897, 46–53. doi: 10.1111/j.1749-6632.1999.tb07877.x

Denver, R. J. (2009). Stress hormones mediate environment-genotype interactions during amphibian development. *Gen. Comp. Endocrinol.* 164, 20–31. doi: 10.1016/j.ygcen.2009.04.016

Denver, R. J. (2013). "Chapter seven – neuroendocrinology of amphibian metamorphosis," in *Current Topics in Developmental Biology*, ed. S. Yun-Bo (New York, NY: Academic Press), 195–227. doi: 10.1016/B978-0-12-385979-2.00007-1

Denver, R. J. (2021). Stress hormones mediate developmental plasticity in vertebrates with complex life cycles. *Neurobiol. Stress* 14:100301. doi: 10.1016/j.ynstr.2021.100301

Denver, R. J., Glennemeier, K. A., and Boorse, G. C. (2002). "28 - Endocrinology of complex life cycles: amphibians," in *Hormones, Brain and Behavior*, eds D. W. Pfaff, A. P. Arnold, S. E. Fahrbach, A. M. Etgen, and R. T. Rubin (San Diego, CA: Academic Press), 469. doi: 10.1016/B978-012532104-4/50030-5

Denver, R. J., Mirhadi, N., and Phillips, M. (1998). Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology* 79, 1859–1872. doi: 10.2307/176694

Drost, H. G., Janitza, P., Grosse, I., and Quint, M. (2017). Cross-kingdom comparison of the developmental hourglass. *Curr. Opin. Genet. Dev.* 45, 69–75. doi: 10.1016/j.gde.2017.03.003

Duboule, D. (1994). Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Development 1994 (Supplement)* 135–142. doi: 10.1242/dev.1994.Supplement.135

Dufty, A. M., Clobert, J., and Møller, A. P. (2002). Hormones, developmental plasticity and adaptation. *Trends Ecol. Evol.* 17, 190–196. doi: 10.1016/S0169-5347(02)02498-9

Emlen, D. J. (1997). Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behav. Ecol. Sociobiol.* 41, 335–341. doi: 10.1007/s002650050393

Emlen, D. J., and Nijhout, H. F. (1999). Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *J. Insect Physiol.* 45, 45–53. doi: 10.1016/S0022-1910(98)00096-1

Emlen, D. J., and Nijhout, H. F. (2001). Hormonal control of male horn length dimorphism in *Onthophagus taurus* (Coleoptera: Scarabaeidae): a second critical period of sensitivity to juvenile hormone. *J. Insect Physiol.* 47, 1045–1054. doi: 10.1016/S0022-1910(01)00084-1

Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I., and Lavine, L. C. (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* 337, 860–864. doi: 10.1126/science.1224286

Flatt, T., Tu, M. P., and Tatar, M. (2005). Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. *BioEssays* 27, 999–1010. doi: 10.1002/bies.20290

Forrest, J. R. (2016). Complex responses of insect phenology to climate change. *Curr. Opin. Insect Sci.* 17, 49–54. doi: 10.1016/j.cois.2016.07.002

Franks, S. J., and Hoffmann, A. A. (2012). Genetics of climate change adaptation. *Annu. Rev. Genet.* 46, 185–208. doi: 10.1146/annurev-genet-110711-155511

Freitas, J. S., Kupsco, A., Diamante, G., Felicio, A. A., Almeida, E. A., and Schlenk, D. (2016). Influence of temperature on the thyroidogenic effects of diuron and its metabolite 3,4-DCA in tadpoles of the American bullfrog (*Lithobates catesbeianus*). *Environ. Sci. Technol.* 50, 13095–13104. doi: 10.1021/acs.est.6b04076

Freitas, M. B., Brown, C. T., and Karasov, W. H. (2017). Warmer temperature modifies effects of polybrominated diphenyl ethers on hormone profiles in leopard frog tadpoles (*Lithobates pipiens*). *Environ. Toxicol. Chem.* 36, 120–127. doi: 10.1002/etc.3506

Frieden, E., and Naile, B. (1955). Biochemistry of Amphibian Metamorphosis: I. Enhancement of Induced Metamorphosis by Gluco-Corticoids. *Science* 121, 37–39. doi: 10.1126/science.121.3132.37

Fuchs, B., Wang, W., Graspeuntner, S., Li, Y., Insua, S., Herbst, E. M., et al. (2014). Regulation of polyp-to-jellyfish transition in *Aurelia aurita*. *Curr. Biol.* 24, 263–273. doi: 10.1016/j.cub.2013.12.003

Fukazawa, H., Hirai, H., Hori, H., Roberts, R. D., Nukaya, H., Ishida, H., et al. (2001). Induction of abalone larval metamorphosis by thyroid hormones. *Fish Sci.* 67, 985–988. doi: 10.1046/j.1444-2906.2001.00351.x

Galis, F., and Metz, J. A. (2001). Testing the vulnerability of the phylotypic stage: on modularity and evolutionary conservation. *J. Exp. Zool.* 291, 195–204. doi: 10.1002/jez.1069

Gaur, A., Zajdel, R. W., Bhatia, R., Isitmangil, G., Denz, C. R., Robertson, D. R., et al. (2001). Expression of HoxA5 in the heart is upregulated during thyroxin-induced metamorphosis of the Mexican axolotl (*Ambystoma mexicanum*). *Cardiovasc. Toxicol.* 1, 225–235. doi: 10.1385/CT:1:3:225

Geminard, C., Rulifson, E. J., and Leopold, P. (2009). Remote control of insulin secretion by fat cells in *Drosophila*. *Cell Metab.* 10, 199–207. doi: 10.1016/j.cmet.2009.08.002

Gibson, G., and Dworkin, I. (2004). Uncovering cryptic genetic variation. *Nat. Rev. Genet.* 5, 681–690. doi: 10.1038/nrg1426

Gilbert, S. F. (2021). Evolutionary developmental biology and sustainability: a biology of resilience. *Evol. Dev.* 23:e12366. doi: 10.1111/ede.12366

Gomez-Mestre, I., and Buchholz, D. R. (2006). Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19021–19026. doi: 10.1073/pnas.0603562103

Goodman, W. G., and Granger, N. A. (2005). "The juvenile hormones," in *Comprehensive Molecular Insect Science*, eds I. Lawrence, L. I. Gilbert, K. Iatrou, and S. S. Gill (Amsterdam: Elsevier), 319–408. doi: 10.1016/B0-44-451924-6/00039-9

Gotoh, H., Cornette, R., Koshikawa, S., Okada, Y., Lavine, L. C., Emlen, D. J., et al. (2011). Juvenile hormone regulates extreme mandible growth in male stag beetles. *PLoS One* 6:e21139. doi: 10.1371/journal.pone.0021139

Gotoh, H., Miyakawa, H., Ishikawa, A., Ishikawa, Y., Sugime, Y., Emlen, D. J., et al. (2014). Developmental link between sex and nutrition; doublesex regulates sex-specific mandible growth via juvenile hormone signaling in stag beetles. *PLoS Genet.* 10:e1004098. doi: 10.1371/journal.pgen.1004098

Gray, K. M., and Janssens, P. A. (1990). Gonadal hormones inhibit the induction of metamorphosis by thyroid hormones in *Xenopus laevis* tadpoles *in vivo*, but not *in vitro*. *Gen. Comp. Endocrinol.* 77, 202–211. doi: 10.1016/0016-6480(90)90304-5

Green, D. A. II, and Kronforst, M. R. (2019). Monarch butterflies use an environmentally sensitive, internal timer to control overwintering dynamics. *Mol. Ecol.* 28, 3642–3655. doi: 10.1111/mec.15178

Greene, E. (1989). A diet-induced developmental polymorphism in a caterpillar. *Science* 243, 643–646. doi: 10.1126/science.243.4891.643

Grether, G. F. (2005). Environmental change, phenotypic plasticity, and genetic compensation. *Am. Nat.* 166, E115–E123. doi: 10.1086/432023

Grunert, L. W., Clarke, J. W., Ahuja, C., Eswaran, H., and Nijhout, H. F. (2015). A quantitative analysis of growth and size regulation in *Manduca sexta*: the physiological basis of variation in size and age at metamorphosis. *PLoS One* 10:e0127988. doi: 10.1371/journal.pone.0127988

Gudernatsch, J. F. (1912). Feeding experiments on tadpoles. *Wilhelm Roux' Arch. Entwicklungsmech. Org.* 35, 457–483. doi: 10.1007/BF02277051

Hatem, N. E., Wang, Z., Nave, K. B., Koyama, T., and Suzuki, Y. (2015). The role of juvenile hormone and insulin/TOR signaling in the growth of *Manduca sexta*. *BMC Biol.* 13:44. doi: 10.1186/s12915-015-0155-z

Hayes, T. B. (1997). Hormonal mechanisms as potential constraints on evolution: examples from the Anura. *Am. Zool.* 37, 482–490. doi: 10.1093/icb/37.6.482

He, L. L., Shin, S. H., Wang, Z., Yuan, I., Weschler, R., Chiou, A., et al. (2020). Mechanism of threshold size assessment: metamorphosis is triggered by the TGF-beta/Activin ligand Myoglianin. *Insect Biochem. Mol. Biol.* 126:103452. doi: 10.1016/j.ibmb.2020.103452

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A.-L., and Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecol. Lett.* 12, 184–195. doi: 10.1111/j.1461-0248.2008.01269.x

Helbing, C. C., Werry, K., Crump, D., Domanski, D., Veldhoen, N., and Bailey, C. M. (2003). Expression profiles of novel thyroid hormone-responsive genes and proteins in the tail of *Xenopus laevis* tadpoles undergoing precocious metamorphosis. *Mol. Endocrinol.* 17, 1395–1409. doi: 10.1210/me.2002-0274

Helm, B. R., Rinehart, J. P., Yocum, G. D., Greenlee, K. J., and Bowsher, J. H. (2017). Metamorphosis is induced by food absence rather than a critical weight in the solitary bee, *Osmia lignaria*. *Proc. Natl. Acad. Sci. U.S.A.* 114, 10924–10929. doi: 10.1073/pnas.1703008114

Herboso, L., Oliveira, M. M., Talamillo, A., Pérez, C., González, M., Martín, D., et al. (2015). Ecdysone promotes growth of imaginal discs through the regulation of Thor in *D. melanogaster*. *Sci. Rep.* 5:12383. doi: 10.1038/srep12383

Heyland, A., and Hodin, J. (2004). Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of nonfeeding development. *Evolution* 58, 524–538. doi: 10.1111/j.0014-3820.2004.tb01676.x

Heyland, A., Price, D. A., Bodnarova-Buganova, M., and Moroz, L. L. (2006). Thyroid hormone metabolism and peroxidase function in two non-chordate animals. *J. Exp. Zool. B Mol. Dev. Evol.* 306, 551–566. doi: 10.1002/jez.b.21113

Hirashima, A., Rauschenbach, I., and Sukhanova, M. (2000). Ecdysteroids in stress responsive and nonresponsive *Drosophila virilis* lines under stress conditions. *Biosci. Biotechnol. Biochem.* 64, 2657–2662. doi: 10.1271/bbb.64.2657

Hirashima, A., Takeya, R., Taniguchi, E., and Eto, M. (1995). Metamorphosis, activity of Juvenile-hormone esterase and alteration of ecdysteroid titres; effects of larval density and various stress on the red flour beetle, *Tribolium freemani* Hinton (Coleoptera: Tenebrionidae). *J. Insect Physiol.* 41, 383–388. doi: 10.1016/0022-1910(94)00138-7

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* 66, 483–492. doi: 10.2307/3544943

Hopkins, W. A. (2007). Amphibians as models for studying environmental change. *ILAR J.* 48, 270–277. doi: 10.1093/ilar.48.3.270

Horne, C. R., Hirst, A. G., and Atkinson, D. (2015). Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species. *Ecol. Lett.* 18, 327–335. doi: 10.1111/ele.12413

Høye, T. T., Post, E., Schmidt, N. M., Trøjelsgaard, K., and Forchhammer, M. C. (2013). Shorter flowering seasons and declining abundance of flower visitors in a warmer Arctic. *Nat. Clim. Change* 3, 759–763. doi: 10.1038/nclimate1909

Hrycaj, S., Chesebro, J., and Popadic, A. (2010). Functional analysis of Scr during embryonic and post-embryonic development in the cockroach, *Periplaneta americana*. *Dev. Biol.* 341, 324–334. doi: 10.1016/j.ydbio.2010.02.018

Huang, Z.-Y., Robinson, G. E., Tobe, S. S., Yagi, K. J., Strambi, C., Strambi, A., et al. (1991). Hormonal regulation of behavioural development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. *J. Insect Physiol.* 37, 733–741. doi: 10.1016/0022-1910(91)90107-B

Ikeya, T., Galic, M., Belawat, P., Nairz, K., and Hafen, E. (2002). Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. *Curr. Biol.* 12, 1293–1300. doi: 10.1016/S0960-9822(02)01043-6

Imasheva, A. G., Bubli, O. A., and Lazebny, O. E. (1994). Variation in wing length in Eurasian natural populations of *Drosophila melanogaster*. *Heredity* 72, 508–514. doi: 10.1038/hdy.1994.68

Intergovernmental Panel on Climate Change (2014). *Climate Change 2013 – The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press. doi: 10.1017/CBO9781107415324

Inui, Y., and Miwa, S. (1985). Thyroid hormone induces metamorphosis of flounder larvae. *Gen. Comp. Endocr.* 60, 450–454. doi: 10.1016/0016-6480(85)90080-2

James, A. C., Azevedo, R. B., and Partridge, L. (1997). Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* 146, 881–890. doi: 10.1093/genetics/146.3.881

Jindra, M. (2019). Where did the pupa come from? The timing of juvenile hormone signalling supports homology between stages of hemimetabolous and holometabolous insects. *Philos. Trans. R. Soc. B* 374:20190064. doi: 10.1098/rstb.2019.0064

Jindra, M., Belles, X., and Shinoda, T. (2015). Molecular basis of juvenile hormone signaling. *Curr. Opin. Insect Sci.* 11, 39–46. doi: 10.1016/j.cois.2015.08.004

Jindra, M., Palli, S. R., and Riddiford, L. M. (2013). The juvenile hormone signaling pathway in insect development. *Annu. Rev. Entomol.* 58, 181–204. doi: 10.1146/annurev-ento-120811-153700

Jones, G., Hanzlik, T., Hammock, B. D., Schooley, D. A., Miller, C. A., Tsai, L. W., et al. (1990). The juvenile hormone titre during the penultimate and ultimate larval stadia of *Trichoplusia ni*. *J. Insect Physiol.* 36, 77–83. doi: 10.1016/0022-1910(90)90177-H

Kaneko, Y., and Hiruma, K. (2014). Short neuropeptide F (sNPF) is a stage-specific suppressor for juvenile hormone biosynthesis by corpora allata, and a critical factor for the initiation of insect metamorphosis. *Dev. Biol.* 393, 312–319. doi: 10.1016/j.ydbio.2014.07.014

Kawahara, A., Baker, B. S., and Tata, J. R. (1991). Developmental and regional expression of thyroid hormone receptor genes during *Xenopus* metamorphosis. *Development* 112, 933–943. doi: 10.1242/dev.112.4.933

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philos. Trans. R. Soc. B* 374:20180176. doi: 10.1098/rstb.2018.0176

Ketterson, E. D., and Nolan, V. Jr. (1999). Adaptation, exaptation, and constraint: a hormonal perspective. *Am. Nat.* 154, S4–S25. doi: 10.1086/303280

Ketterson, E. D., Atwell, J. W., and McGlothlin, J. W. (2009). Phenotypic integration and independence: hormones, performance, and response to environmental change. *Integr. Comp. Biol.* 49, 365–379. doi: 10.1093/icb/icp057

Kijimoto, T., Pespeni, M., Beckers, O., and Moczek, A. P. (2013). Beetle horns and horned beetles: emerging models in developmental evolution and ecology. *Wiley Interdiscip. Rev. Dev. Biol.* 2, 405–418. doi: 10.1002/wdev.81

Kim, J., and Neufeld, T. P. (2015). Dietary sugar promotes systemic TOR activation in *Drosophila* through AKH-dependent selective secretion of Dilp3. *Nat. Commun.* 6:6846. doi: 10.1038/ncomms7846

Klok, C. J., and Harrison, J. F. (2013). The temperature size rule in arthropods: independent of macro-environmental variables but size dependent. *Integr. Comp. Biol.* 53, 557–570. doi: 10.1093/icb/ict075

Koyama, T., and Mirth, C. K. (2016). Growth-Blocking Peptides as nutrition-sensitive signals for insulin secretion and body size regulation. *PLoS Biol.* 14:e1002392. doi: 10.1371/journal.pbio.1002392

Koyama, T., Texada, M. J., Halberg, K. A., and Rewitz, K. (2020). Metabolism and growth adaptation to environmental conditions in *Drosophila*. *Cell. Mol. Life Sci.* 77, 4523–4551. doi: 10.1007/s00018-020-03547-2

Krain, L. P., and Denver, R. J. (2004). Developmental expression and hormonal regulation of glucocorticoid and thyroid hormone receptors during metamorphosis in *Xenopus laevis*. *J. Endocrinol.* 181, 91–104. doi: 10.1677/joe.0.1810091

Kudo, G., and Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology* 94, 2311–2320. doi: 10.1890/12-2003.1

Kulkarni, S. S., and Buchholz, D. R. (2014). Corticosteroid signaling in frog metamorphosis. *Gen. Comp. Endocrinol.* 203, 225–231. doi: 10.1016/j.ygcen.2014.03.036

Kulkarni, S. S., Denver, R. J., Gomez-Mestre, I., and Buchholz, D. R. (2017). Genetic accommodation via modified endocrine signalling explains phenotypic divergence among spadefoot toad species. *Nat. Commun.* 8:993. doi: 10.1038/s41467-017-00996-5

Lafuente, E., and Beldade, P. (2019). Genomics of developmental plasticity in animals. *Front. Genet.* 10:720. doi: 10.3389/fgene.2019.00720

Larsen, D. A., Swanson, P., Dickey, J. T., Rivier, J., and Dickhoff, W. W. (1998). *In vitro* thyrotropin–releasing activity of corticotropin–releasing hormone–family peptides in coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 109, 276–285. doi: 10.1006/gcen.1997.7031

Ledon-Rettig, C. C., Pfennig, D. W., and Crespi, E. J. (2009). Stress hormones and the fitness consequences associated with the transition to a novel diet in larval amphibians. *J. Exp. Biol.* 212, 3743–3750. doi: 10.1242/jeb.034066

Lee, A. K., Sze, C. C., Kim, E. R., and Suzuki, Y. (2013). Developmental coupling of larval and adult stages in a complex life cycle: insights from limb regeneration in the flour beetle, *Tribolium castaneum*. *EvoDevo* 4:20. doi: 10.1186/2041-9139-4-20

Leips, J., and Travis, J. (1994). Metamorphic responses to changing food levels in two species of hylid frogs. *Ecology* 75, 1345–1356. doi: 10.2307/1937459

Lema, S. C. (2020). Hormones, developmental plasticity, and adaptive evolution: endocrine flexibility as a catalyst for 'plasticity-first' phenotypic divergence. *Mol. Cell. Endocrinol.* 502:110678. doi: 10.1016/j.mce.2019.110678

Lema, S. C., and Kitano, J. (2013). Hormones and phenotypic plasticity: implications for the evolution of integrated adaptive phenotypes. *Curr. Zool.* 59, 506–525. doi: 10.1093/czoolo/59.4.506

Levis, N. A., and Pfennig, D. W. (2019). Phenotypic plasticity, canalization, and the origins of novelty: evidence and mechanisms from amphibians. *Semin. Cell Dev. Biol.* 88, 80–90. doi: 10.1016/j.semcdb.2018.01.012

Li, T. R., and White, K. P. (2003). Tissue-specific gene expression and ecdysone-regulated genomic networks in *Drosophila*. *Dev. Cell* 5, 59–72. doi: 10.1016/S1534-5807(03)00192-8

Liu, Y., Sheng, Z., Liu, H., Wen, D., He, Q., Wang, S., et al. (2009). Juvenile hormone counteracts the bHLH-PAS transcription factors MET and GCE to prevent caspase-dependent programmed cell death in *Drosophila*. *Development* 136, 2015–2025. doi: 10.1242/dev.033712

Lombardo, A., and Slack, J. M. (2001). Abdominal B-type Hox gene expression in *Xenopus laevis*. *Mech. Dev.* 106, 191–195. doi: 10.1016/S0925-4773(01)00438-5

Lorenz, C., Opitz, R., Lutz, I., and Kloas, W. (2009). Corticosteroids disrupt amphibian metamorphosis by complex modes of action including increased prolactin expression. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 150, 314–321. doi: 10.1016/j.cbpc.2009.05.013

Losey, J. E., and Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience* 56, 311–323. doi: 10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2

Manzon, R. G., and Denver, R. J. (2004). Regulation of pituitary thyrotropin gene expression during *Xenopus* metamorphosis: negative feedback is functional throughout metamorphosis. *J. Endocrinol.* 182, 273–285. doi: 10.1677/joe.0.1820273

Masumura, M., Satake, S. I., Saegusa, H., and Mizoguchi, A. (2000). Glucose stimulates the release of bombyxin, an insulin-related peptide of the silkworm *Bombyx mori*. *Gen. Comp. Endocrinol.* 118, 393–399. doi: 10.1006/gcen.1999.7438

McGlothlin, J. W., and Ketterson, E. D. (2008). Hormone-mediated suites as adaptations and evolutionary constraints. *Philos. Trans. R. Soc. B* 363, 1611–1620. doi: 10.1098/rstb.2007.0002

Merila, J., and Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* 7, 1–14. doi: 10.1111/eva.12137

Miki, T., Shinohara, T., Chafino, S., Noji, S., and Tomioka, K. (2020). Photoperiod and temperature separately regulate nymphal development through JH and insulin/TOR signaling pathways in an insect. *Proc. Natl. Acad. Sci. U.S.A.* 117, 5525–5531. doi: 10.1073/pnas.1922747117

Mirth, C. K., Tang, H. Y., Makohon-Moore, S. C., Salhadar, S., Gokhale, R. H., Warner, R. D., et al. (2014). Juvenile hormone regulates body size and perturbs insulin signaling in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7018–7023. doi: 10.1073/pnas.1313058111

Mirth, C. K., Truman, J. W., and Riddiford, L. M. (2009). The ecdysone receptor controls the post-critical weight switch to nutrition-independent differentiation in *Drosophila* wing imaginal discs. *Development* 136, 2345–2353. doi: 10.1242/dev.032672

Mirth, C., Truman, J. W., and Riddiford, L. M. (2005). The role of the prothoracic gland in determining critical weight to metamorphosis in *Drosophila melanogaster*. *Curr. Biol.* 15, 1796–1807. doi: 10.1016/j.cub.2005.09.017

Monier, B., Astier, M., Semeriva, M., and Perrin, L. (2005). Steroid-dependent modification of Hox function drives myocyte reprogramming in the *Drosophila* heart. *Development* 132, 5283–5293. doi: 10.1242/dev.02091

Monteiro, A., Tong, X., Bear, A., Liew, S. F., Bhardwaj, S., Wasik, B. R., et al. (2015). Differential expression of ecdysone receptor leads to variation in phenotypic plasticity across serial homologs. *PLoS Genet.* 11:e1005529. doi: 10.1371/journal.pgen.1005529

Moore, M. P., and Martin, R. A. (2019). On the evolution of carry-over effects. *J. Anim. Ecol.* 88, 1832–1844. doi: 10.1111/1365-2656.13081

Moran, N. A. (1994). Adaptation and constraint in the complex life-cycles of animals. *Annu. Rev. Ecol. Evol. Syst.* 25, 573–600. doi: 10.1146/annurev.es.25.110194.003041

Mou, X., Duncan, D. M., Baehrecke, E. H., and Duncan, I. (2012). Control of target gene specificity during metamorphosis by the steroid response gene E93. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2949–2954. doi: 10.1073/pnas.1117559109

Nagamine, K., Ishikawa, Y., and Hoshizaki, S. (2016). Insights into how longicorn beetle larvae determine the timing of metamorphosis: starvation-induced mechanism revisited. *PLoS One* 11:e0158831. doi: 10.1371/journal.pone.0158831

Nässel, D. R., and Broeck, J. V. (2016). Insulin/IGF signaling in *Drosophila* and other insects: factors that regulate production, release and post-release action of the insulin-like peptides. *Cell. Mol. Life Sci.* 73, 271–290. doi: 10.1007/s00018-015-2063-3

Nicieza, A. G. (2000). Interacting effects of predation risk and food availability on larval anuran behaviour and development. *Oecologia* 123, 497–505. doi: 10.1007/s004420000343

Nicolson, S., Denton, D., and Kumar, S. (2015). Ecdysone-mediated programmed cell death in *Drosophila*. *Int. J. Dev. Biol.* 59, 23–32. doi: 10.1387/ijdb.150055sk

Nijhout, H. F. (1975). Threshold size for metamorphosis in tobacco hornworm, *Manduca-sexta* (L). *Biol. Bull.* 149, 214–225. doi: 10.2307/1540491

Nijhout, H. F. (1998). *Insect Hormones*. Princeton, NJ: Princeton University Press.

Nijhout, H. F. (1999). Control mechanisms of polyphenic development in insects. *BioScience* 49, 181–192. doi: 10.2307/1313508

Nijhout, H. F. (2003). Development and evolution of adaptive polyphenisms. *Evol. Dev.* 5, 9–18. doi: 10.1046/j.1525-142X.2003.03003.x

Nijhout, H. F. (2015). Big or fast: two strategies in the developmental control of body size. *BMC Biol.* 13:57. doi: 10.1186/s12915-015-0173-x

Nijhout, H. F., and Grunert, L. W. (2002). Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. *Proc. Natl. Acad. Sci. U.S.A.* 99, 15446–15450. doi: 10.1073/pnas.242548399

Nijhout, H. F., and Williams, C. M. (1974). Control of molting and metamorphosis in tobacco hornworm, *Manduca-sexta* (L) - Cessation of juvenile-hormone secretion as a trigger for pupation. *J. Exp. Biol.* 61, 493–501. doi: 10.1242/jeb.61.2.493

Nijhout, H. F., Kudla, A. M., and Hazelwood, C. C. (2021). Genetic assimilation and accommodation: models and mechanisms. *Curr. Top. Dev. Biol.* 141, 337–369. doi: 10.1016/bs.ctdb.2020.11.006

Nijhout, H. F., Riddiford, L. M., Mirth, C., Shingleton, A. W., Suzuki, Y., and Callier, V. (2014). The developmental control of size in insects. *Wiley Interdiscip. Rev. Dev. Biol.* 3, 113–134. doi: 10.1002/wdev.124

Nijhout, H. F., Smith, W. A., Schachar, I., Subramanian, S., Tobler, A., and Grunert, L. W. (2007). The control of growth and differentiation of the wing imaginal disks of *Manduca sexta*. *Dev. Biol.* 302, 569–576. doi: 10.1016/j.ydbio.2006.10.023

Niki, K., Kikuyama, S., and Yoshizato, K. (1981). Augmentation of nuclear binding capacity for triiodothyronine by aldosterone in tadpole tail. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 57, 271–275. doi: 10.2183/pjab.57.271

Ohhara, Y., Shimada-Niwa, Y., Niwa, R., Kayashima, Y., Hayashi, Y., Akagi, K., et al. (2015). Autocrine regulation of ecdysone synthesis by beta3-octopamine receptor in the prothoracic gland is essential for *Drosophila* metamorphosis. *Proc. Natl. Acad. Sci. U.S.A.* 112, 1452–1457. doi: 10.1073/pnas.1414966112

Park, S., Alfa, R. W., Topper, S. M., Kim, G. E., Kockel, L., and Kim, S. K. (2014). A genetic strategy to measure circulating *Drosophila* insulin reveals genes regulating insulin production and secretion. *PLoS Genet.* 10:e1004555. doi: 10.1371/journal.pgen.1004555

Partridge, L., Barrie, B., Fowler, K., and French, V. (1994). Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* 48, 1269–1276. doi: 10.1111/j.1558-5646.1994.tb05311.x

Patricolo, E., Cammarata, M., and D'Agati, P. (2001). Presence of thyroid hormones in ascidian larvae and their involvement in metamorphosis. *J. Exp. Zool.* 290, 426–430. doi: 10.1002/jez.1084

Patricolo, E., Ortolani, G., and Cascio, A. (1981). The effect of l-thyroxine on the metamorphosis of *Ascidia malaca*. *Cell Tissue Res.* 214, 289–301. doi: 10.1007/BF00249213

Rabah, S. A., Gowan, I. L., Pagnin, M., Osman, N., and Richardson, S. J. (2019). Thyroid hormone distributor proteins during development in vertebrates. *Front. Endocrinol.* 10:506. doi: 10.3389/fendo.2019.00506

Raff, R. A. (1996). *The Shape of Life: Genes, Development and the Evolution of Animal Form*. Chicago, IL: University of Chicago Press. doi: 10.7208/chicago/9780226256573.001.0001

Rauschenbach, I. Y., Lukashina, N. S., Maksimovsky, L. F., and Korochkin, L. I. (1987). Stress-like reaction of *Drosophila* to adverse environmental factors. *J. Comp. Physiol. B* 157, 519–531. doi: 10.1007/BF00691837

Reed, T. E., Schindler, D. E., and Waples, R. S. (2011). Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv. Biol.* 25, 56–63. doi: 10.1111/j.1523-1739.2010.01552.x

Riddiford, L. M. (1996). Juvenile hormone: the status of its "status quo" action. *Arch. Insect. Biochem. Physiol.* 32, 271–286. doi: 10.1002/(SICI)1520-6327(1996)32:3/4<271::AID-ARCH2>3.0.CO;2-W

Riddiford, L. M., Cherbas, P., and Truman, J. W. (2000). Ecdysone receptors and their biological actions. *Vitam. Horm.* 60, 1–73. doi: 10.1016/S0083-6729(00)60016-X

Rodrigues, Y. K., and Beldade, P. (2020). Thermal plasticity in insects' response to climate change and to multifactorial environments. *Front. Ecol. Evol.* 8:271. doi: 10.3389/fevo.2020.00271

Roff, D. (1980). Optimizing development time in a seasonal environment—the ups and downs of clinal variation. *Oecologia* 45, 202–208. doi: 10.1007/BF00346461

Rosenkilde, P., and Ussing, A. P. (1996). What mechanisms control neoteny and regulate induced metamorphosis in urodeles? *Int. J. Dev. Biol.* 40, 665–673.

Rountree, D. B., and Bollenbacher, W. E. (1986). The release of the prothoracicotropic hormone in the tobacco hornworm, *Manduca sexta*, is controlled intrinsically by juvenile hormone. *J. Exp. Biol.* 120, 41–58. doi: 10.1242/jeb.120.1.41

Rountree, D. B., and Nijhout, H. F. (1995). Hormonal control of a seasonal polyphenism in *Precis coenia* (Lepidoptera: Nymphalidae). *J. Insect Physiol.* 41, 987–992. doi: 10.1016/0022-1910(95)00046-W

Sano, H., Nakamura, A., Texada, M. J., Truman, J. W., Ishimoto, H., Kamikouchi, A., et al. (2015). The nutrient-responsive hormone CCHamide-2 controls growth by regulating insulin-like peptides in the brain of *Drosophila melanogaster*. *PLoS Genet.* 11:e1005209. doi: 10.1371/journal.pgen.1005209

Santos, C. G., Humann, F. C., and Hartfelder, K. (2019). Juvenile hormone signaling in insect oogenesis. *Curr. Opin. Insect Sci.* 31, 43–48. doi: 10.1016/j.cois.2018.07.010

Sap, J., Muñoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., et al. (1986). The c-erb-A protein is a high-affinity receptor for thyroid hormone. *Nature* 324, 635–640. doi: 10.1038/324635a0

Schiesari, L., and O'Connor, M. B. (2013). "Chapter Eight - diapause: delaying the developmental clock in response to a changing environment," in *Current Topics in Developmental Biology*, eds A. E. Rougvie and M. B. O'Connor (Cambridge, MA: Academic Press), 213–246. doi: 10.1016/B978-0-12-396968-2.00008-7

Sharma, P., and Patino, R. (2013). Regulation of gonadal sex ratios and pubertal development by the thyroid endocrine system in zebrafish (*Danio rerio*). *Gen. Comp. Endocrinol.* 184, 111–119. doi: 10.1016/j.ygcen.2012.12.018

Shen, Y., Gong, Y.-J., Gu, J., Huang, L.-H., and Feng, Q.-L. (2014). Physiological effect of mild thermal stress and its induction of gene expression in the common cutworm, *Spodoptera litura*. *J. Insect Physiol.* 61, 34–41. doi: 10.1016/j.jinsphys.2013.12.007

Sheridan, J. A., and Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nat. Clim. Change* 1, 401–406. doi: 10.1038/nclimate1259

Shi, Y. B., Wong, J., Puzianowska-Kuznicka, M., and Stolow, M. A. (1996). Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. *BioEssays* 18, 391–399. doi: 10.1002/bies.950180509

Shi, Y.-B. (2000). *Amphibian Metamorphosis: From Morphology to Molecular Biology*. New York, NY: John Wiley & Sons.

Shingleton, A. W., Das, J., Vinicius, L., and Stern, D. L. (2005). The temporal requirements for insulin signaling during development in *Drosophila*. *PLoS Biol.* 3:e289. doi: 10.1371/journal.pbio.0030289

Shkil, F. N., and Smirnov, S. V. (2016). Experimental approach to the hypotheses of heterochronic evolution in lower vertebrates. *Paleontol. J.* 49, 1624–1634. doi: 10.1134/S0031030115140178

Shkil, F. N., Kapitanova, D. V., Borisov, V. B., Abdissa, B., and Smirnov, S. V. (2012). Thyroid hormone in skeletal development of cyprinids: effects and morphological consequences: thyroid hormone in skeletal development. *J. Appl. Ichthyol.* 28, 398–405. doi: 10.1111/j.1439-0426.2012.01992.x

Sim, C., and Denlinger, D. L. (2008). Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6777–6781. doi: 10.1073/pnas.0802067105

Sim, C., and Denlinger, D. L. (2013). Insulin signaling and the regulation of insect diapause. *Front. Physiol.* 4:189. doi: 10.3389/fphys.2013.00189

Smirnov, S. V., Kapitanova, D. V., Borisov, V. B., Abdissa, B., and Shkil, F. N. (2012). Lake Tana large barbs diversity: developmental and hormonal bases. *J. Ichthyol.* 52, 861–880. doi: 10.1134/S0032945212110082

Smith, J. M., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., et al. (1985). Developmental constraints and evolution: a perspective from the Mountain Lake Conference on development and evolution. *Q. Rev. Biol.* 60, 265–287. doi: 10.1086/414425

Smith-Gill, S. J., and Berven, K. A. (1979). Predicting amphibian metamorphosis. *Am. Nat.* 113, 563–585. doi: 10.1086/283413

Sullivan, J. P., Fahrbach, S. E., and Robinson, G. E. (2000). Juvenile hormone paces behavioral development in the adult worker honey bee. *Horm. Behav.* 37, 1–14. doi: 10.1006/hbeh.1999.1552

Suzuki, M. R., and Kikuyama, S. (1983). Corticoids augment nuclear binding capacity for triiodothyronine in bullfrog tadpole tail fins. *Gen. Comp. Endocrinol.* 52, 272–278. doi: 10.1016/0016-6480(83)90122-3

Suzuki, Y., and Nijhout, H. F. (2006). Evolution of a polyphenism by genetic accommodation. *Science* 311, 650–652. doi: 10.1126/science.1118888

Suzuki, Y., and Nijhout, H. F. (2008). Genetic basis of adaptive evolution of a polyphenism by genetic accommodation. *J. Evol. Biol.* 21, 57–66. doi: 10.1111/j.1420-9101.2007.01464.x

Suzuki, Y., McKenna, K. Z., and Nijhout, H. F. (2020). "Regulation of phenotypic plasticity from the perspective of evolutionary developmental biology," in *Phenotypic Switching*, eds H. Levine, M. K. Jolly, P. Kulkarni, and V. Nanjundiah (Cambridge, MA: Academic Press), 403–442. doi: 10.1016/B978-0-12-817996-3.00012-8

Tan, A., and Palli, S. R. (2008). Ecdysone receptor isoforms play distinct roles in controlling molting and metamorphosis in the red flour beetle, *Tribolium castaneum*. *Mol. Cell. Endocrinol.* 291, 42–49. doi: 10.1016/j.mce.2008.05.006

Tang, H. Y., Smith-Caldas, M. S., Driscoll, M. V., Salhadar, S., and Shingleton, A. W. (2011). FOXO regulates organ-specific phenotypic plasticity in *Drosophila*. *PLoS Genet.* 7:e1002373. doi: 10.1371/journal.pgen.1002373

Tauchman, S. J., Lorch, J. M., Orth, A. P., and Goodman, W. G. (2007). Effects of stress on the hemolymph juvenile hormone binding protein titers of *Manduca sexta*. *Insect Biochem. Mol. Biol.* 37, 847–854. doi: 10.1016/j.ibmb.2007.05.015

Taylor, E., and Heyland, A. (2017). Evolution of thyroid hormone signaling in animals: non-genomic and genomic modes of action. *Mol. Cell. Endocrinol.* 459, 14–20. doi: 10.1016/j.mce.2017.05.019

Tomoyasu, Y., Wheeler, S. R., and Denell, R. E. (2005). Ultrabithorax is required for membranous wing identity in the beetle *Tribolium castaneum*. *Nature* 433, 643–647. doi: 10.1038/nature03272

Trenberth, K. E. (2011). Changes in precipitation with climate change. *Clim. Res.* 47, 123–138. doi: 10.3354/cr00953

True, J. R., and Carroll, S. B. (2002). Gene co-option in physiological and morphological evolution. *Annu. Rev. Cell Dev. Biol.* 18, 53–80. doi: 10.1146/annurev.cellbio.18.020402.140619

Truman, J. W. (2019). The evolution of insect metamorphosis. *Curr. Biol.* 29, R1252–R1268. doi: 10.1016/j.cub.2019.10.009

Truman, J. W., and Riddiford, L. M. (2002). Endocrine insights into the evolution of metamorphosis in insects. *Annu. Rev. Entomol.* 47, 467–500. doi: 10.1146/annurev.ento.47.091201.145230

Truman, J. W., and Riddiford, L. M. (2007). The morphostatic actions of juvenile hormone. *Insect Biochem. Mol.* 37, 761–770. doi: 10.1016/j.ibmb.2007.05.011

Truman, J. W., and Riddiford, L. M. (2019). The evolution of insect metamorphosis: a developmental and endocrine view. *Philos. Trans. R. Soc. B* 374:20190070. doi: 10.1098/rstb.2019.0070

Turnock, W. J., Bodnaryk, R. P., and Abramson, D. (1986). Effect of temperature on the rate of pupal-adult development of the noctuid moth, *Mamestra configurata* Wlk.: evidence for differential effects on the initiation of development and subsequent metamorphic development. *Oecologia* 68, 422–427. doi: 10.1007/BF01036750

Vea, I. M., Tanaka, S., Tsuji, T., Shiotsuki, T., Jouraku, A., and Minakuchi, C. (2019). E93 expression and links to the juvenile hormone in hemipteran mealybugs with insights on female neoteny. *Insect Biochem. Mol. Biol.* 104, 65–72. doi: 10.1016/j.ibmb.2018.11.008

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., and Siepel, H. (2021). Shrinking body sizes in response to warming: explanations for the temperature-size rule with special emphasis on the role of oxygen. *Biol. Rev.* 96, 247–268. doi: 10.1111/brv.12653

Wada, H. (2008). Glucocorticoids: mediators of vertebrate ontogenetic transitions. *Gen. Comp. Endocrinol.* 156, 441–453. doi: 10.1016/j.ygcen.2008.02.004

Wang, S., Liu, L., Liu, J., Zhu, W., Tanizaki, Y., Fu, L., et al. (2019). Gene expression program underlying tail resorption during thyroid hormone-dependent metamorphosis of the ornamented pygmy frog *Microhyla fissipes*. *Front. Endocrinol.* 10:11. doi: 10.3389/fendo.2019.00011

Waters, C. N., Zalasiewicz, J., Summerhayes, C., Barnosky, A. D., Poirier, C., Gałuszka, A., et al. (2016). The Anthropocene is functionally and stratigraphically distinct from the Holocene. *Science* 351:aad2622. doi: 10.1126/science.aad2622

Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. I., and Evans, R. M. (1986). The c-erb-A gene encodes a thyroid hormone receptor. *Nature* 324, 641–646. doi: 10.1038/324641a0

Werner, E. E. (1988). "Size, scaling, and the evolution of complex life cycles," in *Size-Structured Populations*, eds B. Ebenman and L. Persson (Berlin: Springer), 60–81. doi: 10.1007/978-3-642-74001-5_6

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. New York, NY: Oxford University Press. doi: 10.1093/oso/9780195122343.001.0001

White, K. P., Rifkin, S. A., Hurban, P., and Hogness, D. S. (1999). Microarray analysis of *Drosophila* development during metamorphosis. *Science* 286, 2179–2184. doi: 10.1126/science.286.5447.2179

Wolpert, L. (1999). From egg to adult to larva. *Evol. Dev.* 1, 3–4. doi: 10.1046/j.1525-142x.1999.00111.x

Wong, J., and Shi, Y.-B. (1995). Coordinated regulation of and transcriptional activation by *Xenopus* thyroid hormone and retinoid X receptors. *J. Biol. Chem.* 270, 18479–18483. doi: 10.1074/jbc.270.31.18479

Wright, M. L., Proctor, K. L., and Alves, C. D. (1999). Hormonal profiles correlated with season, cold, and starvation in *Rana catesbeiana* (bullfrog) tadpoles. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 124, 109–116. doi: 10.1016/S0742-8413(99)00060-2

Xu, J., Sheng, Z., and Palli, S. R. (2013). Juvenile hormone and insulin regulate trehalose homeostasis in the red flour beetle, *Tribolium castaneum*. *PLoS Genet.* 9:e1003535. doi: 10.1371/journal.pgen.1003535

Xu, L. C., Nunes, C., Wang, V. R., Saito, A., Chen, T., Basak, P., et al. (2020). Distinct nutritional and endocrine regulation of prothoracic gland activities underlies divergent life history strategies in *Manduca sexta* and *Drosophila melanogaster*. *Insect Biochem. Mol. Biol.* 119:103335. doi: 10.1016/j.ibmb.2020.103335

Yamamoto, R., Bai, H., Dolezal, A. G., Amdam, G., and Tatar, M. (2013). Juvenile hormone regulation of *Drosophila* aging. *BMC Biol.* 11:85. doi: 10.1186/1741-7007-11-85

Yamanaka, N., Rewitz, K. F., and O'Connor, M. B. (2013). Ecdysone control of developmental transitions: lessons from *Drosophila* research. *Annu. Rev. Entomol.* 58, 497–516. doi: 10.1146/annurev-ento-120811-153608

Yang, A. S. (2001). Modularity, evolvability, and adaptive radiations: a comparison of the hemi- and holometabolous insects. *Evol. Dev.* 3, 59–72. doi: 10.1046/j.1525-142x.2001.003002059.x

Yaoita, Y., and Brown, D. D. (1990). A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. *Genes Dev.* 4, 1917–1924. doi: 10.1101/gad.4.11.1917

Zdarek, J., and Denlinger, D. L. (1975). Action of ecdysoids, juvenoids, and non-hormonal agents on termination of pupal diapause in the flesh fly. *J. Insect Physiol.* 21, 1193–1202. doi: 10.1016/0022-1910(75)90087-6

Zhang, J., and Lazar, M. A. (2000). The mechanism of action of thyroid hormones. *Annu. Rev. Physiol.* 62, 439–466. doi: 10.1146/annurev.physiol.62.1.439

Zhang, L., Cheng, L., Chapman, J. W., Sappington, T. W., Liu, J., Cheng, Y., et al. (2020). Juvenile hormone regulates the shift from migrants to residents in adult oriental armyworm, *Mythimna separata*. *Sci. Rep.* 10:1626. doi: 10.1038/s41598-020-66973-z

Zhang, X. K., and Kahl, M. (1993). Regulation of retinoid and thyroid hormone action through homodimeric and heterodimeric receptors. *Trends Endocrinol. Metab.* 4, 156–162. doi: 10.1016/1043-2760(93)90105-N

Zhao, L., Liu, L., Wang, S., Wang, H., and Jiang, J. (2016). Transcriptome profiles of metamorphosis in the ornamented pygmy frog *Microhyla fissipes* clarify the functions of thyroid hormone receptors in metamorphosis. *Sci. Rep.* 6:27310. doi: 10.1038/srep27310

Zwaan, B. J., Azevedo, R. B., James, A. C., Van 't Land, J., and Partridge, L. (2000). Cellular basis of wing size variation in *Drosophila melanogaster*: a comparison of latitudinal clines on two continents. *Heredity* 84, 338–347. doi: 10.1046/j.1365-2540.2000.00677.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 26 October 2021
doi: 10.3389/fevo.2021.715988

Phenotypic Variation Through Ontogeny: Thyroid Axis Disruption During Larval Development in the Frog *Pleurodema borellii*

Marissa Fabrezi* and Julio César Cruz

Instituto de Bio y Geociencias del NOA (IBIGEO), CCT CONICET Salta-Jujuy, Rosario de Lerma, Salta, Argentina

Studies of the effects of thyroid hormones on larval development in the frog *Xenopus* spp. have provided baseline information to identify developmental constraints and elucidate genetic and hormonal mechanisms driving development, growth, and life history transitions. However, this knowledge requires data based on other anurans to complete a comprehensive approach to the understanding of larval developmental diversity and phenotypic variation through ontogeny. Mesocosm experiments provide realistic data about environmental conditions and timing; this information is useful to describe anuran larval development and/or analyze endocrine disruption. In this study, mesocosm experiments of the larval development of the frog *Pleurodema borellii* were conducted to explore the consequences of thyroid axis disruption; the sensitivity of tadpoles to the methimazole (2.66 mg/l) and thyroxine (T4) (1.66 μg/l) was compared. These concentrations were selected based on previous studies in *Pleurodema borellii.* We test the effects of methimazole and thyroxine on development in early exposure (from beginning of larval development) and late exposure, 18 days after hatching, with doses administered every 48 h. Tadpoles were evaluated 31 days after hatching. Methimazole caused moderate hypertrophy of the thyroid gland, alteration in the growth rates, differentiation without inhibition of development, and an increase of developmental variability. Thyroxine produced slight atrophy of the thyroid gland, accelerated growth rates and differentiation, and minor developmental variability. In tadpoles at stages previous to metamorphose, skull development (differentiation of olfactory capsules, appearance of dermal bones, and cartilage remodeling) seemed to be unaltered by the disruptors. Moreover, similar abnormal morphogenesis converged in specimens under methimazole and thyroxine exposures. Abnormalities occurred in pelvic and pectoral girdles, and vent tube, and could have been originated at the time of differentiation of musculoskeletal tissues of girdles. Our results indicate that premetamorphic stages (Gosner Stages 25–35) are sensitive to minimal thyroid axis disruption, which produces changes in developmental rates; these stages would also be critical for appendicular musculoskeletal morphogenesis to achieve the optimal condition to start metamorphosis.

Keywords: methimazole, thyroid hormone, tadpole, metamorphosis, growth, differentiation, anurans

OPEN ACCESS

Edited by:
Fedor N. Shkil, Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences (RAS), Russia

Reviewed by:
Gayani Senevirathne, Harvard University, United States
Maja Ajdukovic, University of Belgrade, Serbia

****Correspondence:***
Marissa Fabrezi
mfabrezi@conicet.gov.ar

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *27 May 2021*
Accepted: *07 October 2021*
Published: *26 October 2021*

Citation:
Fabrezi M and Cruz JC (2021) Phenotypic Variation Through Ontogeny: Thyroid Axis Disruption During Larval Development in the Frog Pleurodema borellii. Front. Ecol. Evol. 9:715988. doi: 10.3389/fevo.2021.715988

INTRODUCTION

Endocrine disruptors are chemicals that can interfere with endocrine systems. Among vertebrates, the thyroid gland is a vital endocrine gland with special role in the metabolism, growth and development. Thyroid disruptors are well known since some substances, named goitrogens, cause difficulty for the synthesis of thyroid hormones producing the enlargement of the thyroid gland to compensate the inadequate hormone production. Among these substances, the methimazole is used to treat the hyperthyroidism. Differently, the thyroid hormone (thyroxine, T4) is supplied when the thyroid gland doesn't produce enough hormone (i.e., hypothyroidism).

Studies of the effects of thyroid hormones (THs) [i.e., triiodothyronine (T3) and thyroxine (T4)] on anuran larval development have been key to identifying developmental constraints and have provided a framework to improve our understanding of cell behavior and the molecular mechanisms underlying growth and metamorphic remodeling (Shi, 2000; Buchholz, 2017; Rose and Cahill, 2019; Rose, 2021). Most of these studies were based on the frog model system *Xenopus laevis* and/or *Xenopus tropicalis*, and were focused on skull and gut remodeling (Hanken and Summers, 1988; Schreiber et al., 2005; Smirnov and Vassilieva, 2014; Rose et al., 2015; Choi et al., 2017; Rose and Cahill, 2019), tail disappearance (Yaoita, 2019), differentiation of morphological novelties (Senevirathne et al., 2020), and the systemic consequences of the spontaneous absence of thyroid glands (Rot-Nikcevic and Wassersug, 2003, 2004). This model system represents a basal lineage with a whole ontogeny that displays many eco-morphological differences with respect to most anurans; thus, information needs to be complemented with data on anuran larval development diversity to explain adaptive differentiation (Roelants et al., 2011). Further, studies in the direct developer frog *Eleutherodactylus coqui* (Jennings and Hanken, 1998; Callery and Elinson, 2000; Laslo et al., 2019) have proposed the conservation of thyroid gland physiology in absence of typical metamorphic transformations which is observed in other lineages of frogs without free living tadpoles (Naumann et al., 2020).

The knowledge about the anuran thyroid physiology has provided some generalizations for larval development. The normal histomorphology (e.g., size and histology) of the thyroid gland may be divided into phases correlated with larval development (Etkin, 1936) as follows: (a) the gland is small, with follicular cells with poor cytoplasm during limb bud stages and stages of digit differentiation (premetamorphosis); (b) the gland grows and follicular cells become columnar during stages of limb growth (prometamorhosis); and (c) the gland reduces by collapse of follicles, and the epithelium achieves a condition almost squamous (metamorphic climax). In this way, the thyroid gland histology offers an important diagnosis of several histomorphological features modulated by the hypothalamic-pituitary-thyroid (HPT) regulatory axis. Thyroid histology serves as a valuable and sensitive diagnostic to detect the ability of a chemical to interact with the HPT axis (Grim et al., 2009).

Moreover, the expression of enzyme deiodinases on thyroid hormone and thyroid hormone receptors (TRα) during premetamorphosis are involved in limb differentiation (Cai and Brown, 2004) whereas expression of deiodinases and thyroid hormone receptors (TRβ) trigger several morphological changes typical of metamorphosis (Choi et al., 2017). These studies denote differences in temporal sensitivity and receptors between the appendicular system and most larval organs and systems that are lost or remodeled at the metamorphic climax when the levels of hormones increase. Additionally, THs play an important role in the regulation of anuran cranial ontogeny, influencing the timing and sequences of cartilage transformation and bone appearance as well as bone growth. Artificial changes in the TH-level can lead to considerable temporary changes in skull development in some anurans (Smirnov and Vassilieva, 2009; Rose and Cahill, 2019; Rose, 2021). Experimental studies showed that the axial skeleton ossification of most neural arches is not dependent on or only requires a low level of TH. However, TH is necessary to induce ossification in the most posterior arches and the ribs. Similarly in the hind limb skeleton, the cartilaginous precursors of bone elements fail to ossify in TH absence (Smirnov and Vassilieva, 2014). The premetamorphic histomorphology of the thyroid gland and the early sensitivity of limbs suggest the hormone concentrations define rates of development that may accelerate or delay the timing to start the metamorphosis when minimal changes occur.

Mesocosm experiments provide realistic data about environmental conditions and timing; thus, those experiments are useful to describe anuran larval development and are suitable to analyze developmental rates (Skelly and Kiesecker, 2001). Recently, mesocosm experiments of three anuran species (*Pleurodema borellii, Leptodactylus chaquensis,* and *Dermatonotus muelleri*) reported differential responsiveness and sensitivity to endocrine disruptors in tadpoles exposed from premetamorphic stages (Fabrezi et al., 2019). At similar concentrations of T4, developmental rates were modified, as evidenced in body size, histomorphology of the thyroid gland, limb development, and metamorphic transformations were triggered in gut and skull cartilage remodeling in two species (e.g., *Leptodactylus chaquensis* and *Dermatonotus muelleri*), whereas methimazole increased growth rates in *Pleurodema borellii* (Fabrezi et al., 2019).

Pleurodema borellii (Peracca, 1895) is a common frog present in the northwest of Argentina; reproduction and breeding occur during the wet season, eggs are laid in foam nests, tadpoles are typical of ephemeral ponds, with developmental stages identified by standard anuran staging table (Gosner, 1960; Cruz, 2020). Larval development in *P. borellii* shows intraspecific heterochronic variations, since tadpoles belonging to a single clutch complete the embryonic stages at the same time (6 days after fertilization), but larvae develop at different rates; then, after 20 days, tadpoles may reach stages of digit differentiation or remain at limb bud stages, suggesting different individual development under similar environmental variables (Fabrezi et al., 2019; this study).

Here, we present the results of mesocosm experiments in *Pleurodema borellii* larval development to explore the consequences of disruptors (methimazole and thyroxine) on the thyroid gland. Methimazole (CAS Number: 60-56-0) is considered an inhibitor of synthesis of the thyroid

hormone, whereas thyroxine (CAS Number 51-48-9) is an endogenous agonist.

Tadpoles were immersed in predetermined concentrations at the beginning of larval development (early exposure, Gosner Stage 25), and after 18 days of hatching (late exposure). Larval exposure to disruptors began at an earlier development stage than in previous studies and finished later (Degitz et al., 2005; Fabrezi et al., 2019; **Tables 1**, **2**).

We focused on the whole larval development to test the effects of disruptors to change developmental rates, either delaying or stimulating; thyroid gland histomorphology, and the condition under which metamorphosis may be achieved in a non-model species, *Pleurodema borelli*.

MATERIALS AND METHODS

A *Pleurodema borellii* (Peracca, 1895) clutch was deposited in an abandoned swimming pool of the Instituto de Bio y Geociencias Noa (24°59′3.54″S, 65°34′53.13″W) on January 6, 2021. The clutch was transferred to a container and held up to January 11 (Day 0), when the embryos hatch and showed opercular folds covering partially or totally external gills. Specimens were staged following the standard table of Gosner (1960) and Cruz (2020). Then, tadpoles were randomly distributed in groups of 60–70 tadpoles and maintained in 20 plastic containers (40 cm × 40 cm × 30 cm) with 15 l of tap water within a large receptacle placed outdoor (**Figure 1**). Environmental conditions during the experiments varied, with temperatures ranging between 27 and 35°C during the day and 18 and 24°C at night, and rainfall of 106.5 mm distributed over 13 days. Each container was supplied with 1 g of tropical fish food once a day. Tadpole density for each container is considered low, since these species may breed in small ponds with higher densities.

The treatments started 2 days after hatching when most tadpoles were at Gosner Stage 25 with oral disc complete (Cruz, 2020). Four containers were identified

TABLE 1 | Comparison of studies of larval development of anuran species exposed to methimazole.

Study	Species	Initial Stage	Concentrations (mg/l) and days of exposure	Effects on thyroid histopathology and development
Degitz et al. (2005) Laboratory conditions	*Xenopus laevis*	Early exposure: Nieuwkoop and Faber Stage 51≈ Gosner Stage 30 Late exposure: Nieuwkoop and FaberStage 54≈ Gosner Stage 35	6.25, 12.5, 25.0, 50.0, 100 14 days	Hypothyroidism: the severity in the thyroid gland is dependent of concentration. Initial stage had no impact on the concentration at which significant effects on developmental rate are observed. Significant inhibition in development at concentrations above 25.00 mg/l. The span of developmental stage at intermediate concentrations fell between the controls and 100 mg/l treatment.
Fabrezi et al. (2019) Mesocosm conditions	*Pleurodema borellii*	Gosner Stages 22–35	6.6 mg/l 10 days	Moderate hypertrophy (20%) of thyroid gland. Significant increment of growth (Gosner Stages 31–36). The span of developmental stage is similar to the control
	Leptodactylus macrosternum	Gosner Stages 28–30	6.6 mg/l 16 days	Moderate hypertrophy (27%) of thyroid gland. Significant increment of tadpole size. The span of developmental stage is similar to the control

TABLE 2 | Comparison of studies of larval development of anuran species exposed to T4.

Study	Species	Initial stage	Concentrations (μ g/l) and days of exposure	Effects on thyroid histopathology and development
Degitz et al. (2005) Laboratory conditions	*Xenopus laevis*	Early exposure: Nieuwkoop and Faber Stage 51≈ Gosner Stage 30 Late exposure: Nieuwkoop and FaberStage 54≈ Gosner Stage 35	0.25, 0.50, 1.00, 2.00, 4.00 21 days	Increment in mortality at higher concentrations. Decrease in the number of developmental stages related the increment of T4 concentrations. Significant acceleration of development was observed at 2 and 4 μg/l.
Fabrezi et al. (2019) Mesocosm conditions	*Pleurodema borellii*	Gosner Stages 22–35	0.83 10 days	Moderate atrophy of thyroid gland.
	Leptodactylus macrosternum	Gosner Stages 28–30	0.83 16 days	Severe atrophy of thyroid gland. Significant size reduction and decrease in the number of developmental stages (38–39). Acceleration of development. Advanced snout ossification, and hypochord, medial fusion of girdles and beginning of digestive tract transformation (vent tube and oral disc disappearance and empty gut).
	Dermatonotus muelleri	Gosner Stages 29–32	0.83 16 days	Severe atrophy of thyroid gland. Significant size reduction and decrease in the number of developmental stages. Tail shortening and forelimb emergence. Advanced snout ossification, and hypochord, medial fusion of girdles and beginning of digestive tract transformation (vent tube and oral disc disappearance and empty gut). Cartilages of skull and hyobranchial apparatus remodel.

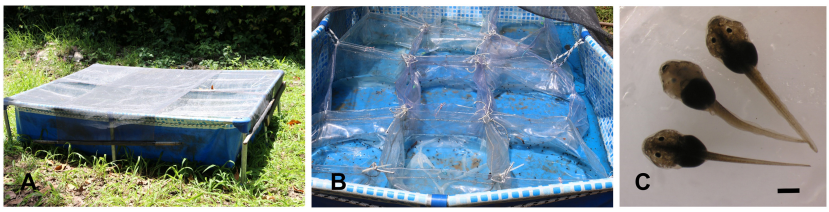


FIGURE 1 | (A) Containers with tadpoles were kept in two large receptacles, which were covered with a net to avoid bird predation. **(B)** Plastic containers (each one was identified with a label was assigned to a treatment) at the beginning of the experiments. **(C)** Tadpoles at Gosner Stage 25. The scale bar is equals 1 mm.

and used as control, the remaining ones were assigned to two experiments (eight containers each): early exposure and late exposure.

Early exposure begins with the first dose of disruptor and tadpoles received a total of 15 doses of disruptor. From these treatments, a group of tadpoles from control and both treatments were collected and fixed after eight doses (18 days after hatching) to describe external morphology and identify larval stages. Early exposure finished 31 days after hatching and 29 days after the first dose of disruptor.

The late exposure experiment started 18 days after hatching and finished 31 days after hatching. Tadpoles of these treatments were at different stages of premetamorphosis, and received seven doses of disruptors during 13 days.

To perform the experiments, the concentrations of methimazole and T4 used by Fabrezi et al. (2019) were considered. **Tables 1**, **2** describe their results in anuran development using different concentrations of methimazole and T4, and starting exposure at developmental stages in which limb bud development is advanced. Based on those studies, we test lower concentrations of methimazole, and intermediate concentrations of T4 beginning earlier; and at similar stages but extending the experiments for more days.

For each exposure, four containers were supplied with 2.66 mg/l of methimazole every 48 h, and four containers were supplied with 1.66 μg/l of T4 every 48 h. Doses were dissolved in tap water. All the containers (a total of 20 treatments) were kept during 31 days after hatching when tadpoles were euthanized and fixed in 4% neutral buffered formaldehyde and Bouin's fixative for further analysis.

The formalin−fixed specimens for morphohistological studies, accessioned as lots, were deposited at the Instituto de Bio y Geociencias del NOA with the following collection numbers: IBIGEO-A 2301-00 (control tadpoles, January 11–29/2021); IBIGEO-A 2302-00 (control tadpoles: January 11–February 11/2021); IBIGEO-A 2303-00 (tadpoles from early methimazole exposure, January 13–29/2021); IBIGEO-A 2304-00 (tadpoles from early T4 exposure, January 13–29/2021); IBIGEO-A 2305-00 (tadpoles from early methimazole exposure, January 11–February 11/2021); IBIGEO-A 2306-00 (tadpoles from early T4 exposure, January 11–February 11/2021); IBIGEO-A 2307-00 (tadpoles from late methimazole exposure, January 29–February); IBIGEO-A 2308-00 (tadpoles from late T4 exposure, January 29–February 11/2021).

Larval growth was inferred from changes in parameters of tadpole size: body length (i.e., the distance between snout and vent tube in ventral view) and total length (i.e., the distance between snout and tip of the tail in lateral view). Measurements were taken with dial calipers (0.02 mm) and are expressed in millimeters. Statistical analysis of body length and total length was based on non-parametric analysis of variance (Kruskal–Wallis) test with multiple comparisons (InfoStat® v.2016).

Larval differentiation was documented based on morphological changes in external and internal morphology. Cleared and double-stained whole−mount specimens (N = 15) were used to examine musculoskeletal variation. Specimens were prepared using the technique of Wassersug (1976). Observations, descriptions, and illustrations were made with a Nikon−SMZ 1000 stereomicroscope equipped with a Nikon Coolpix digital camera. Furthermore, histological serial sections of 7 μm thick of paraffin-embedded pelvis and hind limbs (N = 6) to describe skeleton tissues were stained with Masson's trichrome (Bancroft and Gamble, 2002) and observations were made with a Nikon E200 microscope equipped with a digital camera and Micro metrics SE Premium 4.5 software.

From six specimens fixed in Bouin's fixative, the buccal floor containing the hyobranchial apparatus with adhered thyroid lobes was manually dissected. The samples were dehydrated (by a series of ethanol solutions of increasing concentrations), embedded in paraffin, and sectioned at 5 μm with a Leica RM 2245 rotatory semiautomatic microtome. Sections were stained with hematoxylin and eosin following the protocol of Martoja and Martoja-Pierson (1970). Photomicrographs were obtained with a Nikon E200 microscope equipped with a digital camera and Micro metrics SE Premium 4.5 software.

Histological descriptions and photomicrographs were taken from the widest cross-sectional area of the thyroid glands. Based on descriptions for other taxa (Etkin, 1936; Grim et al., 2009), we considered the following parameters: degree of follicular hypertrophy and hyperplasia, the height of follicular cells, presence of vacuoles in the colloid, and position and degree of condensation of the nuclei. The histomorphology of the thyroid gland was described at the end of experiment in tadpoles at larval Stage 40 (advanced prometamorphosis, Etkin, 1936).

All aspects of the research where approved by the Secretaría de Medio Ambiente y Desarrollo Sustentable, Gobierno de la Provincia de Salta, Argentina, and adhered to the legal requirements of Argentine laws (File number 227–216600/064/2016).

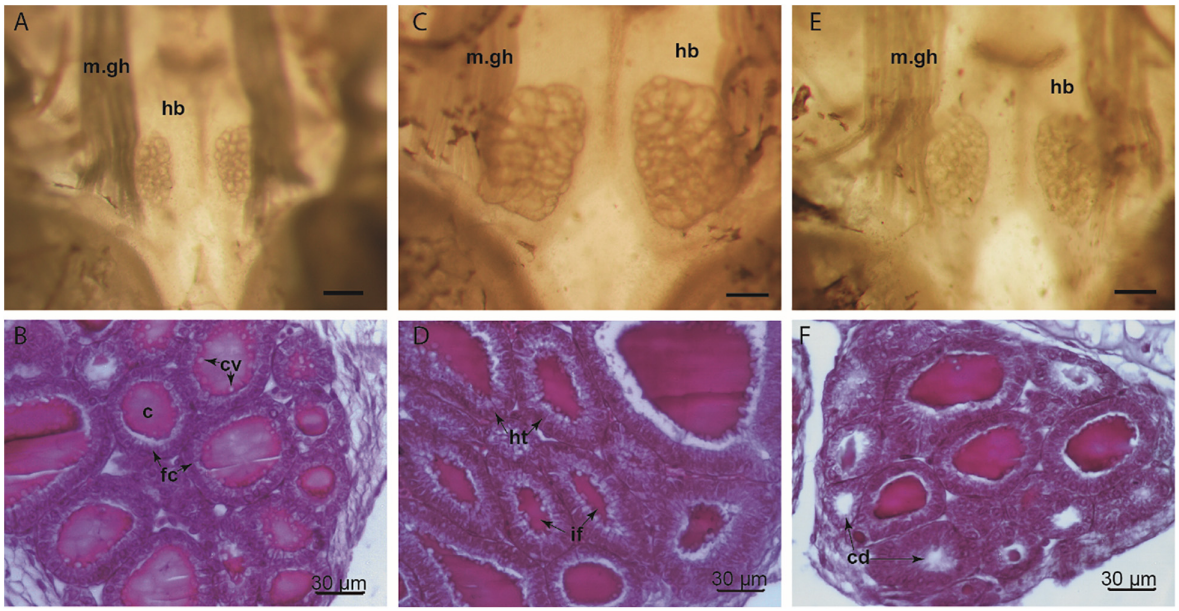


FIGURE 2 | Thyroid gland of *Pleurodema borellii* larvae (Gosner Stage 40, 31 days after hatching), macroscopic and microscopic images. **(A,B)** Control tadpoles showed the typical follicular morphology of the normal thyroid gland, with cuboidal follicular cells and follicular colloid. **(C,D)** Specimens from methimazole early exposure exhibited an overall glandular hypertrophy. The histology revealed an evident follicular cell hypertrophy and irregular follicular morphology. **(E,F)** Specimens from thyroxine early exposure have thyroid glands with follicular lumina less defined relative to the normal gland are also observed. Histological section shows slight depletion of colloid and decrease in the number of chromophobic vacuoles. c, colloid; cd, colloid depletion; cv, chromophobic vacuoles; fc, follicular cells; hb, hypobranchial cartilage; ht, hyperthrophied cells; if, irregular follicles; m.gh, muscle geniohyoideus. Scale bar for macroscopic images = 0.3 mm.

RESULTS

Effects of Disruptors on the Thyroid Gland

The histomorphology of the thyroid gland in advanced prometamorphosis at the end of experiments of early exposure were analyzed. We selected specimens in similar developmental stage (Gosner Stage 40) in order to describe the thyroid gland on the threshold of metamorphosis (**Figure 2**). Cruz (2020) described in *Pleurodema* tadpoles, the follicular number, the colloid volume and the height of the follicular epithelium with their maximum values among Gosner Stages 39–44, reaching the peak between stages 41–42 and concomitant with the maximum volume of the gland.

A considerable increase in overall thyroid gland size was observed in the specimens exposed to methimazole, the glandular lobes were larger with respect to the control specimens, indicating glandular hypertrophy (**Figure 2**). In addition, numerous thyroid follicles exhibiting abnormal morphology (mostly large follicles with colloid content) were observed. The presence of blood cells in interfollicular gaps was greater than in the untreated glands. Follicular cells ranged from tall cubic to columnar, and mild cellular hyperplasia was observed in some follicles. A slight decrease in the number of chromophobic vacuoles was observed inside the colloid.

The thyroid gland of specimens exposed to thyroxine showed slight glandular atrophy relative to untreated specimens. Some heterogeneity in the size of lobes and follicles was observed (**Figure 2**). A large proportion of small follicles was observed, with no colloid content. Only a few larger follicles had colloid content. There were no considerable differences in the height of the follicular cells (**Figure 2**). However, a decrease in the amount of chromophobic vacuoles present inside the colloid was observed with respect to the control specimens.

Growth Rates

Methimazole exposure caused significant differences in growth (**Figure 3**, **4** and **Table 3**). Early exposure showed the increment of body length and total length when tadpoles were evaluated 18 days after hatching and at the end of experiments. Late exposure also produces significant increment of growth. The variability between maximum and minimum values of body size and total length in early treated tadpoles was greater than that of late-treated tadpoles.

T4 exposure altered growth rates (**Figure 3**, **4** and **Table 3**). Early exposure showed the increment of body length and total length when tadpoles were evaluated 18 days after hatching, but at the end of experiments only total length is significantly different from controls. Late exposure also produces significant increment of growth. The variability between maximum and minimum values of body size and total length in T4-treated tadpoles is minor to the controls.

Rates of Differentiation

Tadpoles treated early with methimazole were in premetamorphic larval stages (i.e., Gosner Stages 28 and 36)

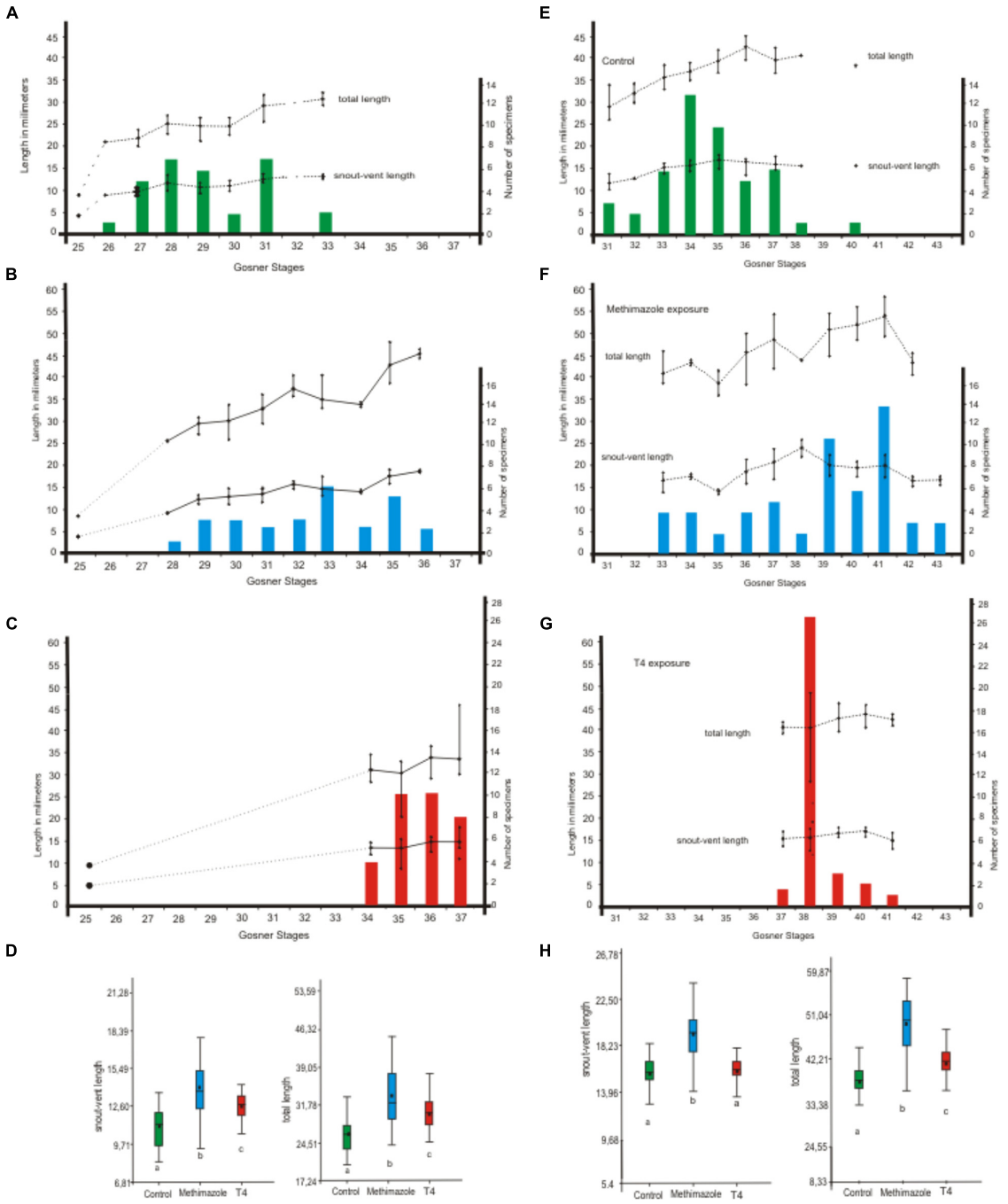


FIGURE 3 | *Pleurodema borellii* size variation of each larval stage exposed early to methimazole and T4. **(A–D)** The experiment began with tadpoles in Gosner Stage 25 ($N = 7$), with an average snout-vent length of 3.30 mm (± 0.35) and an average total length of 8.07 mm (± 0.19). Snout-vent length and total length of each larval stage (bars) after 18 days after hatching were measured to determine growth. Simultaneously, tadpoles were also staged. **(A)** Control specimens. **(B)** Tadpoles exposed to methimazole (eigth doses). **(C)** Tadpoles exposed to T4 (eigth doses). **(D)** Significant differences were found in size variables ($p < 0.05$ for snout-vent length and total length) between each treatment and the control. **(E–H)** Continuity of treatments up to 31 days after hatching (a total of 15 doses). Snout-vent length and total length of each larval stage (bars) at the end of experiments were measured and tadpoles were staged. **(E)** Control specimens. **(F)** Tadpoles exposed to methimazole (15 doses). **(G)** Tadpoles exposed to T4 (15 doses). **(H)** Significant differences were found in size variables ($p < 0.05$ for snout-vent length and total length) between each treatment and the control.

18 days after hatching and reached prometamorphic stages and even metamorphic stages, but some remained at premetamorphic stages (i.e., Gosner Stages 35 and 36) at the end of experiment (31 days after hatching). Early exposure to methimazole accelerated the differentiation respect to the control, although the responses were variable since tadpoles range among

TABLE 3 | Descriptive statistics for snout-vent length (SVL) and total length (TL) of *Pleurodema borellii* during larval stages comprising pre and prometamorphic stages with exposure to methimazole or T4.

Beginnig of experiments	End of exposure	Specimens	N	Mean SVL ± SD (mm)	Mean TL ± SD (mm)
Early exposure	18 days after hatching	Control	26	10.98 ± 1.58 (a)	26.17 ± 3.68 (a)
2 days after hatching		Methimazole	27	13.98 ± 2.36 (b)	33.56 ± 5.67 (b)
		Thyroxine	32	12.55 ± 1.58 (c)	29.95 ± 3.99 (c)
	31 days after hatching	Control	47	15.63 ± 1.66 (a)	37.62 ± 3.70 (a)
		Methimazole	57	19.31 ± 2.41 (a)	49.17 ± 5.73 (b)
		Thiroxine	45	15.89 ± 1.26 (b)	41.24 ± 3.47 (c)
Late exposure	31 days after hatching	Control	47	15.63 ± 1.66 (a)	37.62 ± 3.70 (a)
18 days after hatching		Methimazole	56	16.84 ± 1.58 (b)	43.01 ± 4.36 (b)
		Thiroxine	59	16.59 ± 1.16 (b)	42.86 ± 3.37 (b)

Mean lengths with different letter symbols in parenthesis are significantly different from each other ($P < 0.0001$), as determined by Kruskal Wallis test.

premetamorphic, prometamorphic and metamorphic stages (**Figures 3**, **5**). Tadpoles under late exposure were at advanced premetamorphic stages or early prometamorphic stages (i.e., Gosner Stages 31–37) without reaching metamorphic stages 31 days after hatching (**Figures 4**, **5**).

Early T4 exposure accelerated differentiation and reduced the number of the stages of development (**Figures 3**, **5**). When tadpoles were evaluated 18 days after hatching, most tadpoles were in later premetamorphic stages and earlier prometamorphic stages (four stages). Tadpoles at the end of experiment (31 days after hatching) were in prometamorphic stages (i.e., Gosner Stages 37–40). Tadpoles under late exposure to T4 reached prometamorphic stages (i.e., Gosner Stages 38–41) and metamorphic stages (**Figures 4**, **5**).

Skull Skeleton Differentiation

In tadpoles exposed (early and late exposures) to methimazole and T4, specimens in prometamorphic stages show the differentiation of the nasal cartilages and the appearance of snout bones (nasal, septomaxillary, premaxillary) emerged in the sequences described for most anurans (Trueb, 1993; Fabrezi, 2011), without differences between disruptors (**Figure 6**).

Abnormalities

At the end of experiments, we recorded abnormalities identified as an unusual morphology of the anal tube and hind limb asymmetry (**Figure 7**). The proportion of abnormalities was higher in those specimens under T4 exposures (95% early exposure and 75% late exposure) and near 50% in both treatments under methimazole (**Figure 4**).

The asymmetry of the hind limbs seems to be related to the incomplete development of the acetabulum; thus, the femur does not articulate with the pelvis but is suspended from the pelvis by soft tissues (**Figures 8**, **9**). In the pelvis, the elongation of the ilium is different in each hemigirdle, and in some cases, there is no elongation (**Figure 8**).

The development of the ventral elements of the pectoral girdle is also abnormal in specimens exposed to disruptors, although the glenoid cavity appears well defined and the humerus is articulated (**Figure 8**). In each half of the pectoral girdle, the procoracoids, coracoids, and epicoracoids have an asymmetric development and there is a delay in the endochondral ossification of the coracoid and in the differentiation of the clavicle.

The muscles iliacus externus, and tensor fasciae latae have origins on the iliac shaft and insert on the femur. The pattern described for *Pleurodema borellii* (Fabrezi et al., 2014) is observed in control tadpoles at Gosner Stage 38 (**Figure 8**). In specimens exposed to methimazole (early exposure) at similar stages, the muscle iliacus externus is shorter and compact and the tensor fasciae latae is formed by loose fibers (**Figure 8**). In specimens exposed to T4 (early and late exposures), few and loose fibers of the muscle iliacus externus are attached to the short ilium when it is present (**Figure 8**) or not when the cartilaginous iliac shaft is absent (**Figure 8**).

Histological sections (**Figure 9**) of specimens treated with methimazole revealed: malformed pelvic cartilages, absence of the acetabulum, and disorganized fibers in those muscles with attachments on the pelvis (**Figure 9**). The pelvis is amorphous and rudimentary, and the absence of acetabulum precludes the hind limb articulation. Numerous chondroblasts and reduced extracellular matrix constitute the pelvis and the proximal epiphysis of the femur (**Figure 9**). The histology of the pelvis and femurs of specimens treated with T4 showed similar chondrogenesis to that observed in controls, the hypertrophy of chondrocytes increases toward the middle of diaphysis (**Figure 9**). However, T4 exposure affected processes that model and shape the cartilages and the absence of acetabulum prevents the femur joint.

DISCUSSION

Effects of Disruptors on the Thyroid Gland

The effects of methimazole and T4 on TH synthesis and larval development are well known from experiments involving the model species *Xenopus laevis*. Some generalizations emerged from those studies: they described the concentrations at

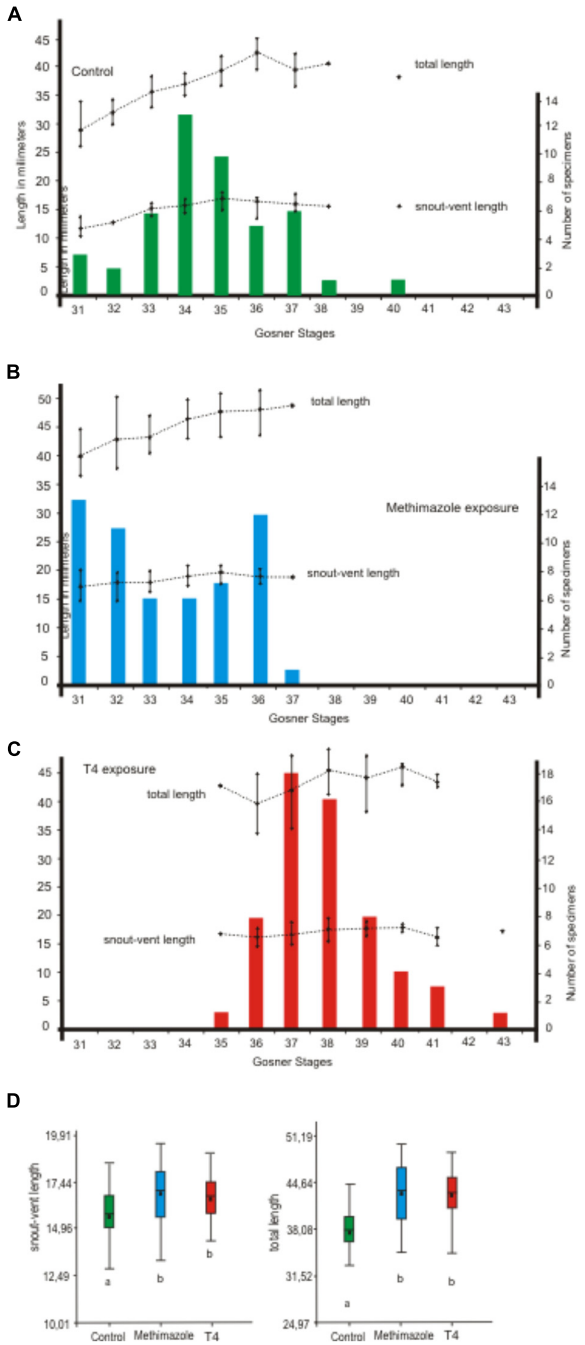


FIGURE 4 | Variation in size of each larval stage of *Pleurodema borellii* during late exposure to methimazole and T4. **(A–D)** The experiment began at 18 after hatching. Tadpoles were exposed for 13 days (seven doses). **(A)** Control specimens. **(B)** Tadpoles exposed to methimazole. **(C)** Tadpoles exposed to T4. **(D)** Significant differences were found in size variables ($p < 0.05$ for snout-vent length and total length) between each treatment and the control. However, no significant differences were found among size variables between tadpoles exposed to methimazole and tadpoles exposed to T4.

which these disruptors cause hypertrophy or atrophy of the thyroid gland, the effects of early or late exposure on development rates, and the consequences of the duration of exposure on sensitivity (Degitz et al., 2005; Opitz et al., 2006; Grim et al., 2009; Smirnov and Vassilieva, 2014; among others). In this study, continuous exposure to low concentration of methimazole and intermediate concentration of T4 for 31 days after hatching in *Pleurodema borellii* produced moderated changes in the thyroid gland histology.

In *Xenopus laevis* (Degitz et al., 2005), the severity of thyroid gland hypertrophy caused by methimazole depends on the concentration, although the minimal concentrations used in those experiments were higher (6.25 mg/l) and the period of exposure was shorter than in our treatments (**Table 1**). Fabrezi et al. (2019) found moderate hypertrophy of the thyroid gland with increment of growth in *Pleurodema borellii* and *Leptodactylus chaquensis* using a similar concentration of methimazole (6.6 mg/l) to those used in experiments of *X. laevis* (Degitz et al., 2005) and 10 and 16 days of exposure, respectively (**Table 1**). Here, we tested a lower concentration of methimazole (2.66 mg/l) starting the exposure in earlier larval stages (early exposure) for more days and we found the expected thyroid gland hypertrophy.

The severity of thyroid gland atrophy caused by T4 depends on the concentration, ranging from 0.25 to 4 μg/l, and the period of exposure (Series numbers 76 and 77—)[1] in *Xenopus laevis*. A low concentration of T4 (0.83 μg/l) caused mild atrophy of the thyroid gland at 10 days of exposure in *Pleurodema borellii* and severe atrophy at 16 days of exposure in *Leptodactylus chaquensis* and *Dermatonotus muelleri* (Fabrezi et al., 2019). Furthermore, *L. chaquensis* and *D. muelleri* showed a notable acceleration of differentiation but not of growth, revealing interspecific differential responsiveness and sensitivity of different tissues to specific hormonal signals. In this study, the effects of medium concentrations (1.66 μg/l) of T4 on the thyroid gland of *Pleurodema borellii* were moderate despite differences in concentrations and days of exposure with the former experiments. Prolonged exposure to T4 may reduce sensitivity, as recorded in *Xenopus laevis* (Degitz et al., 2005).

Changes in Growth and Differentiation Rates

Under natural conditions, *Pleurodema borellii* growth is accelerated at the beginning of larval development (between Gosner Stages 25–30) and then it continues with a moderate increase up to the beginning of metamorphosis. Larval development shows strong heterochronies, since after 20 days of development some tadpoles are in stages of the limb bud and others are reaching the end of premetamorphic stages. Larval development takes 60 days on average (Cruz, 2020).

Acceleration in growth —which was remarkable in prometamorphic larval stages—was observed when tadpoles were exposed early to methimazole (**Figures 3–5**). Furthermore, methimazole did not delay differentiation or inhibit metamorphosis; on the contrary, it was observed

[1]http://www.oecd.org/env/ehs/testing/seriesontestingandassessmentecotoxicitytesting.htm

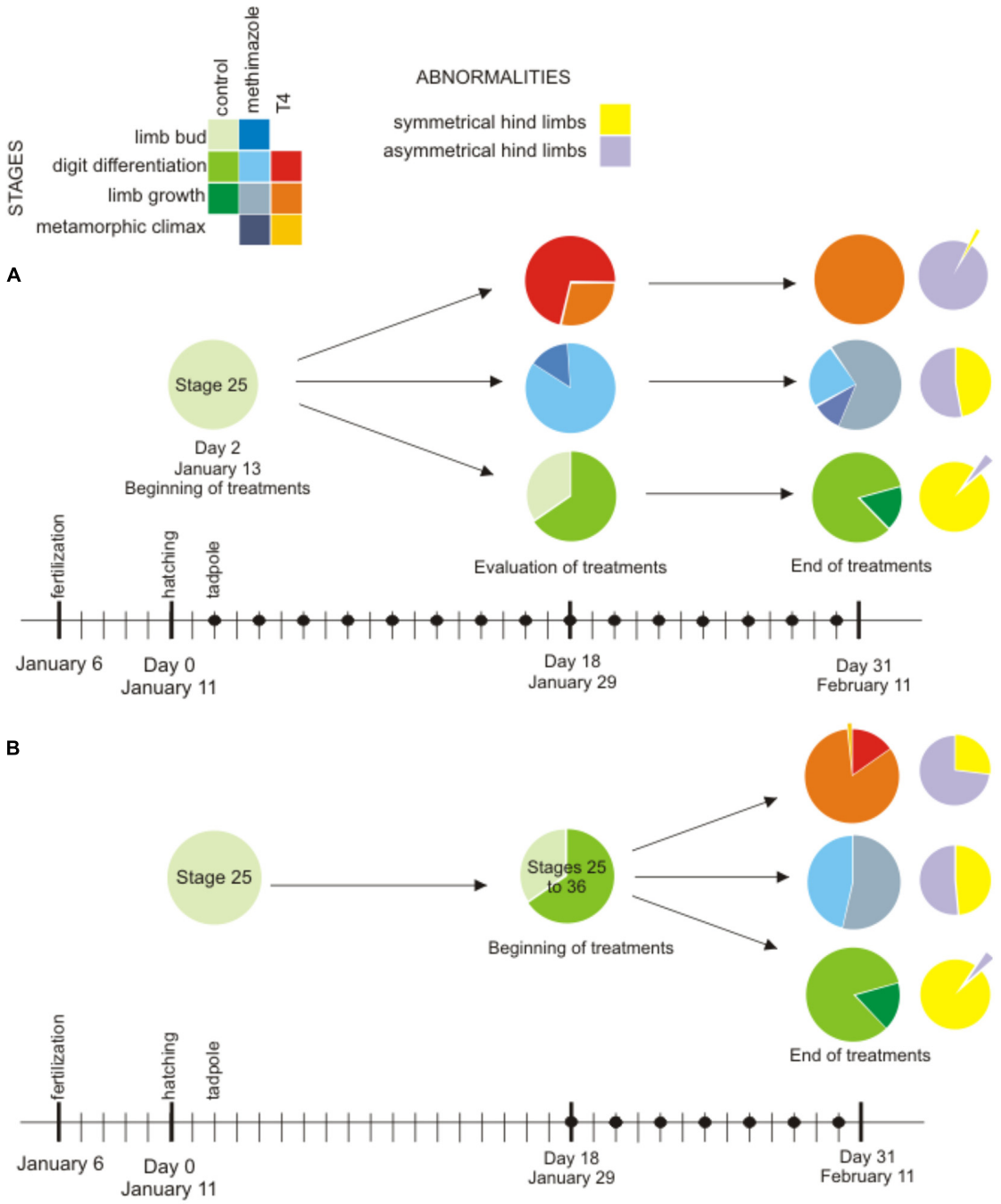


FIGURE 5 | Proportion of differentiation stages of tadpoles during the experiments: premetamorphosis [limb bud stages (Gosner 25–30) and digit differentiation (Gosner 31–36)], prometamorphosis [limb growth (37–41)] and metamorphic climax (42–46) and proportion of abnormalities (identified as hind limbs asymmetries in violet, and normal in yellow) at the end of experiments. **(A)** Early exposure: control in green circles (*N* = 28 at 18 days after hatching; *N* = 60 at 31 days after hatching) with one specimen with abnormal limbs (violet); methimazole in blue circles (*N* = 27 at 18 days after hatching, *N* = 47 at 31 days after hatching), abnormalities appear in the half of tadpoles; and T4 in red and orange circles (*N* = 28 at 16 days of exposure, *N* = 61 at 29 days of exposure), almost all tadpoles present abnormalities (violet) **(B)** Late exposure: control in green circles (*N* = 60 at 31 days after hatching), methimazole in blue circles (*N* = 47 after 14 days of exposure), abnormalities appear in the half of tadpoles, and T4 in red-orange circles (*N* = 61 after 14 days of exposure), abnormalities appear in 75% of tadpoles. Black dots indicate doses.

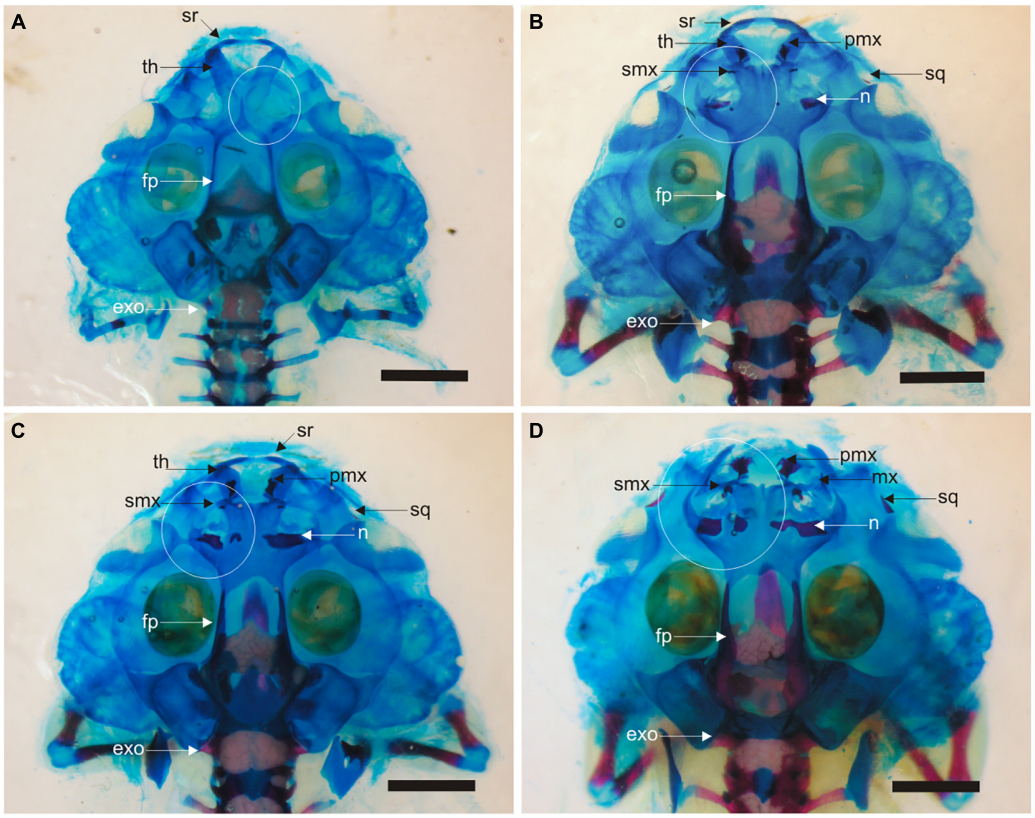


FIGURE 6 | Skull skeleton in tadpoles after 31 days of larval development. **(A)** Control specimen at Gosner Stage 38. Incipient development of cartilages of olfactory capsule denoted by the white circumference. The ossification of frontoparietals and parasphenoid are already present. Accumulation of calcium is observed in the braincase. **(B)** Specimen of early exposure to T4 (Gosner Stage 40). Dorsal cartilages of the olfactory region and dermal ossifications (nasals, premaxillae, maxillae, septomaxillae, and squamosals) have differentiated and erosion of trabecular horns has started. Frontoparietals, parasphenoid and exoccipitals are advanced. Accumulation of calcium is observed in the braincase and vertebral column. **(C)** Specimen of early exposure to methimazole (Gosner Stage 40); same as the specimen described in **(B)**. **(D)** Specimen of early exposure to methimazole (Gosner Stage 41). Erosion of larval structures (suprarostral cartilage and trabecular horns) is advanced. exo, exoccipital; fp, frontoparietal; mx, maxillary; n, nasal; pmx, premaxillary; smx, septomaxillary; sq, squamosal; sr, suprarostral; th, trabecular horn. Scale bar is equal 2 mm.

individual differences, with some tadpoles reaching metamorphosis while others have just reached premetamorphic states. Phuge et al. (2021) reported the ethylenethiourea (a thyroid inhibitor) did not affect metamorphosis in *Sphaerotheca pashchima* and at lower concentrations stimulated metamorphosis in the anuran *Indosylvirana caesari,* and suggested differences in the larval development duration and sensitivity. Our experiments revealed that the stage at which exposure started had an impact on larval differentiation, since tadpoles exposed earlier to methimazole reached metamorphosis whereas tadpoles exposed later remain in prometamorphosis. Then, the effects of disruptor at limb bud stages (i.e., Gosner Stages 25–29) may trigger acceleration of growth and changes in differentiation rates, resulting in the increment of developmental variability and stimulating metamorphosis.

Early exposure to T4 also caused significant variation in growth increase; however, the curve shows a deceleration and reduction of growth variability when prometamorphic stages were reached after 18 days after hatching (**Figure 3**). Early and late T4 exposures also accelerated differentiation, with most tadpoles reaching prometamorphosis after 31 days after hatching. Reduction in the variability of development is noticeable, since the controls spanned nine developmental stages (31–40), whereas tadpoles exposed early to T4 spanned five stages (37–41) at the end of experiments.

Under wild conditions, sustained growth over time is associated with a temperate climate and/or permanent ponds, which provide good environmental conditions to ensure metamorphosis (Emerson, 1988; Roček et al., 2006) or the spontaneous absence of thyroid glands that preclude metamorphosis (Rot-Nikcevic and Wassersug, 2003, 2004). In turn, rapid development is related to unpredictable environmental conditions, in which an abundance of resources suggests that increased protein in the diet enhances either growth or differentiation (Kupferberg, 1997). In *P. borellii*, exposure to methimazole produces variation that resembles the larval development of those species with high developmental variability, like *Pseudis platensis* (Fabrezi et al., 2009; Fabrezi, 2011; Cruz and Fabrezi, 2019), whereas early T4 exposure

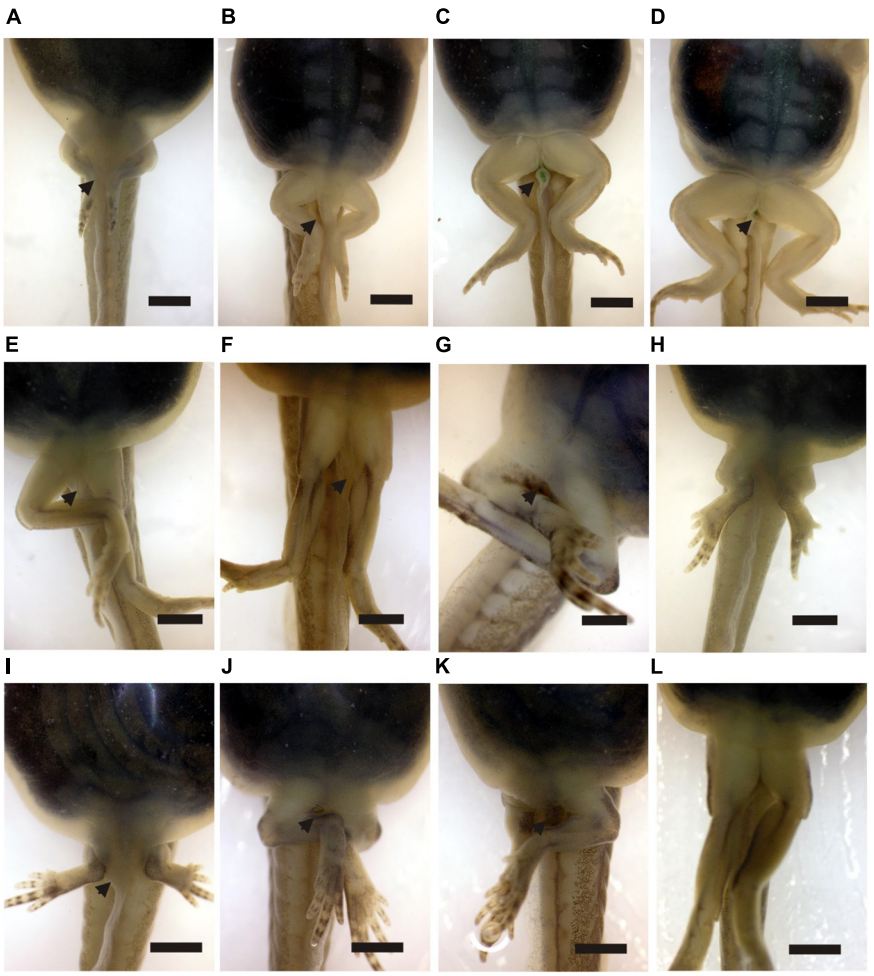


FIGURE 7 | (A–D) Sequence of disappearance of vent tube in *Pleurodema borellii* in normal development observed in Gosner Stages 37, 39, 40, and 41. **(E–H)** Different morphologies of vent tube and asymmetry of legs in specimens exposed to methimazole. **(I–L)** Different morphologies of vent tube and asymmetry of legs in specimens exposed to T4. Scale bar is equal 2 mm.

reduces variation like the larval development of those species that develop rapidly before the pond dries (Fabrezi, 2011; Fabrezi and Cruz, 2014). Furthermore, similar growth and differentiation rates in sister tadpoles forming schools are typical of some species of genus *Leptodactylus* (de Sá et al., 2014). The role of T4 to reduce the variability in larval development was also observed in *Leptodactylus macrosternum* (Fabrezi et al., 2019).

Skull Skeleton Differentiation

Tadpoles exposed at very low or intermediate concentrations of methimazole and T4, respectively, showed internal changes concomitant with the stages reached. Differentiation of the cartilaginous skeleton of olfactory capsules and of bones of the rostral region, and resorption of larval cartilages (suprarostral and trabecular horns) follow the sequence described for most species (Trueb, 1993; Fabrezi, 2011). By contrast, tadpoles of *Dermatonotus muelleri* exposed to lower concentrations of T4 revealed cartilaginous skull remodeling among other metamorphic features even when hind limb development indicated Gosner Stages 38–39 (**Table 2**; Fabrezi et al., 2019).

Rose and Cahill (2019) used thyroid gland inhibitors to isolate the effects of T3 and T4 at specific concentrations, and described changes in growth and differentiation of cranial cartilages during larval development of *Xenopus laevis*. They found differences in the timing in which T4 (earlier) and T3 (later) induced remodeling; absence of dose-dependent responses among stages, and differences of morphological changes when thyroid gland inhibitors were applied. Our results revealed weak inhibition of the thyroid gland activity and supplementation of T4 did not affect sequences of skull bone differentiation.

Abnormalities

Externally, abnormal morphogenesis is observed as an asymmetry of the hind limbs, linear arrangement of the limb segments, and the modification of the vent tube. The combination of these altered morphologies was observed in two control specimens (N = 61), the half of specimens under

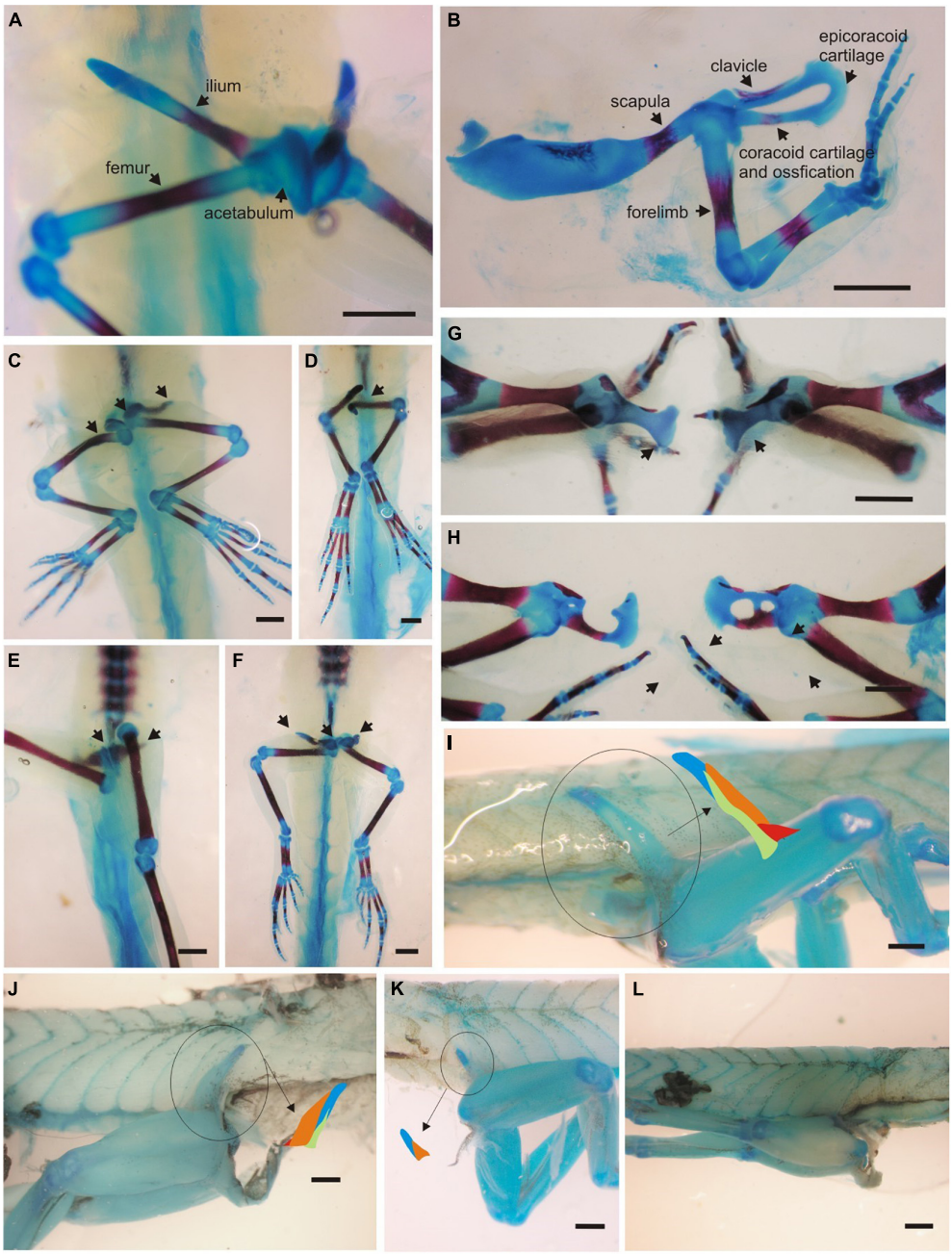


FIGURE 8 | Abnormalities in girdles and limb skeletons and muscles. **(A)** Ventral view of the pelvis in a control specimen (Gosner Stage 40). **(B)** Right pectoral girdle and forelimb in a control specimen (Gosner Stage 40). **(C,D,G)** Girdle and limb skeleton in specimens exposed to methimazole at the end of experiments. **(C)** Femurs hung from rudiments of pelvis, right femur is curved, and the ilion is short. **(D)** Underdevelopment of pelvis and hind limbs joined by soft tissues to hypaxial muscles. **(G)** The coracoid cartilage is present but the procoracoid and the ossification of clavicle are defective. **(E,F,H)** Girdle and limb skeleton in specimens exposed to T4 at the end of experiments. **(E,F)** In both specimens the acetabulum is not well formed and the ilium is short. **(H)** The cartilaginous elements of each half of the girdle (coracoid, procoracoid and epicoracoid) show unequal differentiation and growth. **(I)** Lateral view of pelvis muscles (pelvis to femur) in control specimen at the end of experiment (Gosner Stage 38). The iliac shaft reached the limit between hypaxial and epaxial musculature. The schematic representation described the organization of three pelvic muscles: the muscle iliacus internus (red) originated at the basis of the ilium (blue) and inserted on the femur; the muscle iliacus externus (orange) attached along the anterior half of the iliac shaft and the tensor fasciae latae (green) from the laterointernal surface of the ilium to the thigh. This pattern is observed in the adult of *Pleurodema borellii* (Fabrezi et al., 2014). **(J)** Specimen under early exposure to methimazole after 31 days of larval development (Gosner Stage 38). The iliac shaft reaches the inferior half of hypaxial musculature and the muscles are also short. The tensor fasciae latae have loose fibers. **(K,L)** Specimens (Gosner Stage 38) under early exposure to T4 at the end of experiments. **(K)** The underdevelopment of the short iliac shaft is complemented with a short muscle iliacus externus. **(L)** The specimen without pelvic girdle and absence of pelvic muscles. The thigh is attached by soft connective fibers to hypaxial muscles as is observed in detail in **(K).** Bar is equal to 1 mm.

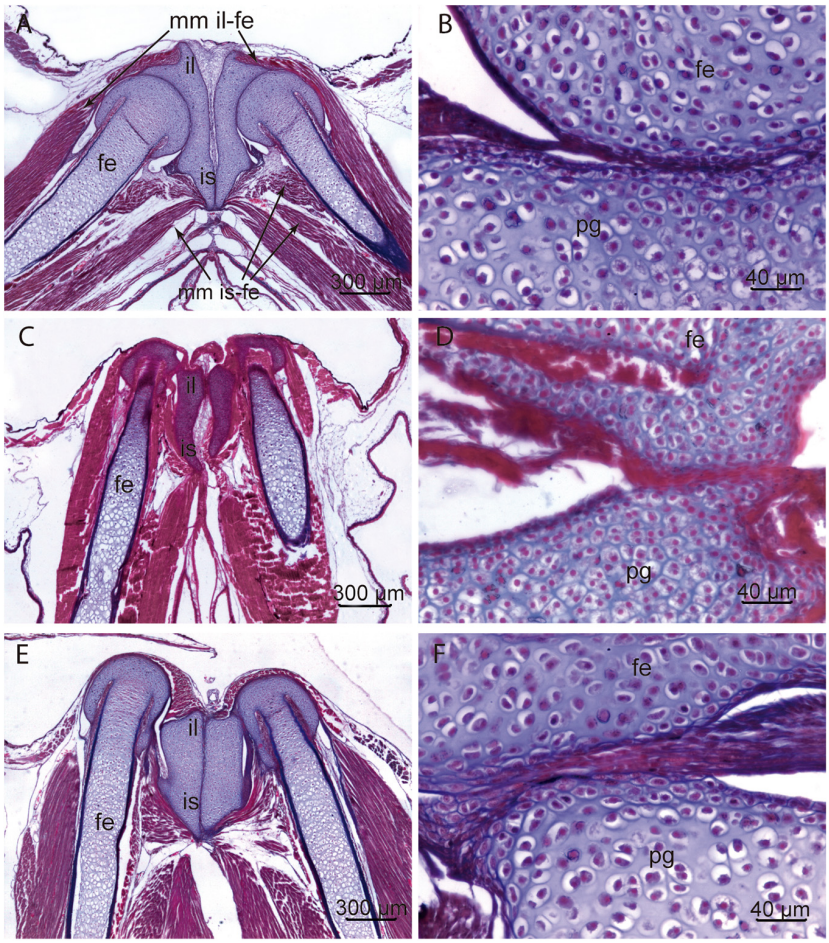


FIGURE 9 | Histology of pelvic girdles. **(A,B)** Control specimen (Gosner Stage 39). Section of the ilium-ischium cartilage. Each hemigirdle contacts with the opposite at the mid-ventral line. Epiphyses of the femur are well defined and fit in the acetabulum. The diaphysis of the femur is formed by hyaline cartilage with hypertrophic chondrocytes and incipient perichondral ossification. The epiphysis of the femur and the pelvic cartilage present abundant chondroblasts rounded by extracellular matrix. The organization of the thigh musculature is advanced. **(C,D)** Methimazole treated specimen (Gosner Stage 39). The rudimentary differentiation of femoral epiphyses and pelvic girdle indicates a delayed local development with the proliferation of chondroblasts, concomitant with a scarce extracellular matrix. The atrophy of muscles attached to the ilium, ischium, and proximal segment of the femur is evident. **(E,F)** Specimen exposed to T4 (Gosner Stage 39). The asymmetry of ilium-ischium cartilage and the absence of acetabulum precludes the articulation of hind limb. The asymmetry is also observed in the associated muscles. The histology of cartilage shows a pattern of chondrogenesis similar to control specimens. fe, femur; il ilium; is, ischium; mm il-fe, muscles originated in the ilium and inserted on the femur muscles; mm is-fe, muscles originated in the ischium and inserted on the femur; pg, pelvic girdle.

methimazole exposures, and almost all T4 specimens from early exposure and the 75% from late exposure (**Figure 5**).

Cruz (2020) described the disappearance of the vent in Gosner Stage 40 as the first external signal of metamorphic change in *P. borellii*. Moreover, Manzano et al. (2013), for this species, reported the differentiation of ischium/pubis at Gosner Stage 33, cavitation of the acetabulum at Gosner Stage 35, and contact of both pubes at Gosner Stage 41. Concomitantly, the development of the muscles with origin in the ischium and ilium (e.g., the M. iliacus internus, M. tensor fasciae latae, M. iliacus externus) which will insert along the femur occurs at Gosner Stages 36–37 when the iliac shaft grows (Fabrezi et al., 2014). The critical stages in which the abnormal morphologies originated are those in which differentiation of the pelvic girdle takes place (Gosner Stages 33–35). Skeletons showed asymmetry of pelvic elements, absence of the acetabulum, and deficient elongation of the iliac shaft with the consequence in the incomplete or failure of muscles differentiation. Furthermore, Van Dijk (1959) described spatially (structural) and temporal relationships among limb-girdles tissues, and hypaxial and abdominal muscles, which could be forming the larval vent tube and the adult cloaca. The abnormal morphogenesis of musculoskeletal elements of the pelvic girdle and adjacent tissues (i.e., vent tube) resulting from the endocrine disruption agree with studies that demonstrated an early hormonal sensitivity of limb mesenchyme at limb bud stages (Cai and Brown, 2004) which could be extended to subsequent stages (e.g., when the cartilages of the femur and pelvic girdle are differentiating, and the organization of the thigh musculature begins).

Methimazole and T4 affected the morphogenesis of the early anlages of pelvic girdles (ischium-pubis and ilium). However, exposure to T4 did not affect chondrogenesis but the shape and growth of the pelvic elements. This fact is consistent with studies that have demonstrated the role of T4 in specific mechanisms regulating cartilage shape and growth (Rose et al., 2015; Rose and Cahill, 2019).

The thyroid hormones are fundamental during anuran metamorphosis. However, tail locomotion (Wassersug, 1975; Handrigan and Wassersug, 2007) and quadruped locomotion (Ročková and Roček, 2005; Manzano et al., 2013; Fabrezi et al., 2014) imply different tissues and developmental processes: tail locomotion disappears and quadruped morphology appears without remodeling. We observed certain parallelism in the response to methimazole and T4 suggesting the occurrence of a temporal and local sensitivity to the TH in the tissues precursors of limb and girdles during premetamorphic stages (25–35). Furthermore, thyroid gland inhibition by methimazole in *Xenopus subtropicalis* at larval stages of limb differentiation also prevents the formation of the hypochord, which is part of the urostyle (Senevirathne et al., 2020).

To conclude, we have analyzed endocrine disruption by testing two thyroid active substances (methimazole and T4) during larval development of the frog *Pleurodema borellii* in mesocosm experiments. We found the concentrations and exposure time affect thyroid gland histomorphology without suppression of glandular activity. The sensitivity of larval stages of premetamorphosis (Gosner Stages 25–36) to disruptors leads to modification of growth and differentiation rates. These changes in developmental rates resemble patterns of larval development of other anuran species. Even when antagonistic disruptors were tested, exposure to a low concentration of methimazole and intermediate concentration of T4 produced convergent abnormal morphogenesis in pelvic and pectoral girdles, and the sequence of disappearance of the vent tube revealing the role of thyroid axis signals acting appendicular morphogenesis much before metamorphosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Secretaría de Medio Ambiente y Desarrollo Sustentable, Gobierno de la Provincia de Salta, Argentina, and adhered to the legal requirements of Argentine laws (File number 227–216600/064/2016).

AUTHOR CONTRIBUTIONS

MF contributed to the conception, design, performed the experiments and morphological records, and wrote the first draft of the manuscript. JC was responsible for histology and statistics and wrote sections of the manuscript. MF and JC contributed to manuscript revision, read, and approved the submitted version. Both authors contributed to the article and approved the submitted version.

FUNDING

MF and JC were funded by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). This study was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2018-1383) to MF. The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

ACKNOWLEDGMENTS

MF was grateful to Veronica Laura Lozano for some helpful suggestions for experimental mesocosm designs some years ago. The permissions to collect and maintain the animals in the laboratory were obtained from the Secretaría de Medio Ambiente y Desarrollo Sustentable, Gobierno de la Provincia de Salta, Argentina, and adhered to the legal requirements of Argentine laws (File number 227–216600/064/2016). *Pleurodema borellii* is a frog commonly available with category of least concerned and stable populations for IUCN.

REFERENCES

Bancroft, J. D., and Gamble, M. (2002). *Theory and Practice of Histological Techniques*. Amsterdam: Elsevier Health Sciences.

Buchholz, D. R. (2017). *Xenopus* metamorphosis as a model to study thyroid hormone receptor function during vertebrate developmental transitions. *Mol. Cell. Endocrinol.* 459, 64–70. doi: 10.1016/j.mce.2017.03.020

Cai, L., and Brown, D. D. (2004). Expression of type II iodothyronine deiodinase marks the time that a tissue responds to thyroid hormone induced metamorphosis in *Xenopus laevis*. *Dev. Biol.* 266, 87–95. doi: 10.1016/j.ydbio.2003.10.005

Callery, E. M., and Elinson, E. M. (2000). Thyroid hormone-dependent metamorphosis in a direct developing frog. *Proc. Natl. Acad. Sci. U.S.A.* 14, 2615–2620. doi: 10.1073/pnas.050501097

Choi, J., Ishizuya-Oka, A., and Buchholz, D. R. (2017). Growth, development, and intestinal remodeling occurs in the absence of thyroid hormone receptor α in tadpoles of *Xenopus tropicalis*. *Endocrinology* 158, 1623–1633. doi: 10.1210/en.2016-1955

Cruz, J. C. (2020). Histo-morphology of the thyroid gland during the larval development of *Pleurodema borellii* (Anura, Leptodactylidae). *Acta Zool.* 1–9. doi: 10.1111/azo.12354

Cruz, J. C., and Fabrezi, M. (2019). Histology and microscopic anatomy of the thyroid gland during the larval development of *Pseudis platensis* (Anura, Hylidae). *J. Morphol.* 281, 122–134. doi: 10.1002/jmor.21085

de Sá, R. O., Grant, T., Camargo, A., Heyer, W. R., Ponssa, M. L., and Stanley, E. (2014). Systematics of the neotropical genus *Leptodactylus* Fitzinger, 1826 (Anura: Leptodactylidae): phylogeny, the relevance of non-molecular evidence, and species accounts. *S. Am. J. Herpetol.* 9, S1–S128. doi: 10.2994/SAJHD-13-00022.1

Degitz, S. J., Holcombe, W. G., Flynn, K. M., Kosian, P. A., Korte, J. J., and Tietge, J. E. (2005). Progress towards development of an amphibian-based thyroid screening assay using *Xenopus laevis*. Organismal and thyroidal responses to the model compounds 6-propylthiouracil, methimazole, and thyroxine. *Toxicol. Sci.* 87, 353–364. doi: 10.1093/toxsci/kfi246

Emerson, S. B. (1988). The giant tadpole of *Pseudis paradoxa*. *Biol. J. Linn. Soc.* 34, 93–104. doi: 0.1111/j.1095-8312.1988.tb01951.x

Etkin, W. (1936). The phenomena of anuran metamorphosis. III. The development of the thyroid gland. *J. Morphol.* 59, 69–89. doi: 0.1111/j.1095-8312.1988.tb01951.x

Fabrezi, M. (2011). Heterochrony in growth and development in anurans from the Chaco of South America. *Evol. Biol.* 38, 390–411. doi: 10.1007/s11692-011-9128-5

Fabrezi, M., and Cruz, J. C. (2014). Ontogeny of the thyroid glands during larval development of South American horned frogs (Anura, Ceratophryidae). *Evol. Biol.* 41, 606–618. doi: 10.1007/s11692-014-9292-5

Fabrezi, M., Lozano, V. L., and Cruz, J. C. (2019). Differences in responsiveness and sensitivity to exogenous disruptors of the thyroid gland in three anuran species. *J. Exp. Zool. B Mol. Dev. Evol.* 332, 279–293. doi: 10.1002/jez.b.22908

Fabrezi, M., Manzano, A. S., Abdala, V., and Lobo, F. (2014). Anuran locomotion: ontogeny and morphological variation of a distinctive set of muscles. *Evol. Biol.* 41, 308–326. doi: 10.1007/s11692-014-9270-y

Fabrezi, M., Quinzio, S. I., and Goldberg, J. (2009). The giant tadpole and delayed metamorphosis of *Pseudis platensis* Gallardo, 1961 (Anura. Hylidae). *J. Herpetol.* 43, 228–243. doi: 10.1007/s11692-014-9292-5

Gosner, K. (1960). A simplified table for staging anurans embryos and larvae, with notes on identification. *Herpetologica* 16, 183–190.

Grim, K. C., Wolfe, M., Braunbeck, T., Iguchi, T., Ohta, Y., Ttooi, Y., et al. (2009). Thyroid histopathology assessments for the amphibian metamorphosis assay to detect thyroid-active substances. *Toxicol. Pathol.* 37, 415–424. doi: 10.1177/0192623309335063

Handrigan, G. R., and Wassersug, R. J. (2007). The anuran *Bauplan*: a review of the adaptive, developmental, and genetic underpinnings of frog and tadpole morphology. *Biol. Rev.* 82, 1–25. doi: 10.1111/j.1469-185X.2006.00001.x

Hanken, J., and Summers, C. H. (1988). Skull development during anuran metamorphosis: III. Role of thyroid hormone in chondrogenesis. *J. Exp. Zool.* 246, 156–170. doi: 10.1002/jez.1402460208

Jennings, D. H., and Hanken, J. (1998). Mechanistic basis of life history evolution in anuran amphibians: thyroid gland development in the direct-developing frog, *Eleutherodactylus coqui*. *Gen. Comp. Endocr.* 111, 225–232. doi: 10.1006/gcen.1998.7111

Kupferberg, S. J. (1997). The role of larval diet in anuran metamorphosis. *Am. Zool.* 37, 146–159. doi: 10.1093/icb/37.2.146

Laslo, M., Denver, R. J., and Hanken, J. (2019). Evolutionary conservation of thyroid hormone receptor and deiodinase expression dynamics in ovo in a direct-developing frog, *Eleutherodactylus coqui*. *Front. Endocrinol.* 10:307. doi: 10.3389/fendo.2019.00307

Manzano, A., Abdala, V., Ponssa, M. L., and Soliz, M. (2013). Ontogeny and tissue differentiation of the pelvic girdle and hind limbs of anurans. *Acta Zool.* 94, 420–436. doi: 10.1111/j.1463-6395.2012.00571.x

Martoja, R., and Martoja-Pierson, M. (1970). *Técnicas de Histología Animal*. Barcelona: Toray-Masson.

Naumann, B., Schweiger, S., Hammel, J. U., and Müller, H. (2020). Parallel evolution of direct development in frogs – skin and thyroid gland development in African squeaker frogs (Anura: Arthroleptidae: *Arthroleptis*). *Dev. Dyn.* 250, 584–600. doi: 10.1101/2020.09.07.286476

Opitz, R., Hartmann, S., Blank, T., Braunbeck, T., Lutz, I., and Kloas, W. (2006). Evaluation of histological and molecular endpoints for enhanced detection of thyroid system disruption in *Xenopus laevis* tadpoles. *Toxicol. Sci.* 90, 337–348. doi: 10.1093/toxsci/kfj083

Phuge, S., Sequeira, A., and Pandit, R. (2021). Effect of ethylenethiourea on metamorphosis and ovary development: a comparative study of three larval frogs. *J. Exp. Zool. A Ecol. Integr. Physiol.* 335, 469–476. doi: 10.1002/jez.2464

Roček, Z., Böttcher, R., and Wassersug, R. J. (2006). Gigantism in the tadpoles of the Neogene frog *Paleobatrachus*. *Paleobiology* 32, 666–675. doi: 10.1666/05073.1

Ročková, H., and Roček, Z. (2005). Development of the pelvis and posterior part of the vertebral column in the Anura. *J. Anat.* 206, 17–35. doi: 10.1111/j.0021-8782.2005.00366.x

Roelants, K., Haas, A., and Bossuyt, F. (2011). Anuran radiations and the evolution of tadpole morphospace. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8731–8736. doi: 10.1073/pnas.1100633108

Rose, C. S. (2021). Amphibian hormones, calcium, physiology, bone weight, and lung use call for inclusive approach to understanding ossification sequence evolution. *Front. Ecol. Evol.* 9:620971. doi: 10.3389/fevo.2021.620971

Rose, C. S., and Cahill, J. W. (2019). How thyroid hormones and their inhibitors affect cartilage growth and shape in the frog *Xenopus laevis*. *J. Anat.* 234, 89–105. doi: 10.1111/joa.12897

Rose, C. S., Murawinski, D., and Horne, V. (2015). Deconstructing cartilage shape and size into contributions from embryogenesis, metamorphosis, and tadpole and frog growth. *J. Anat.* 226, 575–595. doi: 10.1111/joa.12303

Rot-Nikcevic, I., and Wassersug, R. J. (2004). Arrested development in *Xenopus laevis* tadpoles: how size constrains metamorphosis. *J. Exp. Biol.* 207, 2133–2145. doi: 10.1242/jeb.01002

Rot-Nikcevic, I., and Wassersug, R. J. (2003). Tissue sensitivity to thyroid hormone in athyroid *Xenopus laevis* larvae. *Dev. Growth Differ.* 45, 321–325. doi: 10.1046/j.1440-169x.2003.00700.x

Schreiber, A. M., Liquan, C., and Brown, D. D. (2005). Remodeling of the intestine during metamorphosis of *Xenopus laevis*. *Proc. Natl. Acad. Sci. U.S.A.* 102, 3720–3725. doi: 10.1073/pnas.0409868102

Senevirathne, G., Baumgart, S., Shubin, N., Hanken, J., and Shubin, N. H. (2020). Ontogeny of the anuran urostyle and the developmental context of evolutionary novelty. *Proc. Natl. Acad. Sci. U.S.A.* 117, 3034–3044. doi: 10.1073/pnas.1917506117

Shi, Y.-B. (2000). *Amphibian Metamorphosis: From Morphology to Molecular Biology*, 1st Edn. New York, NY: Wiley-Liss, 288.

Skelly, D. K., and Kiesecker, J. M. (2001). Venue and outcome in ecological experiments: manipulations of larval anurans. *Oikos* 94, 198–208. doi: 10.1034/j.1600-0706.2001.t01-1-11105.x

Smirnov, S. V., and Vassilieva, A. B. (2014). Thyroid hormones in the skeletogenesis and accessory sources of endogenous hormones in *Xenopus laevis* (Amphibia; Anura) ontogeny: experimental evidence. *Dokl. Biol. Sci.* 455, 136–138. doi: 10.1134/S0012496614020185

Smirnov, S. V., and Vassilieva, A. B. (2009). Number of ossification centers in the anuran cranial bones depends upon the rate of development: experimental evidence. *Russ. J. Herpetol.* 16, 167–176.

Trueb, L. (1993). "Patterns of diversity in the Lissamphibian skull," in *The Vertebrate Skull: Patterns of Structural and Systematic Diversity*, Vol. 2, eds J. Hanken, and B. K. Hall (Chicago, IL: University of Chicago Press), 255–343.

Van Dijk, D. E. (1959). On the cloacal region of Anura in particular of larval *Ascaphus*. *Ann. Univ. Stell.* 35A, 169–249.

Wassersug, R. J. (1975). The adaptive significance of the tadpole stage with comments on the maintenance of complex life cycles in anurans. *Am. Zool.* 15, 405–417. doi: 10.1093/icb/15.2.405

Wassersug, R. J. (1976). A procedure for differential staining of cartilage and bone in whole formalin fixed vertebrates. *Stain Technol.* 51, 131–134. doi: 10.3109/10520297609116684

Yaoita, Y. (2019). Tail resorption during metamorphosis in *Xenopus* tadpoles. *Front. Endocrinol.* 10:143. doi: 10.3389/fendo.2019.00143

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 04 November 2021
doi: 10.3389/fevo.2021.733947

Increasing Hormonal Control of Skeletal Development: An Evolutionary Trend in Amphibians

Anna B. Vassilieva and Sergei V. Smirnov*

A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia

The biphasic life history of amphibians includes metamorphosis, a complex developmental event that involves drastic changes in the morphology, physiology and biochemistry accompanying the transition from the larval to adult stage of development. Thyroid hormones (THs) are widely known to orchestrate this remodeling and, in particular, to mediate the development of the bony skeleton, which is a model system in evolutionary morphological studies of amphibians. Detailed experimental studies of the role of THs in the craniogenesis of diverse urodelan amphibians revealed that (i) these hormones affect both the timing and sequence of bone formation, (ii) TH involvement increases in parallel with the increase in divergence between larval and adult skull morphology, and (iii) among urodelans, TH-involvement in skull development changes from a minimum in basal salamanders (Hynobiidae) to the most pronounced in derived ones (Salamandridae and Plethodontidae). Given the increasing regulatory function of THs in urodelan evolution, we hypothesized a stronger involvement of THs in the control of skeletogenesis in anurans with their most complex and dramatic metamorphosis among all amphibians. Our experimental study of skeletal development in the hypo- and hyperthyroid yellow-bellied toad (*Bombina variegata*: Bombinatoridae) supports the greater involvement of THs in the mediation of all stages of anuran cranial and postcranial bones formation. Similar to urodelans, *B. variegata* displays enhancing TH involvement in the development of cranial bones that arise during larval ontogeny: while the hormonal impact on early larval ossifications is minimal, the skull bones forming during metamorphosis are strictly TH-inducible. However, in contrast to urodelans, all cranial bones, including the earliest to form, are TH-dependent in *B. variegata*; moreover, the development of all elements of the axial and limb skeleton is affected by THs. The more accentuated hormonal control of skeletogenesis in *B. variegata* demonstrates the advanced regulatory and inductive function of THs in the orchestration of anuran metamorphosis. Based on these findings, we discuss (i) changes in THs function in amphibian evolution and (ii) the role of THs in the evolution of life histories in amphibians.

Keywords: *Bombina*, heterochronies, life history, metamorphosis, skeletogenesis, thyroid hormones

OPEN ACCESS

Edited by:
Sarah Kelly McMenamin, Boston College, United States

Reviewed by:
Pedro Martinez, University of Barcelona, Spain
Daniel Buchholz, University of Cincinnati, United States

****Correspondence:***
Anna B. Vassilieva
vassil.anna@gmail.com

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *30 June 2021*
Accepted: *05 October 2021*
Published: *04 November 2021*

Citation:
Vassilieva AB and Smirnov SV (2021) Increasing Hormonal Control of Skeletal Development: An Evolutionary Trend in Amphibians. Front. Ecol. Evol. 9:733947. doi: 10.3389/fevo.2021.733947

INTRODUCTION

The amphibian life cycle consisting of water-dwelling larvae and terrestrial adults is unique among vertebrate animals. The main event in amphibian development is metamorphosis, a cascade of transformations at the molecular, biochemical, physiological, and morphological levels (for reviews, see, e.g., Fritzsch, 1990; Furlow and Neff, 2006; Brown and Cai, 2007). The extent of

this transformation differs in the different amphibian lineages. Caecilians, a very specialized group of fossorial or aquatic amphibians, display only moderate morphological reorganization limited mainly to the loss of gills and rebuilding of the palatal region of the skull and hyobranchium (Wake, 1989; Reiss, 1996). At metamorphosis, urodelans retain the overall larval body plan, although undergo profound changes in the external and internal morphology. In them, resorption of the external gills and caudal fins is accompanied by changes in the skull structure: larval provisory bones disappear, and adult bones appear (Rose, 2003). In anurans, metamorphic transformation is extremely profound and results in the remodeling of the body plan: fish-like aquatic larvae transform into tailless terrestrial animals adapted to jumping. At metamorphosis, the larval cartilaginous jaw apparatus is replaced by an adult bony apparatus, suspensorium undergoes rebuilding, and the jaw joint shifts posteriorly (Roček, 2003).

Based on these differences in the degree of metamorphic transformations, the evolution of amphibian metamorphosis was hypothesized to be a transition from slow, gradual, and ill-defined metamorphosis in the recent amphibians' ancestors, temnospondyls, to explosive, necrobiotic anuran metamorphosis with complete disappearance of some larval elements and/or complete remodeling of the others (Reiss, 2002; Schoch, 2009).

Thyroid hormones (THs) are well known to play a key role in the mediation of amphibian metamorphosis; mechanisms and functions of thyroid hormone signaling are widely described in numerous publications (for reviews, see, e.g., Galton, 1992; Tata, 1999; Fort et al., 2007; Grimaldi et al., 2013). The development and functional dynamics of the thyroid gland have been studied in a number of anuran and urodelan species; typically, in biphasic amphibians completing metamorphosis, the level of THs is low in early larval stages, rises closer to metamorphosis, reaches its peak at metamorphic climax when most transformation occurs and then lowers after full metamorphic transformation (for anurans, see, e.g., Regards et al., 1978; Weil, 1986; Weber et al., 1994; for urodelans, Eagleson and McKeown, 1978; Larras-Regard et al., 1981; Alberch et al., 1986). The role of THs in the evolutionary diversification of amphibian developmental patterns (metamorphosis, neoteny, and direct development) is rather well documented (Laudet, 2011). However, while our knowledge on the function of the thyroid axis at the level of thyroid receptors, gene expression and interplay with different endocrine factors, among others, is rather extensive, the role of THs in "the last link," morphogenesis, remains rather poorly studied.

TH-mediation in the development of the amphibian skeleton is also relatively poorly studied. This is extremely surprising since the skeleton is a traditional model system for evo-devo investigation. Thus, in amphibians, most studies on different heterochronies (now considered one of the main mechanisms of evolutionary changes) are based on data from skeletal morphology (e.g., Reilly et al., 1997; Schlosser, 2001; Sheil et al., 2014).

Although skeletal changes accompanying amphibian metamorphosis are proposed to be TH-mediated, concrete studies aimed at investigating this phenomenon are rather rare. Apodans remain unstudied in this respect. Compared with them, urodelans (salamanders) seem to be rather well studied. Thus, dysfunction of the thyroid axis was revealed to account for the absence of several bones in neotenic salamanders retaining larval cranial morphology as an adult morphology (Larsen, 1968; Rosenkilde and Ussing, 1996; Rose, 2003). Thyroid axis involvement in the cranial development of plethodontids and salamandrids was estimated in a series of experiments with exogenous TH and goitrogen treatment of larvae developing in a regime of high and low TH levels or hypophysectomized larvae (Rose, 1995a,b, 1996; Clemen and Greven, 2018; Ajduković et al., 2021).

In our laboratory, the role of THs in the skeletal development of salamanders has been studied during the last two decades. For this aim, changes in skeletal morphology (timing and sequence of bone appearance) were compared in larvae reared under different TH regimes: normal (in control larvae), low (under treatment with a goitrogen, thiourea, causing TH-deficiency), and high (under treatment with exogenous TH, triidothyronine at different concentrations). This standard protocol of the experiment was applied to both the basal and derived salamanders: the Siberian newt *Salamandrella keyserlingii* (Hynobiidae), axolotl *Ambystoma mexicanum* (Ambystomatidae), the Ribbed newt *Pleurodeles waltl* and Smooth newt *Lissotriton vulgaris* (Salamandridae) (Smirnov and Vassilieva, 2003, 2005; Smirnov et al., 2011, 2020). Accumulated evidence suggests that urodelan evolution is accompanied by an increasing TH-impact on cranial metamorphic remodeling (Smirnov, 2006).

Concerning anurans, similar experimental studies are relatively scarce. Although the pattern of their metamorphosis proposes an important role of THs in the orchestration of numerous complex transformations occurring in synchrony, our knowledge on the role of THs in anuran skeletal development remains fragmentary. Thus, TH-treatment was shown to accelerate the development of several cranial bones in *Lithobates pipiens* (Ranidae) and in the Oriental fire-bellied toad *Bombina orientalis* (Bombinatoridae) (Kemp and Hoyt, 1965a,b; Hanken and Hall, 1988b). Additionally, treatment with goitrogens inhibiting thyroid function or exogenous thyroid hormones resulted in changes in the rate of metamorphic transformations in the chondrocranium of *B. orientalis,* Clawed frog *Xenopus laevis* (Pipidae), and direct-developing frog Puerto Rican Coqui *Eleutherodactylus coqui* (Eleutherodactylidae) (Hanken and Summers, 1988; Callery and Elinson, 2000; Rose and Cahill, 2019). Our preliminary experiments revealed that in *X. laevis* and the Common frog *Rana temporaria* (Ranidae), development of most cranial bones is dependent upon the TH-level (Vassilieva and Smirnov, 2007; Smirnov and Vassilieva, 2014). Recent experiments involving parallel TH and goitrogen treatment of several non-model leptodactylid frogs revealed, correspondingly, accelerating and inhibiting effects on some cranial bones (Fabrezi et al., 2019). Moreover, THs seem to influence the development of the postcranial skeleton since ossification of limbs and axial skeleton was shown to be TH-mediated (Kemp and Hoyt, 1969; Fabrezi et al., 2019; Senevirathne et al., 2020).

Although this evidence does not provide a complete picture of the role of THs in skeletal development, it proposes that THs are involved in the mediation of metamorphic transformation to a greater extent in anurans than in urodelans. To test this hypothesis, we experimentally studied the role of THs in the skeletal development of the Yellow-bellied toad *Bombina variegata* (Bombinatoridae). To make the results comparable, we used the same experimental protocol both for this toad and salamanders previously studied by us.

The reason we chose this toad to study is that (i) the morphology and skeletal development of *Bombina* were previously described in detail (e.g., Maglia and Púgener, 1998; Lukas and Olsson, 2020; Roček et al., 2021), (ii) *B. orientalis* was used in pioneering experimental studies on the TH-mediation of cranial development in anurans (Hanken and Hall, 1988b; Hanken and Summers, 1988), and (iii) *B. variegata* belongs to the basal anuran family.

Based on our observations and published data on TH-involvement in the skeletal development of anurans and urodelans, we discuss (i) the evolution of TH-function in the amphibian phylogeny and (ii) the putative role of THs in the evolution of amphibian life histories.

MATERIALS AND METHODS

The ontogenetic series of *Bombina variegata* larvae were obtained and reared under laboratory conditions in two sets of experiments using different concentrations of TH. Egg clutches for Experiment I performed in 2007 derived from the breeding group of five males and five females of *B. variegata* obtained from trade. Breeding was stimulated by injections of the adults with chorionic gonadotropin. Clutches for Experiment II performed in 2019 derived from spontaneous spawning of a *B. variegata* breeding group (four males and three females) housed in the batrachological section in the Moscow Zoo (Moscow, Russia). Larvae were reared in aquaria with different experimental media: (i) clear tap water (controls); (ii) alkaline solutions of 3,3^1,5-triiodothyronine (T_3; Sigma Chemical Co., Germany) at concentrations of 2×10^{-8} M, 2×10^{-9} M, and 2×10^{-10} M; and (iii) 0.02% and 0.04% solutions of goitrogen thiourea (TU; Solins, Russia). Previously, similar concentrations of T_3 and TU were used in experimental studies of the TH-effect on skeletogenesis in urodelan (Smirnov and Vassilieva, 2003, 2005; Smirnov et al., 2011, 2020) and anuran amphibians (Vassilieva and Smirnov, 2007; Smirnov and Vassilieva, 2014). In Experiment I, treatment of the different groups of larvae with T_3 and TU started at 6 and 7 days posthatching (dph), respectively, soon after the beginning of exogenous feeding. In Experiment II, T_3-treatment of tadpoles started at six dph and at 15 dph. In total, 206 and 106 specimens were used in Experiments I and II, respectively; for details, see **Table 1**.

The animals from every experimental and control group were kept at the same density (120 L aquaria, each initially containing approximately 30 larvae) and temperature 18–22°C under a natural light regime. One-half of the rearing medium was replaced every day. The larvae were fed *ad libitum* with chopped and scalded common nettle leaves (*Urtica dioica*). The larvae were reared until the end of metamorphosis (normal and TH-induced) or until the end of the experiment for the TU-treated individuals (over 120 days for several specimens). The larvae were sampled at regular intervals (every day or every 2nd day for controls and TU-treated larvae, every day for TH-treated larvae, and every week for TU-treated larvae older than 2 months) and fixed in 10% neutral buffered formalin. The larvae were staged following Gosner (1960), and the snout-vent length (SVL) was measured with an electronic caliper to the nearest 0.1 mm. One specimen, a mature male (SVL 41.2 mm) among breeding group (Experiment I), was used to examine adult skeletal morphology. All specimens were prepared as skeletal whole mounts. They were stained with Alizarin red to detect calcium deposits and cleared in KOH; additionally, three to five specimens from the control and experimental groups were stained with Alcian blue for cartilage following the protocol described by Wassersug (1976). The whole mounts were analyzed for skeleton development under a stereomicroscope (Olympus SZX7) with digital photo attachment. The timing of ossification was recorded as the first observable sign of Alizarin red staining. The terminology used for the bones and cartilages generally follows Maglia and Púgener (1998). For actual taxonomy, we followed an updated database by Frost (2021).

All procedures were carried out in accordance with the Severtsov Institute's Animal Ethics Committee.

RESULTS

Larval Development Under Different Hormonal Regimes

Larvae hatch after 6 days of embryonic development and start exogenous feeding on 4–5 dph. Control tadpoles start to leave the water at 30–31 dph, and the first of them complete metamorphosis at 33–34 dph.

At 2×10^{-10} M T_3, developing tadpoles do not differ externally from the norm, and the rate of metamorphosis is only slightly accelerated.

At 2×10^{-9} M T_3, larval development is strongly accelerated, resulting in precocious metamorphosis: the first metamorphs leave the water and attain St. 46 approximately 10 days before the norm, being markedly smaller in size (see **Table 1**). The external metamorphic changes of the head, tail, and skin structures anticipate Gosner's staging based on the development of the limbs: by St. 38, the tail fins start to reduce, the cloacal tail piece is absent, the keratodonts and mouth sheaths are lost, the snout shortens and the eyes become enlarged and more salient.

At 2×10^{-8} M T_3, metamorphic changes of the skin structures (reduction of tail fins, cloacal tail piece, and opercular fold) and mouthparts (loss of keratodonts and mouth sheaths) as well as tail resorption, head transformation (shortening of the snout, eyes and nostrils bulging), and larval gut remodeling, are greatly accelerated. The discrepancies between the staging based on limb development (differentiation of the digits) and head transformation attain several stages: e.g., after 14 days of treatment, at 20 dph, tadpoles with hindlimb differentiation

TABLE 1 | Specimens of *Bombina variegata* examined in the experimental study.

Experimental group	Beginning of stimulation (dph)	Experiment duration (dph)	Attained developmental stage	Number of specimens	SVL range (mm)
Experiment I (2007)					
Norm I	–	37	46	63	6.0–19.5
T_3 2×10^{-10} M	6	34	46	23	10.0–18.0
T_3 2×10^{-9} M	6	27	46	44	8.5–13.5
Thiourea 0.02%	7	75	43–44	34	9.0–19.0
Thiourea 0.04%	7	123	38–39	42	9.0–22.0
Experiment II (2019)					
Norm II	–	34	46	53	8.0–18.5
T_3 2×10^{-8} M early	6	20	35	24	5.0–6.6
T_3 2×10^{-8} M late	15	22	37/42	29	5.3–11.5

dph, days post hatching; SVL, snout-vent length.

corresponding to Stage 36 or 37 display the erupted forelimbs, oral disk loss and mouth width corresponding to Stage 42. These tadpoles stop feeding and do not develop further; at this stage, the experiment is terminated.

In goitrogen-treated (TU 0.02%) larvae, development is markedly retarded. The most advanced individuals reach St. 43–44 and begin to leave the water at 69–70 dph, but none of them complete metamorphosis during the experiment. Several individuals arrest development at St. 38. No specific changes in the external morphological features are observed in them compared with the norm.

Tadpoles treated with 0.04% TU stop developing at the late midlarval stages (38–39), and none of them begin any metamorphic transformations during the experiment. Many individuals display lateral curvatures of the posterior part of the vertebral column or tail.

Bony Skull Development Under Different Hormonal Regimes

Control Groups

The adult osteology and normal skeletal development of *B. variegata* revealed in our study are mostly consistent with those of *B. orientalis*, described in detail by Hanken and Hall (1984, 1988a) and Maglia and Púgener (1998). Adult morphology differs mainly in the absence of the interfrontal bone in the skull roof in *B. variegata* and the absence of the fusions of vertebrae I + II and VII + VIII, found in *B. orientalis* (Maglia and Púgener, 1998) but not observed in our adult specimen.

The typical sequence and staging of skeletal ossification in normally developing larvae are summarized in **Table 2**. The first signs of ossifications appear at the early St. 36, in approximately two-week-old, fast growing tadpoles. Cranial ossification usually starts with the dermal bone parasphenoid underlying the neurocranium. This is followed by the paired dermal frontoparietal in the skull roof. This midlarval skull development is completed by the formation of the first endochondral bone, the paired exoccipital, which appears behind the well chondrified otic capsule lateral to the foramen magnum.

The premetamorphic phase of development starting at St. 39 is characterized by the appearance of several paired dermal bones in the rostral part of the skull. The premaxilla arises on its frontal surface. The facial process of this bone is the first to form; it persists as a single ossified portion of the premaxilla until the onset of metamorphosis (**Figure 1**). The septomaxilla forms as a small semicircular ossicle close to the narial opening. The next bones to appear, the vomer on the skull base anterior to the parasphenoid and the nasal on the dorsal surface of the nasal capsules, form almost synchronously.

The metamorphic period beginning with the appearance of the forelimbs at St. 42 is characterized by a drastic head transformation and the formation of several dermal bones. The squamosal on the lateral side of the palatoquadrate and the angulosplenial on the ventral surface of Meckel's cartilage arise simultaneously. At the same time, the dental process of the premaxilla forms ventral to the facial process and then fuses to the latter, forming an entire bone. The maxilla appears lateral to the premaxilla in the upper jaw and grows toward the corners of the laterally expanding mouth opening. The dentary arises in the lower jaw medial and rostral to the angulosplenial. The quadratojugal forms on the lateral surface of the palatoquadrate near the jaw joint; next, the pterygoid appears on the inner surface of the same cartilage in the oral cavity roof. Finally, the endochondral prootic bone appears in the anterior part of the otic capsule; by metamorphosis completion, the otic capsule becomes partially ossified due to the expanding growth of the exoccipital and prootic in its posterior and anterior parts, respectively. Toward the end of metamorphosis, at St. 46, the only hyobranchial ossification, the endochondral os thyroideum appears as paired thin rod-like anlagen, but in our series, it is present in only one of five postmetamorphic animals.

In the samples studied, the endochondral mentomeckelian and sphenethmoid fail to appear in the young metamorphs; their formation is shifted to postmetamorphic development.

The sequence of skeletal development is almost invariable: in both control groups, only in a single specimen does the

TABLE 2 | Sequence of skeletal ossification in *Bombina variegata* listed as the earliest bones appearance (normal development).

Stage	Skull	Axial skeleton	Hind limb and pelvic girdle	Forelimb and pectoral girdle
36	Parasphenoid Frontoparietal	First neural arches	Femur Fibula Tibia Tarsals	Humerus Ulna Radius
37	Exoccipital	Neural arches I–VIII Neural arch IX First centra	Ilium First metatarsals	Cleithrum First metacarpals [Coracoid, clavicle] Scapula
38		Neural arch X Hypochord	First phalanges	First phalanges
39	Premaxilla	Neural arch XI		
40	Septomaxilla	Ribs	All phalanges	All phalanges
41	[Vomer, nasal]			
42	[Squamosal, angulosplenial]		Ischium	
43	Maxilla Dentary			
44	Quadratojugal			
45	Pterygoid Prootic			
46	Os thyroideum	Urostyle formed		

Elements in [brackets] appear simultaneously or the sequence of their appearance is not resolved.

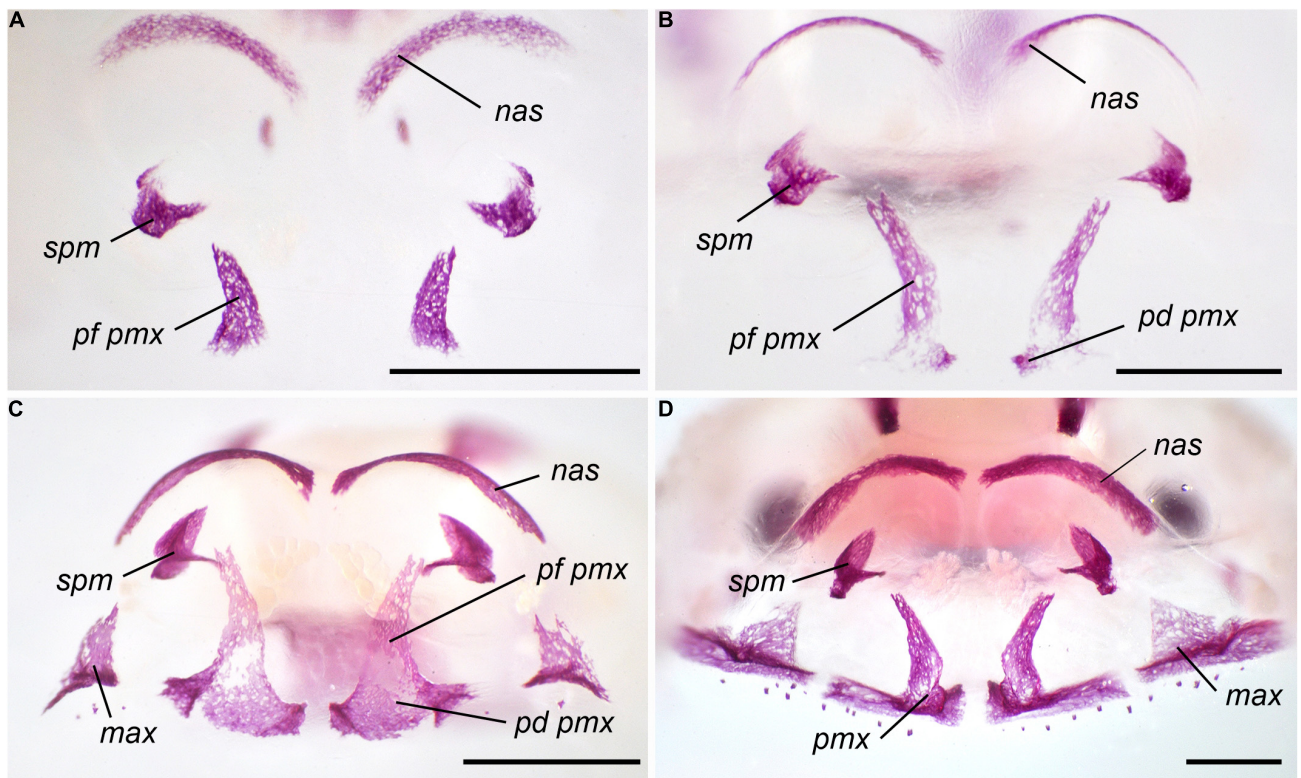


FIGURE 1 | Normal development of the premaxilla in *Bombina variegata*. **(A)** Stage 37; **(B)** Stage 42; **(C)** Stage 44; **(D)** Stage 45. Abbreviations: *max,* maxilla; *nas,* nasal; *pd pmax,* dental process of the premaxilla; *pf pmx,* facial process of the premaxilla; *pmx,* premaxilla; *spm,* septomaxilla. Scale bar 1 mm.

parasphenoid appear after the frontoparietals. The observed variability between Norm I and Norm II is limited mostly to temporal differences; thus, in Norm I, ossification starts on the 14th dph and is completed in 23 days, by 37 dph, in contrast to the 20th dph and 14 days, by 34 dph, respectively, in Norm II (**Table 3**).

TABLE 3 | Timing (the earliest registration in dph) of cranial bones appearance in larval *Bombina variegata* under different experimental regimes.

Bone	Experiment 2007					Experiment 2019		
	Norm I	T_3		TU		Norm II	T_3	
		2×10^{-10} M	2×10^{-9} M	0.02%	0.04%		2×10^{-8} M early	2×10^{-8} M late
Parasphenoid	14	14	14	17	22	22	17	22
Frontoparietal	14	14	14	17	22	20	17	22
Exoccipital	18	17	15	20	59	22	–	24
Premaxilla	23	19	15	48	–	24	17	22
Septomaxilla	26	22	18	48	–	26	17	22
Vomer	28	19	15	52	–	28	18	24
Nasal	28	23	18	52	–	28	–	–
Angulosplenial	29	23	18	52	–	28	18	24
Squamosal	29	25	21	52	–	28	18	24
Maxilla	30	29	22	60	–	28	18	24
Dentary	30	33	22	63	–	28	17	24
Quadratojugal	32	33	27	–	–	32	18	24
Pterygoid	33	34	26	–	–	34	–	–
Prootic	33	34	–	–	–	34	–	–
Os thyroideum	37	–	–	–	–	34	–	–

T_3-Treated Tadpoles

Changes in the timing and sequence of cranial bone appearance in T_3-treated tadpoles are summarized in **Tables 3**, **4**.

At the lowest T_3 concentration, 2×10^{-10} M, the timing of the appearance of the first skull bones, parasphenoid and frontoparietal, remains unchanged compared with the norm. Other ossifications normally arising in the midlarval, premtamorphic, and early metamorphic periods (exoccipital, premaxilla, septomaxilla, vomer, nasal, angulosplenial, and squamosal) appear somewhat precociously compared with the norm (**Table 3**). Additionally, they are slightly shifted to earlier developmental stages: the premaxilla and vomer appear in Stages 37–38, and the angulosplenial, squamosal, and nasal appear in Stages 39–40. Moreover, the sequence of bone appearance is somewhat altered: the vomer arises precociously, before the septomaxilla, nasal, and angulosplenial, thus changing its sequence position from 6–7 to 5 (**Table 4**). Bones normally appearing at late metamorphosis, in St. 43–46 (maxilla, dentary, quadratojugal, pterygoid, and prootic) form in the same sequence and at approximately the same time as in controls (Norm I), except for the os thyroideum, which fails to appear during the experiment.

At the higher T_3 concentration of 2×10^{-9} M, no changes in the timing and stage of the appearance of the parasphenoid and frontoparietals are detected, whereas the formation of all remaining cranial bones is strongly affected in both timing and sequence. They form faster than both in the norm and under T_3 2×10^{-10} M and mostly at earlier developmental stages. Thus, the premaxilla and vomer arise simultaneously with the slightly accelerated exoccipital at St. 37, followed during the same stage by the simultaneously forming septomaxilla, nasal, and angulosplenial and then squamosal, dentary and maxilla (St. 37–38). The appearance of the pterygoid varies within stages 38–40 and slightly anticipates the quadratojugal, thus changing its sequence position from 13 to 12 (**Table 4**). The prootic and os thyroideum do not form in this group by the end of metamorphosis. The developmental pattern of individual bones is somewhat affected: in the developing premaxilla, the dental process either almost immediately follows the facial process, or its separate anlage is not observed, and the premaxilla seems to appear as an entire bone. Often, the vomer develops from two separate ossifications, the medial and lateral centers. In single specimens, the nasal, squamosal, and pterygoid also ossify from two anlagen.

At the highest T_3 concentration, 2×10^{-8} M, beginning from early developmental stages (6 dph), the timing and sequence of bone appearance are strongly altered (**Tables 3**, **4**). Unlike the norm, the first to appear are the premaxilla and dentary (**Figure 2**), which change their sequence positions from 4 and 11, respectively, to 1-2 in both. They are closely followed by the frontoparietal (shifting from 1–2 to 3) and, almost immediately, by the parasphenoid and septomaxilla (shifting from 1–2 and 5, respectively, to 4–5 in both). The appearance of the vomer, greatly accelerated relative to the norm, is anticipated by the more or less simultaneous formation of squamosal, angulosplenial, maxilla (which fuses laterally to the premaxilla, **Figure 3A**), and quadratojugal. By shifting its sequence position from 6–7 to 10, the vomer becomes the last bone to form, since the remaining several dermal and endochondral bones fail to appear by the end of the experiment.

At 2×10^{-8} M T_3 beginning from the midlarval stages (15 dph), the appearance of the earliest bones normally arising at St. 36–37 (parasphenoid, frontoparietal, and exoccipital) remains mostly unchanged. Other bones display a marked acceleration of their appearance (**Table 3**) as well as alterations in the order of their appearance: the premaxilla

TABLE 4 | Sequence of cranial bones appearance in larval *Bombina variegata* under different experimental regimes.

Sequence position	Norm I–II	T_3 2 × 10^{-10} M	T_3 2 × 10^{-9} M	T_3 2 × 10^{-8} M early	TU 0.02%
1	Parasphenoid	[Parasphenoid	[Parasphenoid	[Premaxilla	[Parasphenoid
2	Frontoparietal	Frontoparietal]	Frontoparietal]	Dentary]	Frontoparietal]
3	Exoccipital	Exoccipital	[Exoccipital	Frontoparietal	Exoccipital
4	Premaxilla	Premaxilla	Premaxilla	[Parasphenoid	[Premaxilla
5	Septomaxilla	Vomer	Vomer]	Septomaxilla]	Septomaxilla]
6	[Vomer	Septomaxilla	[Septomaxilla	[Squamosal	[Vomer
7	Nasal]	[Nasal	Nasal	Angulosplenial	Nasal
8	[Squamosal	Angulosplenial]	Angulosplenial]	Maxilla	Squamosal
9	Angulosplenial]	Squamosal	Squamosal	Quadratojugal]	Angulosplenial]
10	Maxilla	Maxilla	[Dentary	Vomer	Maxilla
11	Dentary	Dentary	Maxilla]	*Exoccipital,*	Dentary
12	Quadratojugal	Quadratojugal	Pterygoid	*Nasal, Pterygoid,*	*Quadratojugal,*
13	Pterygoid	[Pterygoid	Quadratojugal	*Prootic, and Os*	*Pterygoid*,
14	Prootic	Prootic]	*Prootic and Os*	*thyroideum do*	*Prootic, and Os*
15	Os thyroideum	*Os thyroideum does not form*	*thyroideum do not form*	*not form*	*thyroideum do not form*

Bones in [brackets] appear simultaneously or the sequence of their appearance is not resolved.

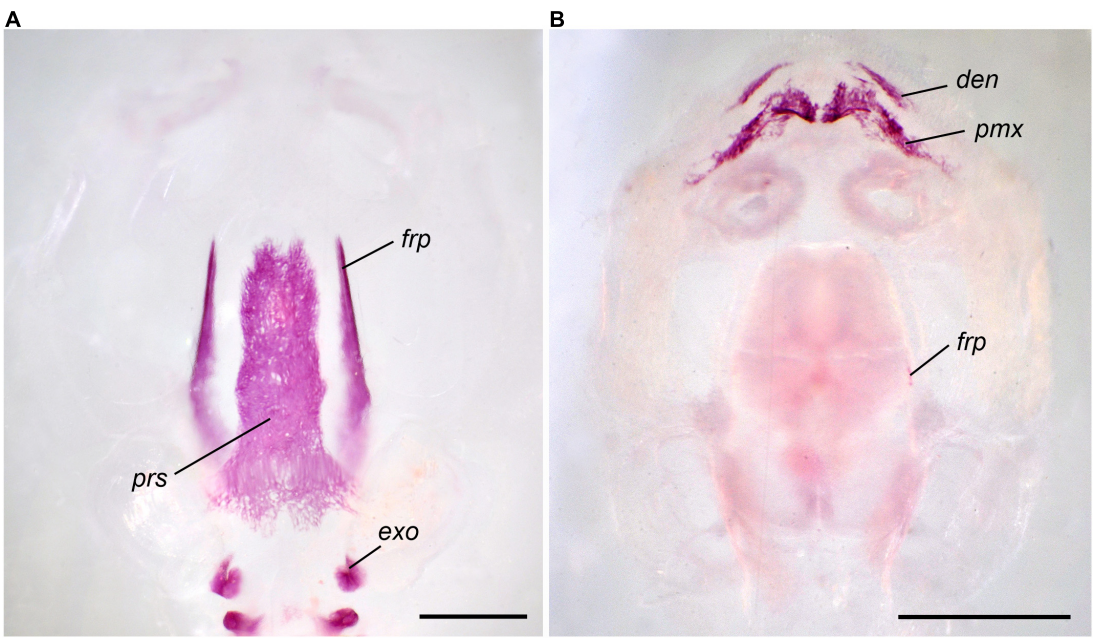


FIGURE 2 | First cranial bones to appear in *Bombina variegata:* **(A)** in norm II, Stage 37, 24 dph; **(B)** in T_3 2 × 10^{-8} M (early), Stage 35, 17 dph. Abbreviations: *den,* dentary; *exo,* exoccipital; *frp,* frontoparietal; *pmx,* premaxilla; *prs,* parasphenoid. Scale bar 1 mm.

and septomaxilla immediately follow the parasphenoid and frontoparietal, thus appearing before the exoccipital. The squamosal, maxilla, angulosplenial, dentary, and quadratojugal arise nearly simultaneously, followed immediately by the vomer. The remaining bones (nasal, prootic, pterygoid, and os thyroideum) do not appear during the experiment. Some bones display changed pattern of development. Thus, in contrast to the norm, the formation of the premaxilla begins with its dental part and not with the facial process (**Figure 4**). Additionally, the premaxilla fuses with the maxilla

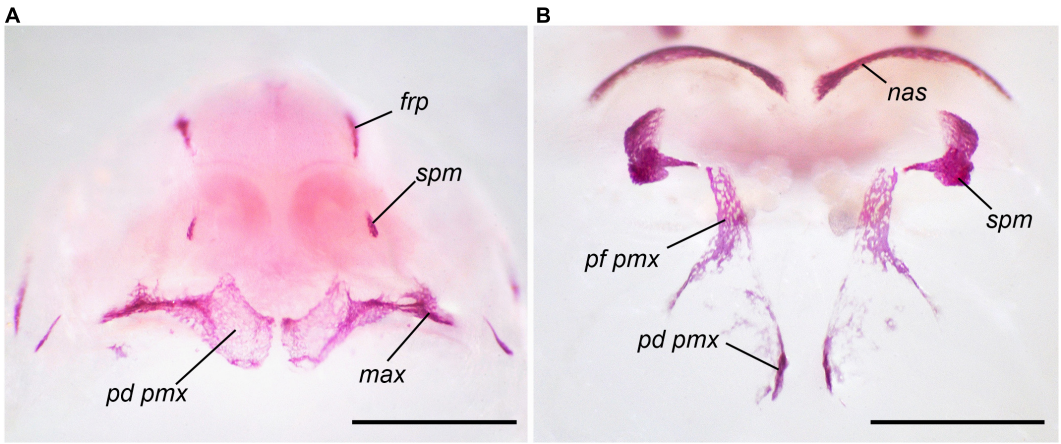


FIGURE 3 | Development of the premaxilla in *Bombina variegata*: **(A)** in T_3 2×10^{-8} M (early), Stage 35, 18 dph; **(B)** in TU 0.02%, Stage 43, 75 dph. For abbreviations, see **Figures 1**, **2**. Scale bar 1 mm.

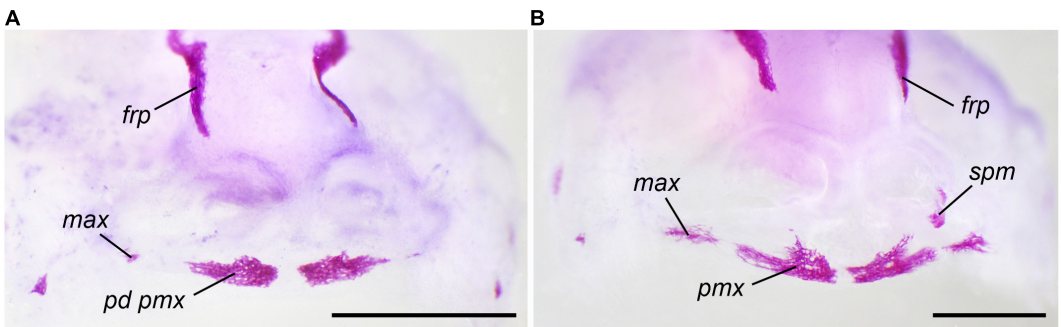


FIGURE 4 | Development of the premaxilla and maxilla in *Bombina variegata* at T_3 2×10^{-8} M (late): **(A)** Stage 36, 22 dph **(B)** Stage 37, 24 dph. For abbreviations, see **Figures 1**, **2**. Scale bar 1 mm.

on each side of the skull to form a paired compound bone that expands laterally and occupies the entire upper jaw. The facial processes of both the premaxilla and maxilla remain low and underdeveloped. Multiple centers of ossification are detected in the developing parasphenoid and frontoparietal bones (**Figure 5**).

TU-Treated Tadpoles

At TU 0.02%, bones forming in the midlarval, premetamorphic and early metamorphic periods appear in the same sequence as in the norm, but the timing of their appearance is delayed (**Tables 3**, **4**). Bones normally appearing close to metamorphosis (quadratojugal, pterygoid, prootic, and os thyroideum) fail to form even after four months of the experiment. Certain dermal bones display multiple ossification centers in their development; e.g., two ossified anlagen are usually detected in the angulosplenial (the medial and the lateral one); in the premaxilla, the facial process and dental portion remain separate almost until the end of the experiment (**Figure 3B**).

At TU 0.04%, only three of the first appearing bones (parasphenoid, frontoparietal, and exoccipital) arise, and the appearance of the last is strongly delayed relative to the norm (**Table 3**). Multiple ossification centers usually appear in the developing frontoparietal and parasphenoid.

Effect of T_3-Treatment on the Larval Chondrocranium

The pattern of remodeling of the larval chondrocranium and hyobranchium in *B. variegata* at 2×10^{-8} M T_3 (Experiment II, treatment started at 6 and 15 dph) is mostly consistent with that in TH-treated *B. orientalis*, described in detail by Hanken and Summers (1988).

Tadpoles sampled after 5, 7, 9, and 11 days of T_3-treatment display gradual metamorphic transformations of the larval cartilaginous elements of the skull and hyobranchium well before such remodeling starts in the norm. The timing of these transformations does not depend upon the stage and time of the onset of the treatment. In both groups (with early and late beginning of T_3-administration), the first signs of the remodeling (partial resorption of the medial part of the suprarostral cartilages and the rostral ends of the trabecular horns, and the disappearance of the joint between the infrarostral and Meckelian cartilages) are clearly apparent

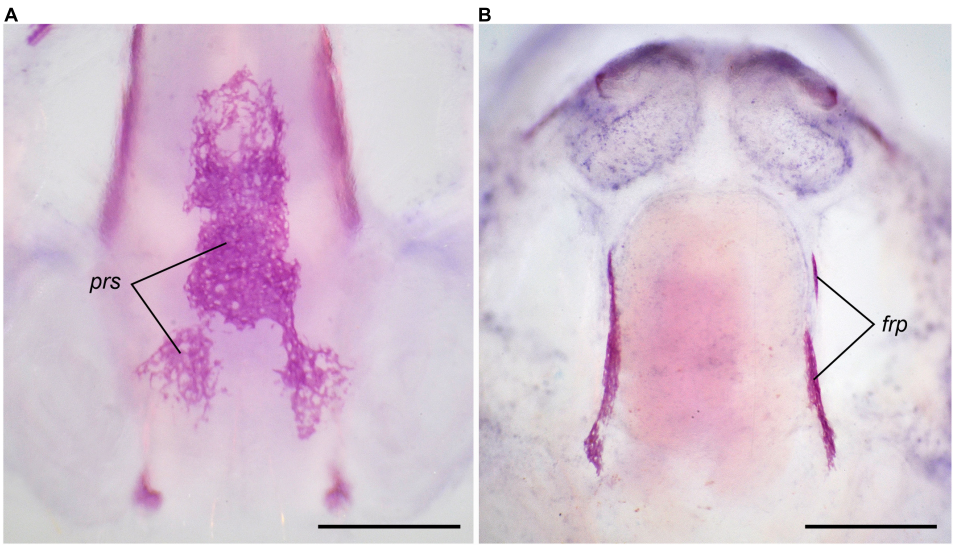


FIGURE 5 | Multiple ossification centers in *Bombina variegata* at T_3 2×10^{-8} M (late): **(A)** in the parasphenoid, Stage 37, 24 dph **(B)** in the frontoparietal, Stage 36, 22 dph. For abbreviations, see **Figures 1**, **2**. Scale bar 1 mm.

after 5 days of treatment (11 dph and 20 dph, respectively). After 9 days of T_3-administration, in comparison to the norm (**Figures 6A,B**) of the same age, the T_3-treated tadpoles display a transitional stage of cartilage remodeling normally characteristic of early metamorphic St. 42–43. In them, the suprarostral cartilage is mostly resorbed, retaining only the rudiments of the lateral alae; the trabecular horns are strongly eroded; palatoquadrate displays partial erosion of the anterior quadratocranial commissures and ascending processes; a thin laminar cartilage appears on the place of the future roof of the nasal capsule, forming the primordium of the cartilaginous tectum nasi; infrarostral cartilage fuses with Meckelian cartilage, forming a single cartilaginous mandible (**Figure 6C**); in the hyobranchium, the basihyal (copula I) is resorbed, and the ceratobranchial cartilages become thinner and lose the transverse commissures (**Figure 6D**). After 11 days of T_3-treatment, the suprarostral cartilage and trabecular horns disappear entirely, and the lower jaw achieves its metamorphic shape. However, remodeling of the palatoquadrate is somewhat delayed; although the subocular arcs are eroded, the anterior quadratocranial commissures and ascending processes still persist; therefore, the joint with the Meckelian cartilage is not displaced backward, and the transformed lower jaw protrudes forward of the upper jaw (**Figure 6E**). In the hyobranchium, the resorption of the ceratohyal is in progress (**Figure 6F**).

Development of the Postcranial Bony Skeleton Under Different Hormonal Regimes

In normal development, ossification of the axial skeleton begins with the basal parts of neural arches II–IV, more or less simultaneously with the formation of the first cranial bones at early St. 36 (**Table 2**). Neural arch of the vertebra I starts to ossify with some delay, when the arches of vertebrae II–V are already ossified. During the midlarval period, all neural arches of the presacral (I–VIII) and sacral (IX) vertebrae become ossified, and signs of ossification in the centra appear. By the end of St. 38, the neural arch of postsacral vertebra X ossifies simultaneously with the appearance of a thin elongated hypochord plate ventral to the notochord. During the premetamorphic period, the neural arch of the second postsacral vertebra (XI) ossifies and start to slightly elongate in the posterior direction. Soon, the neural arches of vertebrae X and XI fuse together, forming the primordium of the coccyx. By the end of metamorphosis, the coccygeal vertebrae fuse with the hypochord, completing the formation of the urostyle. During the metamorphic stages, the ribs fused to vertebrae II–IV progressively ossify.

Under T_3-treatment, elements of the axial skeleton ossify earlier relative to the norm (**Table 5**). Under TU-treatment, a delay in the ossification of the vertebrae is observed; the most delayed vertebrae under TU 0.02% are vertebrae X–XI forming the coccyx. Under TU 0.04%, the retardation of the development of vertebrae I–X is more pronounced. Ossifications normally appearing in premetamorphic and metamorphic periods (hypochord, vertebra XI, and ribs) do not arise at TU 0.04% until the end of the experiment and the urostyle does not form (**Table 5**).

In the normal development of the appendicular skeleton, forelimb formation is slightly delayed relative to hindlimb formation (see **Table 5**) but usually occurs at the same developmental stages (**Table 2**). Most bony elements of the pectoral and pelvic girdles form during the midlarval stages except for the ischium, which appears only close to metamorphosis, after all foot and hand phalanges are already more or less ossified.

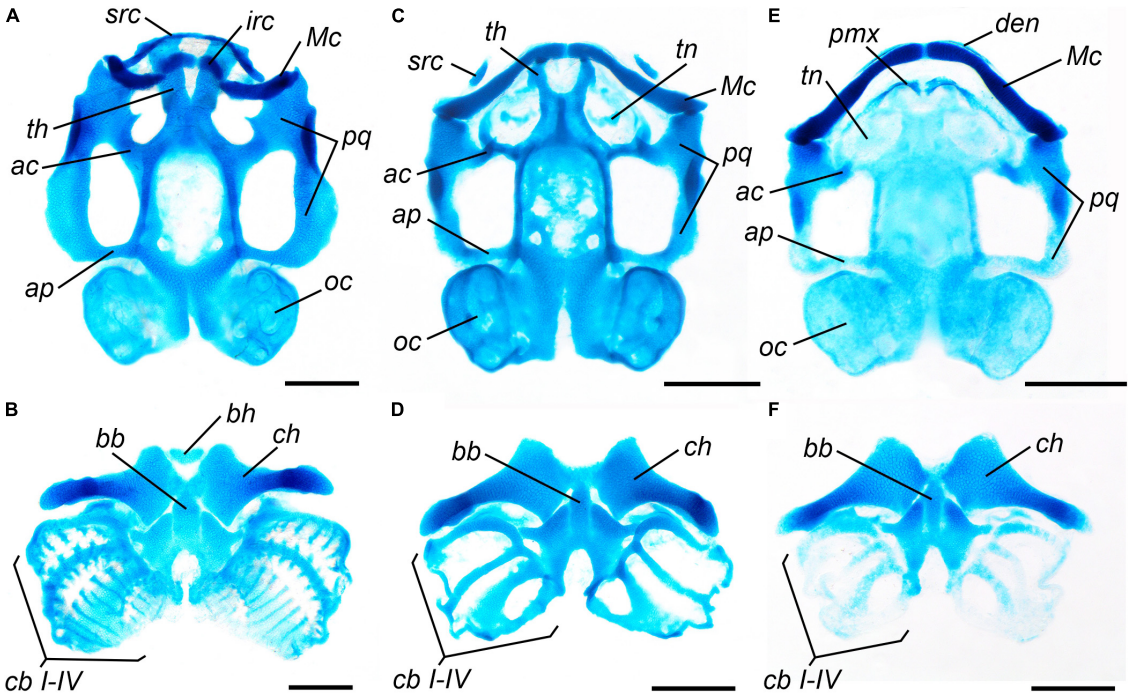


FIGURE 6 | Larval chondrocranium and hyobranchium of *Bombina variegata* in normally developing and TH-treated tadpoles: **(A,B)** norm, Stage 35, 15 dph; **(C,D)** after 9 days of treatment with T_3 2×10^{-8} M, limb development corresponding to Stage 35, 15 dph; **(E,F)** after 11 days of treatment with T_3 2×10^{-8} M, limb development corresponding to Stage 35, 17 dph. Abbreviations: *ac*, anterior commissure of palatoquadrate; *ap*, ascending process of palatoquadrate; *bb*, basibranchial; *bh*, basihyal; *cb*, ceratobranchials; *ch*, ceratohyal; *den*, dentary; *irc*, infrarostral cartilage; *Mc*, Meckelian cartilage; *oc*, otic capsule; *pmx*, premaxilla; *pq*, palatoquadrate; *src*, suprarostral cartilage; *tn*, tectum nasi; *th*, trabecular horns. Scale bar 1 mm.

At 2×10^{-8} M T_3 beginning from 6 dph, appendicular and axial skeleton do not show signs of ossification before the end of the experiment. At 2×10^{-9} M T_3, ossification of all elements of the appendicular skeleton is accelerated compared with the norm (**Table 5**). In contrast, under TU-treatment (0.02%), the latter is retarded. Higher TU concentration (0.04%) has a more profound effect on more later-forming appendicular ossifications (such as first foot and hand phalanges); the last appearing bones, normally ossifying close to metamorphosis, such as distal phalanges of digits I on the hand and foot and the ischium, do not form during the experiment. If these bones appear (especially the autopodial elements), they remain rudimentary as thin ossified rings in the middle of the cartilaginous matrices. Among elements of the pectoral girdle, the endochondral scapula and coracoid display a more profound reaction to TU-treatment, resulting in some changes in the sequence of bone appearance.

DISCUSSION

The Role of Thyroid Hormones in Skeletal Development in *Bombina variegata*

Normal Development of the Skeleton

Overall, the revealed ossification sequence in the skull and postcranial skeleton during the normal development of *B. variegata* is similar to that of *B. orientalis* studied by Hanken and Hall (1984, 1988a) and Maglia and Púgener (1998). The degree of variability in the timing and sequence of cranial bone appearance is comparable with that found in *B. orientalis* (Hanken and Hall, 1988a). On a large-scale comparison within anurans with a biphasic life history, *B. variegata* displays a rather typical cranial ossification sequence, with the frontoparietal, parasphenoid, and exoccipital arising first and dentary, quadratojugal, and pterygoid are among the last bones to arise (Trueb and Alberch, 1985; Weisbecker and Mitgutsch, 2010). At the same time, the unusually early appearance of the vomer and late appearance of the prootic in comparison with the majority of studied anuran species may be considered characteristic features of the genus *Bombina*.

Effects of T_3- and TU-Treatment on Cranial Development in *Bombina variegata*

In all experimental groups, the sequence of skull ossification is more or less truncated. Actually, the sole cranial ossifications in *B. variegata* to form under all hormonal regimes are two dermal bones, **frontoparietal** and **parasphenoid**. In most cases, they appear more or less simultaneously and occupy the first two positions in the ossification sequence (except at the highest T_3 concentration), thus displaying only a limited reaction to the TH-level changes: their development is slightly accelerated by a high TH-dosage and slightly retarded

TABLE 5 | Timing (the earliest registration in dph) of the postcranial skeletal elements appearance in larval *Bombina variegata* under different experimental conditions (Experiment I).

Skeletal elements	Norm I	T_3 2 × 10^{-9} M	TU 0.02%	TU 0.04%
		Limbs and limb girdles		
Femur	14	12	22	22
Fibula, tibia	14	13	22	22
Tarsals	17	14	25	24
Metatarsals	18	14	30	30
Ilium	19	14	30	30
First foot phalanges	19	17	38	51
All foot phalanges	23	19	48	–
Ischium	28	25	52	–
Humerus	18	14	27	22
Ulna, radius	18	14	30	24
Cleithrum	19	15	37	45
Coracoid	19	15	38	79
Clavicle	19	15	37	45
Scapula	19	18	38	116
Metacarpals	19	15	37	45
First hand phalanges	19	17	38	51
All hand phalanges	24	19	48	–
		Axial skeleton		
Presacral vertebrae I–VIII	17	14	24	27
Sacral IX	19	14	24	37
Postsacral X	19	14	33	37
Postsacral XI	23	15	53	–
Hypochord	23	15	38	–
Centra	23	18	38	–
Ribs	25	22	52	–
Urostyle	35	27	–	–

by TU. All other bones are more affected by T_3- or TU-treatment. The formation of the **exoccipital** is somewhat accelerated under TH-action depending upon the dosage and retarded under TU-treatment and very significantly at the high goitrogen dosage. At the highest T_3-dosage and early onset of treatment, this bone fails to form. Although exoccipital is among the early ossifications and normally appears 3rd in the ossification sequence, it probably requires sufficient differentiation of the preceding cartilaginous structures. It is noteworthy that the development in this experimental group is abbreviated to 20 dph, i.e., terminating earlier than the exoccipital forms in the norm. When exposure to high dosages of T_3 begins later, at mid-larval stages, the exoccipital appears, although it is preceded by some dermal ossifications (premaxilla and septomaxilla), which develop later in the norm.

The latest bones in the normal sequence, the dermal **quadratojugal** and **pterygoid** and the endochondral **prootic** and **os thyroideum**, fail to appear under partial inhibition of skull development with lower TU- dosage when craniogenesis is blocked at the mid-metamorphic stage. Under T_3-treatment, the number of bones that fail to appear increases as the hormone dosage increases (**Table 4**); presumably, they do not have sufficient time to form because of the general shortening of larval development, especially under high T_3 dosages. Other cranial bones display dosage-dependent acceleration of their development under T_3-treatment and retardation under moderate TU-inhibition.

A relatively low dosage of T_3 (2×10^{-10} M) causes some acceleration of the formation of bones appearing at the initial stages of craniogenesis (except for the earliest parasphenoid and frontoparietal) but does not affect the appearance of bones arising in its final stages (from Stage 43 during normal development). This observation suggests that this concentration of exogenous T_3 raises internal TH levels in larval tissues above the normal physiological level characteristic of the midlarval and premetamorphic stages and stimulates accelerated skull ossification. At the same time, this level apparently does not exceed the T_3 peak characteristic of the stages at which drastic metamorphic rearrangements begin; therefore, it does not affect the formation of ossifications occurring at the metamorphic period of development. A higher concentration of T_3 (2×10^{-9} M) causes a greater acceleration of the development of most cranial bones, as well as a more marked alteration of their normal ossification sequence. Thus, in normal development in *B. variegata*, the **vomer** and **nasal** arise simultaneously, but the vomer responds more drastically to the action of exogenous T_3: its formation is more accelerated, and it visibly shifts to earlier positions in the sequence. Nasal, although accelerated in development, lags behind that of the vomer. The highest concentration of T_3 (2×10^{-8} M) has the most profound effect on craniogenesis, causing the precocious formation of most ossifications (including the earliest ones) compared with the norm and the most significant alterations of the normal sequence, with even relatively late bones (such as **dentary**) shifting to early positions. This indicates that bones arising after the beginning of metamorphosis strongly depend on THs in their development, but the degree of TH-responsivity varies among bones. It is worth noting that even different constituent parts of certain bones may exhibit different reactions to TH-changes. In the developing premaxilla, the dental process appears precociously under high T_3 concentrations and postpones its appearance under TU-treatment. Its facial process develops in TU-treated tadpoles and does not show evident acceleration in TH-treated tadpoles.

All cranial bones, which display the most profound reaction to TH-level changes, are associated with the palatoquadrate and Meckelian cartilages, and all of them both in normal and TH-induced development appear only after these cartilages start to remodel. This pattern of development suggests the occurrence of a certain interplay between dermal bones and adjacent cartilages. Three hypotheses may be proposed. First, it is precociously induced cartilage remodeling that induces the precocious bone development. Second, it is THs that precociously induce bones,

whereas cartilages provide only a necessary framework for them. Third, both cartilage and THs are involved in the induction of bone appearance. To elucidate the main inducing factor, further studies are needed.

Observation of the development of other bones supports the occurrence of a certain cartilage-bone interplay. Normally, in the **premaxilla,** its facial process appears first, followed by the dental process. In TH-treated tadpoles, the dental process precedes the facial process. In anurans, the developing facial process of the premaxilla abuts the superior prenasal cartilage appearing at prometamorphosis (Roček, 2003; Pugener and Maglia, 2007). In our experiment, early tadpoles were TH-treated before the formation of this cartilage resulting in the delayed appearance of the facial process or its absence. Also, in anurans, bones associated with the nasal capsule always ossify after the adjacent parts of the capsule are chondrified (Pugener and Maglia, 2007). The floor of the nasal capsule (solum nasi) chondrifies early, whereas chondrification of its roof (tectum nasi) is a late event that is only moderately accelerated by exogenous TH. Consequently, although normally the nasal and vomer in *B. variegata* appear simultaneously on the tectum nasi and solum nasi, respectively, in the TH-treated larvae the vomer greatly precedes the nasal. The ascending process of the **maxilla** also remains rudimentary at high T_3 dosages, presumably due to the delayed development of the lateral wall of the nasal capsule invested by this process.

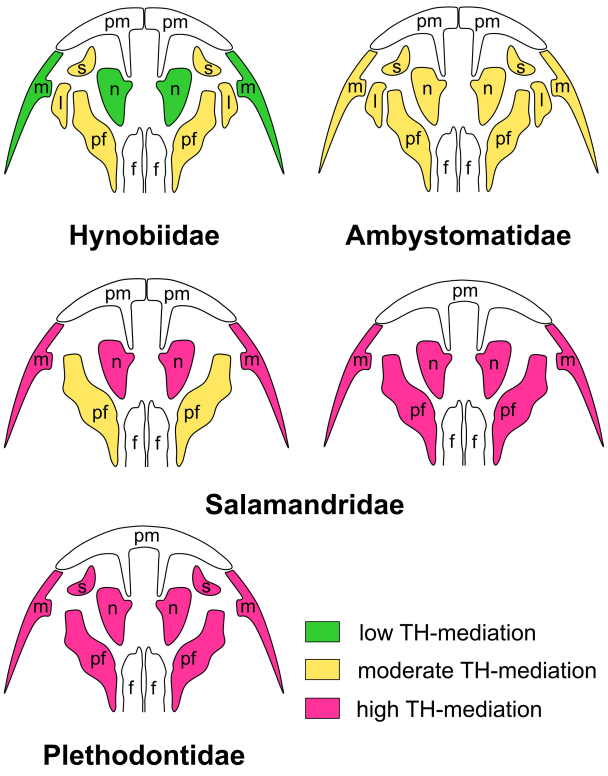


FIGURE 7 | Levels of TH-mediation of the development of late-larval cranial bones in experimentally studied urodelan amphibians treated with exogenous triiodothyronine, T_3, and thiourea, TU: hynobiid *Salamandrella keyserlingii* (after Smirnov et al., 2011); ambystomatid *Ambystoma mexicanum* (after Smirnov and Vassilieva, 2005); salamandrids *Pleurodeles waltl* (after Smirnov et al., 2020) and *Lissotriton vulgaris* (after Smirnov and Vassilieva, 2003); and perennibranchiate plethodontids treated with thyroxine, T_4 (after Rose, 1995a,b, 1996, 2003). "Low TH-mediation" implies that the bone forms normally in all specimens under TH-deficiency and is only slightly accelerated and delayed under T_3- and TU-treatment, respectively; "moderate TH-mediation" implies that the bone appears in all specimens under TH-deficiency but is markedly accelerated under T_3-treatment and delayed (and possibly deviant) under TU-treatment; "high TH-mediation" implies that the bone is markedly accelerated under T_3-treatment and does not form (or is rudimentary) in all specimens under TU-treatment. Abbreviations: l, lacrimal; m, maxilla; n, nasal; pf, prefrontal; pm, premaxilla; s, septomaxilla.

The Role of Thyroid Hormones in the Skeletogenesis of Urodelans and Anurans

Bony Skull

Urodelans, experimentally studied to date, display a consistent pattern of cranial ossification. First to appear are tooth-bearing bones (premaxilla, dentary, vomer, coronoid, and palatopterygoid), followed by midlarval bones (frontal, parietal, orbitosphenoid, and quadratojugal), and then late-appearing bones (maxilla, nasal, prefrontal, and septomaxilla), which arise in the premetamorphic stages or during metamorphosis. At metamorphosis, the salamander skull undergoes considerable remodeling: (i) the coronoid and palatine portion of the palatopterygoid (or palatopterygoid entirely in plethodontids) resorb, and (ii) the larval vomer transforms into the adult vomer. Anteriorly, vomer forms outgrowths extending toward the premaxilla and maxilla. Posteriorly, the vomer develops a tooth-bearing caudal process (vomerine bar), extending along a lateral edge of the parasphenoid. In plethodontids, this bar transforms into a separate bony element bearing the parashenoid tooth patch.

In *S. keyserlingi, A. mexicanum, L. vulgaris*, and *P. waltl*, the first bones to appear arise at the same time and sequence under different hormonal conditions (Smirnov and Vassilieva, 2003, 2005; Smirnov et al., 2011, 2020). Midlarval bones display some acceleration and retardation under TH and TU treatments, respectively. The effect of TH-changes is profound in the late-appearing dermal bones, maxilla, nasal, and prefrontal (and lacrimal and septomaxilla in *S. keyserlingi* and *A. mexicanum*) (**Figure 7**). The most evident reaction is revealed in metamorphic palate remodeling and coronoid resorption. These events are greatly accelerated in TH-treated animals and fail to occur in TU-treated animals. The latter, even after a year and half of the experiment, retain the palatopterygoid and coronoid accompanied by a larval vomer without outgrowths toward the upper jaw bones and posterior vomerine bar. Earlier, Rose (1995a,b) revealed late-appearing bones (maxilla, nasal, prefrontal, and septomaxilla) and metamorphic transformation of the vomer and resorption of the palatopterygoid and coronoid to be greatly accelerated under TH-treatment in the plethodontid *Eurycea bislineata*. As in our experiments, the degree of acceleration depended upon the applied TH-dosage and the stage of larval development at which animals

were TH-treated. However, the effect of thyroid deficiency on cranial development has not been studied and remains unknown in plethodontids.

These findings indicate that (i) THs are involved in the cranial ossification of salamanders and (ii) skull development is accompanied by changes in the degree of TH-responsivity of cranial bones. The appearance of early bones seems to be not reactive to THs, and midlarval bones display slight TH-responsivity, which becomes evident in late-appearing bones (maxilla, nasal, prefrontal, and septomaxilla) and obligate for metamorphic cranial remodeling. Interestingly, whereas the appearance of early bones does not depend upon TH-level, their further development and morphological differentiation become TH-inducible. Thus, in *P. waltl,* changes in the TH-level do not affect the timing of the premaxillar appearance, while later formation of its outgrowth toward the vomer is strictly induced by THs (Smirnov et al., 2020).

In our experiments, because of different reactions to TH-changes, T_3-treated salamanders displayed changes in the sequence of bone appearance: highly TH-responsive bones, normally appearing at the late larval stage or at metamorphosis, arose precociously and preceded the appearance of bones, which normally arise prior to them. In nature, a similar phenomenon seems to occur in ambystomatids. The developing neotenic *A. mexicanum* differs from closely related metamorphosing congeneric *A. tigrinum* in (i) a precocious TH-surge (Larras-Regard, 1985; Rosenkilde, 1985) and (ii) a precocious appearance of the TH-mediated nasal bone (Rose, 2003; Smirnov and Vassilieva, 2005).

Additionally, in our experiments, because of different bone reactions to TH-changes, TH-treated animals exhibited a mixture of early larval and metamorphic features. In salamanders, changes in the TH-level do not influence the timing and rate of the early larval period of development of the coronoid, palatopterygoid and vomer. Their further metamorphic transformations are obligatory TH-mediated and are greatly accelerated under TH-treatment. Consequently, TH-induced precocious transformation begins when the TH-independent period of these bones' development is not yet completed. The earlier exogenous THs are applied, the more underdeveloped these bones are at the beginning of metamorphic transformation. This resulted, e.g., in the appearance of vomer consisting of the underdeveloped larval (anterior) portion and adult (posterior) portion.

In nature, a similar developmental pattern was observed in the direct-developing plethodontid *Desmognathus aeneus* (Marks, 2000) (see below).

Although late-appearing bones display obvious reactions to TH-changes in all salamanders studied in this respect, "degree" of their reaction differs. Thus, under a similar TH-treatment, they show moderate acceleration in *S. keyserlingi* but considerable acceleration in *L. vulgaris* and *P. waltl*. Similar differences were recorded in TU-treated salamanders. In *S. keyserlingi*, TH-deficiency results in the postponed appearance of these bones. In *L. vulgaris* and *P. waltl* under TH-deficiency, the same bones are greatly delayed in appearance and remain rudimentary or fail to appear at all. A similar phenomenon is displayed by neotenic perennibranchiate plethodontids, which, because of the likely thyroid dysfunction, fail to metamorphose and retain larval cranial morphology: they lack late-appearing bones (maxilla, nasal, prefrontal, and septomaxilla) and palate remodeling (Rose, 1995c, 1996). However, these events (at least some of them) occur under exogenous TH-treatment (Rose, 1995a, 1996, 2003). In summary, these observations indicate that the same bones differ in their TH-reactivity: bones that are rather moderately TH-reactive in the basal salamander *S. keyserlingi* are TH-inducible in more derived urodelans.

Observations of *B. variegata* show that the involvement of THs in the regulation of skeletal development in anurans largely follows the same patterns that have been revealed in urodelans, especially with regard to the bony skull. In both salamanders and frogs, THs play a key role in the realization of the complete craniogenesis sequence: when larvae develop under the TH-deficiency, this sequence is truncated via the underdevelopment of late ossifications normally forming during the premetamorphic phase or metamorphic climax. Additionally, the involvement of THs in skull bones development become enhanced in both urodelans and anurans during ontogeny: while the TH excess or deficiency have only a regulatory impact (if ever) on the development of the earliest bones in the cranial ossification sequence, affecting the timing of their appearance, the later bones appearing at metamorphosis are strictly TH-inducible. Cranial bones that form normally in the stages when the level of endogenous THs is low show the least TH-reactivity (**Figure 8**). Bones, which normally develop closer to and/or during the metamorphic climax, are TH-sensitive: they do not develop at reduced TH-levels, while their appearance is markedly accelerated when the TH-level is increased. These bones require higher TH-levels for their development, and the closer to the metamorphic climax they appear, the higher the level.

Induced TH-changes may cause similar changes in the developmental pattern of the same bones in frogs and salamanders. Thus, under high TH-levels, the premaxilla and maxilla tend to fuse in *L. vulgaris* (Smirnov and Vassilieva, 2003), *P. waltl* (Smirnov et al., 2020), and *B. variegata* (this study; see **Figure 3A**). In both urodelans and anurans, constituent parts of the same bone may exhibit different reactions to changes in TH-levels, as exemplified by the premaxilla in *P. waltl* (Smirnov et al., 2020) and *B. variegata* (this study). Additionally, under TH-changes, in both anurans and urodelans, the appearance of multiple ossification centers was observed in bones normally developing from the sole center. For example, in *P. waltl* specimens with craniogenesis suppressed by the TU, the nasal is often represented by numerous ossicles (Smirnov et al., 2010, 2020). In anurans, under TU- and T_3-treatment, multiple ossification centers were recorded in the frontoparietal, parasphenoid, vomer, angulosplenial, premaxilla, pterygoid, and others (Smirnov and Vassilieva, 2009). Presumably, this phenomenon is related to the induced alterations in the rate of differentiation, mineralization, and fusion of bone primordia caused by changes in the normal hormonal regime. In some cases, it confirms the complex evolutionary origin of the bone. In particular, in urodelans, the frontal was shown to have originated via fusion of the three separate ossification

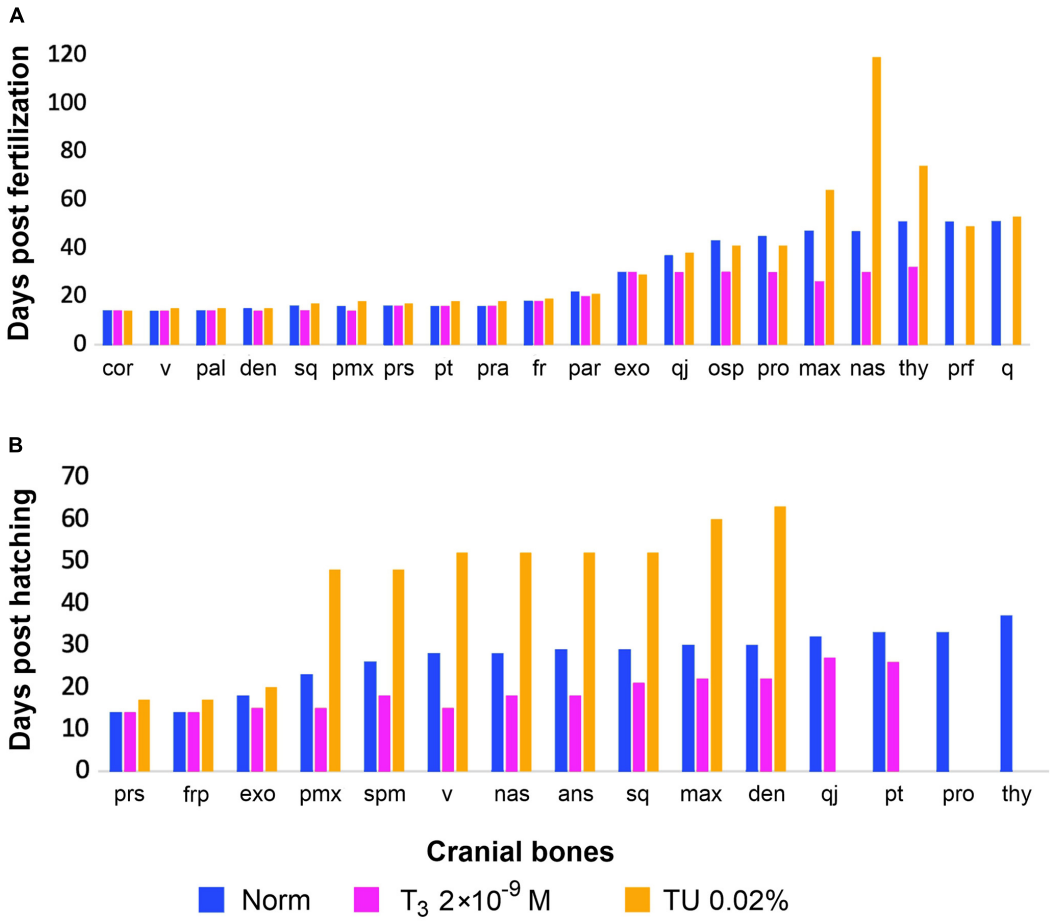


FIGURE 8 | Timing of cranial bones development (earliest appearance) under same experimental hormonal regimes (in norm, under T_3 and TU-treatment), **(A)** in *Pleurodeles waltl* (after Smirnov et al., 2020) and **(B)** in *Bombina variegata* (this study). Abbreviations: cor, coronoid; den, dentary; exo, exoccipital; fr, frontal; max, maxilla; nas, nasal; osp, orbitosphenoid; pal, palatine; par, parietal; pmx, premaxilla; pra, prearticular; prf, prefrontal; pro, prootic; prs, parasphenoid; pt, pterygoid; q, quadrate; qj, quadratojugal; sq, squamosal; thy, os thyroideum; v, vomer.

centers (Lebedkina, 1979, 2004). In *B. variegata,* formation of the frontoparietal from several early fusing anlagen was also confirmed by histological studies (Čihák et al., 2003).

Sporadic individual variability in the number of ossification centers observed in the developing bones in anurans (Wiens, 1989; Hall and Larsen, 1998; Banbury and Maglia, 2006) seems to result from individual changes in thyroid axis function.

Appendicular Skeleton

Experimental studies of the impact of THs on the development of the appendicular skeleton in urodelans are rather scarce. Brown (1997) demonstrated that exogenous T_4 promotes accelerated limb elongation in axolotl, but did not report the effect of THs on the timing of limb bone ossification; the various goitrogens in this study were found to not interfere with limb development. In *Lissotriton vulgaris*, the timing of limb and limb girdle formation under exogenous T_3- and TU-treatment does not differ from the norm (Smirnov and Vassilieva, 2003). In contrast, in *B. variegata*, all ossifications of the appendicular skeleton display some TH-responsivity that is displayed in the timing of their appearance (**Figure 9**). Moreover, treatment with a high dosage of TU inhibits the ossification of the latest elements of the limb skeleton, i.e., the last phalanges of the fingers and toes and the ischium. However, the development of the appendicular skeleton is clearly less TH-controlled than that of the skull: if high TU-dosages block cranial ossification at rather early larval stages, the ossification of the limb skeleton progresses much further to an almost complete metamorphic set of bones. In parallel, under treatment with high T_3-dosages from early larval stages, tadpoles display an obvious dissociation between head and limb development resulting in the appearance of animals with a mixture of a metamorphosing skull and hyobranchium and early-larval limbs.

Previous studies have shown that in anurans, THs are strictly required for normal limb formation (Brown et al., 2005). THs participate in the differentiation of limb cartilage and accelerate limb bone ossification (Kemp and Hoyt, 1969; Brown et al., 2005). However, it is not clear whether all stages of limb skeleton development are TH-dependent. On the one hand, experiments show that under TH-deficiency caused

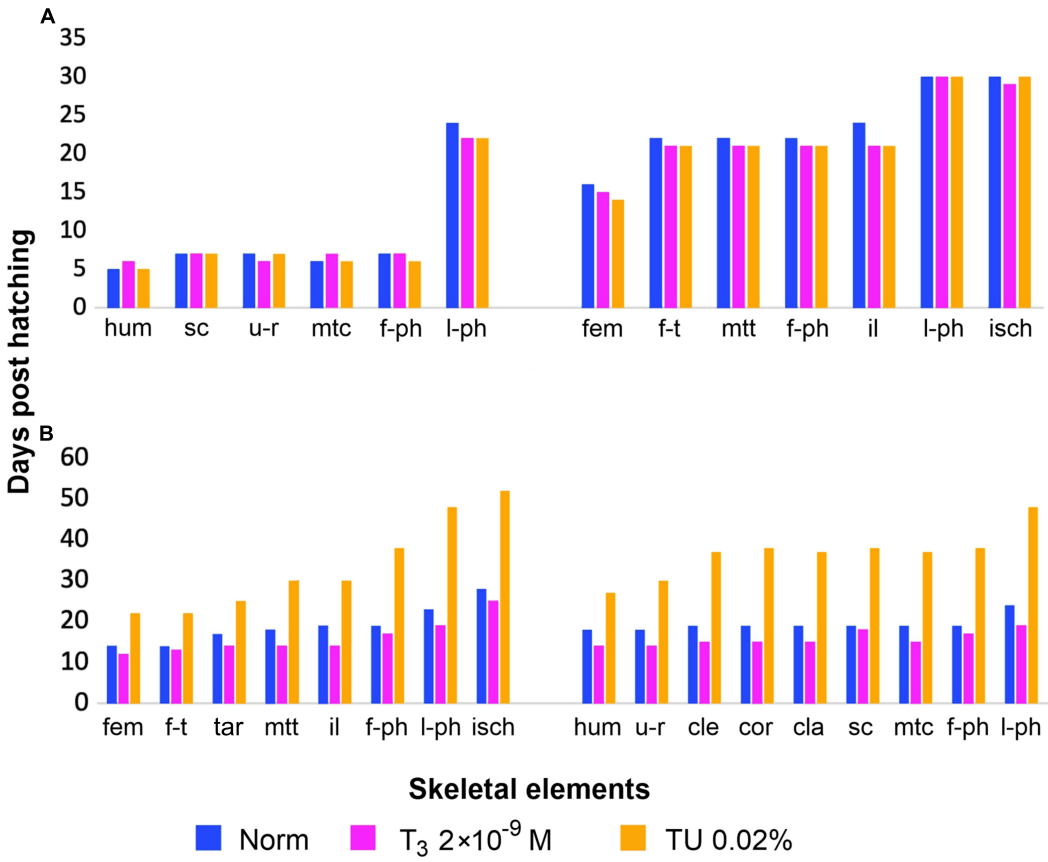


FIGURE 9 | Timing of appendicular bones development (earliest appearance) under same experimental hormonal regimes (in norm, under T_3 and TU-treatment), **(A)** in *Lissotriton vulgaris* (after Smirnov and Vassilieva, 2003) and **(B)** in *Bombina variegata* (this study). Abbreviations: cla, clavicle; cle, cleithrum; cor, coracoid; fem, femur; f-ph, first phalanges; f-t, fibula and tibia; tar, tarsals; hum, humerus; il, ilium; isch, ischium; l-ph, last phalanges; mtc, metacarpals; mtt, metatarsals; sc, scapula; u-r, ulna and radius.

by methimazole treatment beginning in early larval stages, the hind limbs remain bud-like in *Xenopus* tadpoles (Brown et al., 2005). On the other hand, in giant athyroid *Xenopus* tadpoles, the development of the appendicular bony skeleton is arrested at more advanced stages, and their ossification state is rather advanced relative to their length (Kerney et al., 2010). Other studies have shown that the disruption of thyroid axis at various levels allows limb cartilage to differentiate but prevents elongation (Mitsui et al., 2006; Choi et al., 2017). Our observations on *B. variegata* indicate that even under strong TH-suppression, larval limbs attain full digits differentiation and form almost all ossifications, but the longitudinal growth of the bones strictly requires increased TH-levels. On the other hand, in *Pelobates fuscus* (Pelobatidae), tadpoles treated with TU 0.04% hind limbs remain bud-like even after six months of the experiment (Smirnov, unpublished observations). The controversy of these data suggests that anurans can differ in the degree of TH-involvement of limb development.

Axial Skeleton

The formation of the ossified presacral and sacral vertebrae in the studied anurans appears to be only partially TH-affected. In *Xenopus laevis*, they develop in naturally athyroid giant tadpoles and experimentally goitrogen-treated larvae (Kerney et al., 2010; Smirnov and Vassilieva, 2014). In *B. variegata*, TH-changes affect only the timing of their development (see **Table 5**). The posterior part of the anuran axial skeleton is formed by the urostyle, a structure that forms from the fused postsacral vertebrae (coccyx) and the ossified hypochord. This is a unique anuran feature that is thought to play a key role in the formation of the postmetamorphic anuran body plan (Handrigan and Wassersug, 2007a). A recent study showed that the development of the urostyle, especially its hypochordal part, is directly affected by THs, since in *Xenopus* tadpoles treated with methimazole, the formation of coccygeal vertebrae was incomplete, and the hypochord was totally absent (Senevirathne et al., 2020). However, our results show that in *B. variegata* both coccyx and hypochord are highly TH-reactive: they markedly accelerate their development under treatment with moderate T_3 dosages, but under TU-treatment with a moderate dosage, their ossification is delayed, plus the coccygeal and hypochordal parts of the urostyle remain unfused by the end of the experiment; under TU-treatment with high dosage, only primordia of the neural arches X appear, but not vertebra XI

or hypochord. As in the appendicular skeleton, the above data indicate the possible interspecific variability in axial skeleton TH-mediation among anurans.

Our observations on *B. variegata* suggest that only a low level of THs is necessary for the development of the presacral and sacral vertebrae, whereas the urostyle beginning to form in the premetamorphic period needs a high TH-level to develop. Frogs of the Asian family Megophryidae display a striking example of necrobiotic metamorphosis in the axial skeleton. The stream-dwelling tadpoles of many megophryid genera develop caudal vertebrae (up to 30 in *Leptobrachella*), which disappear during metamorphosis along with tail resorption (Handrigan and Wassersug, 2007b; Handrigan et al., 2007). It is reasonable to assume that resorption of these caudal vertebrae is also TH-inducible, as are other remodeling processes in anuran metamorphosis.

Thyroid Hormone-Regulation of Amphibian Skeletal Development and Decoupling of Larval and Adult Morphology

Earlier, experimental embryologists (Corsin, 1967; Medvedeva, 1975; Lebedkina, 1986, among others) revealed the involvement of tissue-inductive interactions in the mediation of cranial bones development in salamanders. Comparison of this non-hormonal and thyroid hormonal mediation of skull development shows that in *S. keyserlingi,* extirpation of the nasal sac resulted in the failure of the nasal bone to appear (Lebedkina, 1986), whereas TH-impact is limited to the timing of this bone appearance (Smirnov and Vasil'eva, 2002; Smirnov et al., 2011). In salamandrids, as exemplified by *Ichthyosaura alpestris*, extirpation of the nasal placode did not prevent the appearance of the nasal bone but influenced its shape (Hall, 1999b, p. 147), whereas THs exerted a dominant impact on its appearance and development in *L. vulgaris* and *P. waltl* (Smirnov and Vassilieva, 2003; Smirnov et al., 2020).

Similarly, in *S. keyserlingi,* extirpation of the nasolacrimal duct prevented the appearance of the lacrimal bone (Medvedeva, 1975), whereas THs influenced only the timing of its appearance (Smirnov et al., 2011). In *Ambystoma mexicanum*, both the nasolacrimal duct and THs exerted a dominant impact on the appearance and development of the lacrimal (Medvedeva, 1986; Smirnov and Vassilieva, 2005). In salamandrids (e.g., *P. waltl, Triturus karelinii,* and *Ishthyosaura alpestris*), a separate lacrimal is absent, although it participates in the complex bone prefrontolacrimal (Medvedeva, 1975; Lebedkina, 1979, 2004; Vater, 2007). In *P. waltl*, the nasolacrimal duct influenced only the shape of the lacrimal portion of this bone (Medvedeva, 1975), whereas THs were necessary for its appearance (Smirnov et al., 2020). In *L. vulgaris*, no impact of the nasolacrimal duct was revealed (Medvedeva, 1975), whereas TH played a dominant role in this bone development (Smirnov and Vassilieva, 2003).

Septomaxilla in *S. keyserlingi* did not appear in the absence of the nasolacrimal duct (Medvedeva, 1975), whereas TH-changes influenced the timing of its appearance (Smirnov et al., 2011). In *A. mexicanum*, both the nasolacrimal duct and THs were necessary for the appearance of this bone (Medvedeva, 1986; Smirnov and Vassilieva, 2005). In *E. bislineata,* septomaxilla is TH-inducible and appears to be independent of the nasolacrimal duct, as it may normally develop in its absence (Rose, 1995b).

These findings indicate that in the basal salamander, *S. keyserlingi*, inductive tissue interactions exert a dominant impact on the development of late-appearing cranial bones, whereas the role of THs is limited to influencing the timing of their appearance. In contrast, in more derived salamanders, as exemplified by *A. mexicanum, L. vulgaris, P. waltl*, and *E. bislineata*, THs induce the development of these bones, whereas non-hormonal mediation plays a minor role.

As stated by Alberch (1989), the evolution of the primarily gradual development in amphibians was accompanied by a concentration of morphological transformations on a limited ontogenetic stage, determined as metamorphosis. At the same time, experimental and histological studies on amphibians of diverse lineages by Corsin (1967), Medvedeva (1975, 1986), Lebedkina (1979, 2004) showed the progressive attenuation of some of inductive tissue interactions and the disintegration of inductive cascades at the transition from basal to advanced urodelans.

Although it is widely recognized that amphibian ontogeny is regulated by both non-hormonal and hormonal factors (Rose and Reiss, 1993; Hall, 1999a), it may be assumed that in salamander evolution, the decrease in the role of tissue interactions provided grounds for the enhancement of hormonal regulation and induction of skeletogenetic events. In anurans, given the TH-dependence of not only complete cranial development but also postcranial development, hormonal control is likely a major mechanism mediating skeletal ontogeny. The transition to hormonal control facilitates the relative independence of larval and adult cranial morphologies and, in parallel, permits the synchronization of developmental transformations. Without strong tissue interrelationships, cranial bones become released from larval constraints, and vice versa, larval elements become free from adult constraints; therefore, larval and adult morphology can be readily dissociated.

Among salamanders, this dissociation is evident in the biphasic plethodontids, which display the maximum TH-involvement in the mediation of skull development. In contrast, both TH-involvement and developmental differences in cranial morphology are relatively slight in hynobiids (Smirnov, 2006).

In anurans, whose skeletal development is mainly mediated by THs, the difference is enormous if skulls are compared between tadpoles and frogs. Moreover, anuran larvae display a wide range of cranial morphologies due to the acquisition of numerous larval-specific features adaptive to a variety of trophic specializations, such as suspension-feeding, detritophagy or predation (Haas and Richards, 1998; Haas, 2003; Vera Candioti, 2007, to name a few).

In caecilians, the third lineage of extant amphibians, cranial development is a gradual process, as exemplified by the basal *Epicrionops* sp. (Reiss, 1996). In this apodan, the adult skull differs from the late larval skull, mainly in the "increase in the area of dermal bones" (Rose and Reiss, 1993, p. 303). The only evident metamorphic event is the fusion of the maxilla and palatine

accompanied by the division of the pterygoid into two portions, although the latter is an individually variable feature not shared by other caecilians (Reiss, 1996).

A gradual pattern of cranial development with only a few slight differences in larval and adult morphologies is revealed in the putative ancestors of recent amphibians, dissorophoid temnospondyls, for whom detailed ontogenetic series are known (Schoch, 1998, 2002; Boy and Sues, 2000). Thus, in *Apateon gracilis* (Branchiosauridae), all cranial bones appeared during the larval period, and the palate did not undergo metamorphic rearrangement (Schoch and Fröbisch, 2006). However, both caecilians and branchiosaurids display profound metamorphic transformation of the hyobranchium (Boy and Sues, 2000; Wake, 2003).

Given the revealed correlation between the level of TH-involvement and the degree of larval/adult cranial dissociation in frogs and salamanders, THs seem to play a minor (if ever) role in the mediation of skull development in both apodans and temnospondyls.

The transition to hormonal mediation enhances the independence of larval and adult developmental programmes, thus creating preconditions for necrobiotic metamorphosis. Usually, within amphibians, necrobiotic metamorphosis is considered a characteristic of anurans, which undergo drastic metamorphic changes in the body plan. In anurans, larval specializations (e.g., the suprarostral cartilages and trabecular horns in the skull and tail in the locomotory system) resorb during the larval-adult transition rather than rearrange into adult ones. Although in urodelans, the body plan does not change fundamentally during larval-adult transformation, the latter also includes necrobiotic processes, as exemplified by the resorption of provisory bones (coronoid, palatine, larval vomer, and their dentitions). By repeated vital staining, Lebedkina (1979, 2004) showed that larval vomer in salamanders does not rearrange into adult bone but resorbs completely and becomes replaced by adult vomer differing in the microstructure of bony tissue. The remodeling of the larval hyobranchium in metamorphosing urodelans also implies the resorption of some cartilaginous elements of the hyobranchium and the transformation of others (Rose, 2003). However, in the plethodontid *Eurycea bislineata*, adult ceratobranchial cartilage was shown to develop *de novo* rather than rearranged (Alberch and Gale, 1986). All these necrobiotic events in both frogs and salamanders are strictly TH-dependent.

The Increasing Role of Thyroid Hormones in the Regulation of Amphibian Development: Implications for Life History Evolution

We hypothesize that THs play a key role in the major evolutionary repatterning of amphibian ontogeny and life history.

Direct Development

The transition to hormonal control of the adult portion of the skeletal developmental programme allows larval and adult cranial morphologies to be readily dissociated as exemplified by direct-developing and neotenic salamanders lacking larval and adult skull morphology, respectively. Given the strong TH-impact on cranial development revealed in the salamanders of the family Plethodontidae by Rose (1995a,b), it is no wonder that this salamander lineage demonstrates the largest diversity of cranial developmental patterns. Some plethodontids, e.g., *Eurycea bislineata* studied in detail by Rose (1995a,b,c), are biphasic and display both larval and adult skull morphology. Several species are neotenic (the so-called perennibranchiates) and lack adult cranial morphology. Most plethodontids are direct-developers and lack larval skull morphology. Whereas questions such as whether direct development is primitive or derived in plethodontids and how many times it has arisen are widely discussed (Chippindale et al., 2004; Mueller et al., 2004; Bonett et al., 2005, 2014; Beachy et al., 2017), knowledge on the skeletal development of direct-developers is fragmentary (Alberch and Alberch, 1981; Hanken, 1984; Ehmcke and Clemen, 2000; Dulcey Cala et al., 2009). To date, in most detail, the cranial ontogeny of a direct-developing salamander has been described by Marks (2000) in *Desmognathus aeneus*. In this salamander, in contrast to biphasic plethodontids, the usual sequence of bone appearance is altered (e.g., the maxilla appears precociously), larval bones, palatopterygoid and coronoid, do not appear, and larval vomer is greatly underdeveloped, whereas the adult vomerine portion appears precociously and precedes the appearance of several bones, including the nasal and septomaxilla.

Although direct development has been hypothesized to involve a shift toward precocious activation of the thyroid gland (Lynn, 1961; Matsuda, 1987), little evidence of TH-dependence in direct-developing embryos of plethodontids has been found (Dent, 1942). Under these circumstances, it is worth noting that the pattern of cranial development recorded in *D. aeneus* is remarkably similar to that observed in the TH-treated early larvae of *L. vulgaris*. In this newt, TH-treatment, which mimics the precocious natural TH-peak, resulted in the precocious appearance of the maxilla and posterior (adult) portion of the vomer. Both appeared when larval vomer, coronoid, and palatopterygoid remained underdeveloped and one-third of cranial bones were still absent (Smirnov and Vassilieva, 2003).

Although this evidence provides only indirect support for the hypothesis that direct development in plethodontids evolved via precocious TH-activity, the similarity of cranial development in *D. aeneus* to that observed in TH-treated *L. vulgaris* larvae seems rather prospective.

Among anurans, direct development is widespread and has arisen independently multiple times in a wide variety of families (Duellman and Trueb, 1986; Gomez-Mestre et al., 2012; Bardua et al., 2021). Most knowledge on direct-developing frogs comes from studies of *Eleutherodactylus coqui* (Eleutherodactylidae). Skeletal development of this frog displays profound ontogenetic repatterning: (i) larval-specific features such as the suprarostral cartilage and trabecular horns never form; (ii) Meckel's, infrarostral, and palatoquadrate cartilages, as well as the hyobranchium since their appearance resemble a mid-metamorphic stage of development in metamorphosing

anurans; and (iii) the skull ossification sequence is unusual in that bones associated with the suspensorium (squamosal) and jaws (angulosplenial, premaxilla, dentary, and maxilla) appear precociously (Hanken et al., 1992).

Initially, this ontogenetic repatterning was hypothesized to occur due to the emancipation of developmental processes from TH-dependence (Hanken et al., 1997). However, further experimental studies have shown that *E. coqui* undergoes ontogenetic transformation, "cryptic metamorphosis," which closely resembles the metamorphosis of biphasic frogs and is largely orchestrated by THs (Jennings and Hanken, 1998; Callery and Elinson, 2000).

The thyroid activity and TH profile in *E. coqui* display the same pattern as in biphasic anurans: their significant rise coincides with the phase of the most significant morphological rearrangements and is followed by a decline (Hanken et al., 1992; Laslo et al., 2019). The main difference is that in *E. coqui,* the TH-rise occurs in an embryo.

There is little research on skeletal ontogeny in direct-developing frogs other than *E. coqui.* Those that were studied display different degrees of ontogenetic repatterning, which is less pronounced than in *E. coqui,* but all of them exhibit precocious appearance of at least some bones associated with the suspensorium and jaws, as found in some Neotropical frogs of the families Craugastoridae and Brachycephalidae (Vera Candioti et al., 2020), as well as in Asian tree frogs (Rhacophoridae) (Kerney et al., 2007; Vassilieva, 2017). A similar trend was also revealed in *Eleutherodactylus guentheri* (Lynn and Lutz, 1946), *E. nubicola* (Lynn, 1942), and *E. ricordii* (Hughes, 1959).

Remarkably, a similar sequence change, i.e., the precocious appearance of bones, which contribute to the upper and lower jaws and suspensorium, is observed in a biphasic anuran, *B. variegata*, under experimental treatment with high TH-dosages (this study) (**Figure 10**). Previously, the ontogenetic cranial pattern in TH-treated early newt larvae was revealed to resemble closely that of the direct-developing salamander (see above).The similarity of these phenomena (likeness of the direct-developer and TH-treated biphasic amphibian) in salamanders and frogs seems more than mere coincidence. Rather, it seems to support the hypothesis that direct development in anurans and urodelans evolved via precocious TH-activity.

Paedomorphosis

In urodelans with their decoupled somatic and reproductive development, heterochronies leading to paedomorphic underdevelopment are widespread: neotenic forms (i.e., larval reproducers) are found in most recent families of Caudata. The development of the skeleton, in particular the skull, in paedomorphic urodelans shows signs of arrest at different stages, ranging from mid-larval to almost metamorphic. Paedomorphic signs are displayed in the loss of a more or less extensive set of cranial bones, as well as in the retention of the provisory larval bones (coronoid and palatine) and in the incomplete (if ever) remodeling of the larval palate. Five families (Plethodontidae, Cryptobranchidae, Sirenidae, Proteidae, and Amphiumidae) contain obligate neotenics. The last four are represented exclusively by neotenic species.

Neotenic (perennibranchiate) plethodontids lack late-appearing bones (e.g., maxilla and septomaxilla) and retain larval vomer, palatopterygoid, and coronoid (Rose, 1999, 2003). This cranial underdevelopment seems to result from thyroid dysfunction since TH-treatment induced resorption of the coronoid in *Eurycea wallacei* (Dundee, 1962) and the appearance of the maxilla and septomaxilla in *Eurycea rathbuni* (Dundee, 1957). It is worth noting that these two salamanders are obligate neotenics, whereas other perennibranchiate plethodontids retain the capacity to metamorphose and naturally metamorphosed individuals are occasionally found, e.g., *Gyrinophilus palleucus* according to Yeatman and Miller (1985) and *Eurycea tynerensis* (Bonett et al., 2014).

Dysfunction of the thyroid axis has also been proposed (Rose, 1996, 1999, 2003) to be responsible for the underdevelopment of the skull in the obligate neotenic salamanders *Andrias japonicus, A. davidianus* and *Cryptobranchus alleganiensis* (Cryptobranchidae); *Siren intermedia, S. lacertina*, and *Pseudobranchus striatus* (Sirenidae); and *Necturus maculosus* and *Proteus anguinus* (Proteidae), which display skull morphology ranging from almost completely adult in cryptobranchids to mostly larval in proteids (Larsen, 1963; Reilly and Altig, 1996; Rose, 2003). Although TH-synthesis dysfunction seems possible, all attempts to induce cranial transformation via TH-treatment were unsuccessful, but Safi et al. (1997a,b) proposed other mechanisms of the disruption of thyroid axis function in proteids. Consequently, other reasons for cranial underdevelopment are not excluded. In the basal *A. japonicus,* which undergoes metamorphic skull remodeling with the resorption of the palatine and coronoid, late-appearing bones such as the septomaxilla and lacrimal fail to arise (Rose, 2003). It is these bones that in the basal cryptobranchoid salamanders, *S. keyserlingii* and *Ranodon sibiricus* (Hynobiidae), are obligately induced by the nasolacrimal duct and do not develop in its absence (Medvedeva, 1975). Since cryptobranchids lack the nasolacrimal duct, it seems highly likely that its absence prevents the development of these bones rather than some hormonal factor.

Paedomorphic skeletal modifications are also widely distributed among anurans, although skeletal underdevelopment is less pronounced in them. In frogs, sexual development is strictly TH-dependent, and reproduction is impossible in animals that do not metamorphose (Hayes, 1997). Although ovary maturation was observed in giant athyroid *Xenopus* tadpoles (Rot-Nikcevic and Wassersug, 2004) and in long-living overwintering *Pelobates fuscus* tadpoles (Smirnov, 1992), anuran larval reproducers have never been found. Therefore, the dependence of anuran development upon THs prevents the evolution of neoteny. Since frogs need metamorphosis to reproduce, arrest of development at stages preceding the metamorphic climax is impossible. In parallel, loss of highly TH-mediated bones associated with metamorphosis is also impossible. Bones that are subjected to paedomorphic loss or underdevelopment are among the last-appearing bones. They arise after the TH-peak and, as exemplified by *B. variegata* (this study), seem to be less TH-controlled. Usually, quadratojugal, palatine, and stapes, and sometimes vomer and sphenethmoid

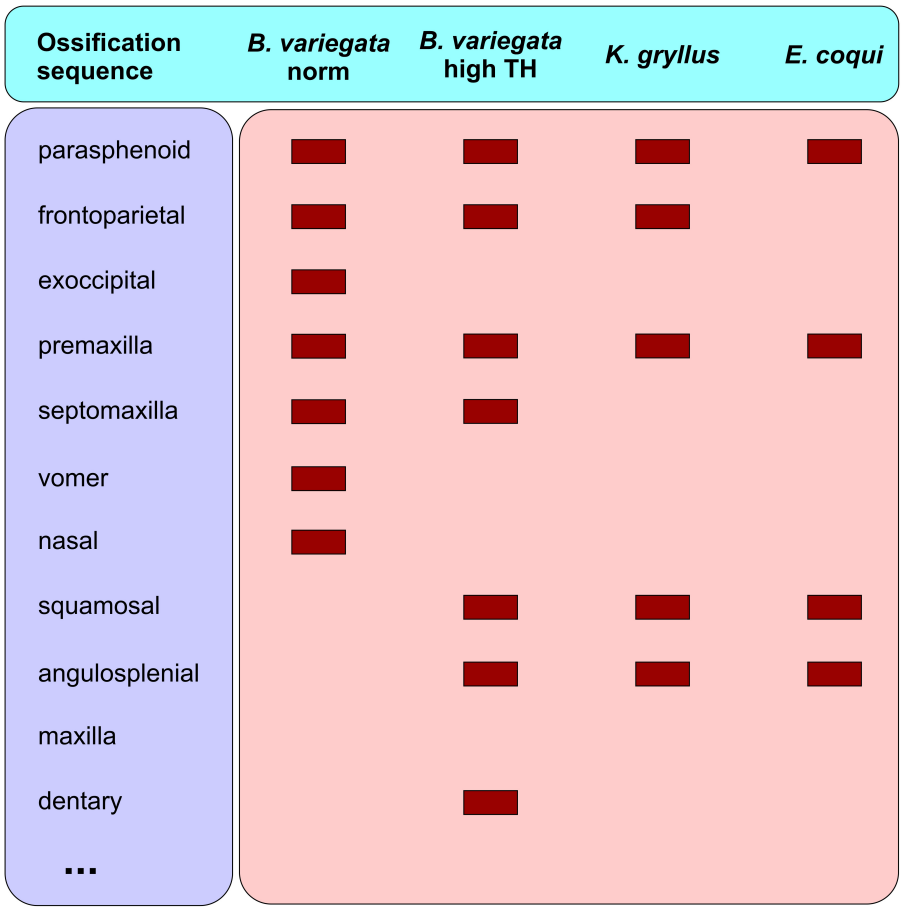


FIGURE 10 | First cranial bones in *Bombina variegata* (under normal conditions and under high-dosage T_3-treatment beginning from 6 dph) and in direct developing frogs, *Kurixalus gryllus* (after Vassilieva, 2017) and *Eleutherodactylus coqui* (after Hanken et al., 1992).

are lost in paedomorphic frogs (Davies, 1989; Yeh, 2002). It is these bones that usually arise last in the cranial ossification sequence (Weisbecker and Mitgutsch, 2010).

THYROID HORMONES AND SKELETOGENESIS IN AMPHIBIANS: CONCLUDING REMARKS AND PERSPECTIVES

A number of studies, including this review, show that TH-changes, artificially caused in developing salamanders, result in skeletal changes. A similar phenomenon is displayed by naturally developing urodeles. The most profound evidence of TH-involvement in the diversification of skeletal morphology in salamanders is shown by neotenic species. Facultative neoteny, a common event among salamanders, results from thyroid axis dysfunction, as proven by artificially induced metamorphosis in TH-treated neotenics.

Facultative neotenics do not complete metamorphosis and, as adults, retain larval features in the skull and hyobranchial apparatus. The neotenic individuals of *Batrachuperus londongensis* (Hynobiidae) display an incomplete resorption of palatine and retain extra ceratobranchials in the hyobranchium (Jiang et al., 2018). In the neotenic *Dicamptodon tenebrosus* (Dicamptodontidae), cranial development arrests at a mid to late larval stage: the vomer and palatopterygoid are larval, a rudimentary toothless coronoid is retained, the nasal is absent and the septomaxilla is variably present (Rose, 2003). In the ambystomatid salamander, *Ambystoma talpoideum,* neotenic individuals completely retain larval hyobranchium, although their palatopterygoid may disintegrate (Reilly, 1987). Among Salamandridae, neotenic (paedotypic) individuals of *Lissotriton vulgaris* and *Ichthyosaura alpestris* usually retain a complete or only partially resorbed palatopterygoid in parallel with the larval vomer and hyobranchium. Additionally, sometimes they lack the nasal and prefrontal (Djorović and Kalezić, 2000; Ivanović et al., 2014). Neotenic *Notophthalmus viridiscens* newts display the occurrence of a completely adult skull accompanied by a hyobranchium differing in the degree of transformation. The latter may (i) retain the completely larval morphology (but with ossified primarily cartilaginous elements), (ii) attain almost complete metamorphic remodeling, or (iii) show an intermediate morphology (Reilly, 1987). Neotenic plethodontids

display the most underdeveloped morphology: they lack the maxilla, nasal, prefrontal, and septomaxilla and retain the larval vomer, palatopterygoid, and coronoid (Rose, 2003). In normal, biphasic development, the appearance of these lacking bones and remodeling of the palate and hyobranchium are associated with the metamorphic climax and are TH-inducible.

Since no larval reproducers are known among anurans, TH-involvement in the diversification of anuran larval development as well as of adult morphology is much less evident than in urodeles. Our study has shown that changes in thyroid function (i) influence the rate and length of larval development, (ii) cause changes in the ossification sequence, (iii) provoke changes in the pattern of bone formation, (iv) induce the appearance of additional centers of ossification, (v) facilitate bones fusion, and (vi) may result in their underdevelopment or loss. Consequently, the occurrence of such events in the developing frog may suggest the occurrence of changes in thyroid axis function.

For example, *Pseudys platensis* (Hylidae) has a very prolonged larval development several months long (Downie et al., 2009; Fabrezi et al., 2009) and is uncommon for the studied anuran developmental events: this frog completes the cranial ossification sequence and differentiation of the plectral apparatus of the middle ear prior to the end of metamorphosis (Fabrezi and Goldberg, 2009). This precocious skeletal formation suggests a precocious increase in TH-level. This seems to be the case since the profile of thyroid gland activity in *P. platensis* larvae rises in the prometamorphic phase, i.e., well before the metamorphic climax (Cruz and Fabrezi, 2020).

In contrast, frogs of *Lepidobatrachus* species (Ceratophryidae) are characterized by larval development shortened to less than three weeks (Ruibal and Thomas, 1988; Fabrezi, 2011). Predatory tadpoles of *Lepidobatrachus* are distinguished by a mixture of larval, mid-metamorphic, and adult features, including skeleton (Ruibal and Thomas, 1988; Hanken, 1993; Fabrezi and Quinzio, 2008). The peculiarities of *Lepidobatrachus* morphology were explained by "premature metamorphosis" (Hanken, 1993), which was suggested to result from an early rise in TH-level (Fabrezi and Cruz, 2014). However, since histological examination did not reveal any distinctive peak in thyroid gland activity in *Lepidobatrachus* tadpoles, it was hypothesized that the TH increase was attained due to maternal THs or live prey (Fabrezi and Cruz, 2014).

In adult anurans, variability in the relative lengths of hind limbs in two species of the *Telmatobius* genus (Telmatobiidae) was proposed to result from environmentally (via temperature) caused differences in the reaction to TH-influence (Barrionuevo, 2020).

Morphological changes artificially caused by TH-changes in *B. variegata* (this study) are similar to those usually found in naturally developing amphibians. However, this similarity does not automatically indicate the latter to result from changes in thyroid physiology. TH-involvement remains hypothetical until the experimental test. In our study, TH-involvement, degree of TH-influence, level of TH-sensitivity, morphological consequences of TH-changes and their dependence upon the timing and dosage of TH- or TU-administration, among others, were evaluated with a combination of artificially induced TH-deficiency and TH-treatment. Variable parameters in this study were TH and TU concentrations, timing of their administration and stage of animal development. Additionally, salamanders previously studied using the same experimental protocol differ in their phylogenetic position from basal in *S. keyserlingi* (Hynobiidae) to more derived in *P. waltl* and *L. vulgaris* (Salamandridae).

Thyroid hormones are intensively studied, and new experimental methods will doubtless appear, but the experimental approach used in our study seems adequate for assigned targets and may be applied in future research. Such research will benefit if it (i) is not limited to model species (e.g., *Xenopus* and *Pleurodeles*) but includes various species differing in phylogenetic position, life cycle, developmental trajectory, and ecological adaptations; (ii) studies both bones and cartilages and the interplay between them; and (iii) uses in parallel different agents influencing different processes in the thyroid pathway. The last point is important since deviation in any process included in the thyroid pathway may influence thyroid physiology and cause consequent changes in development and morphology. It is worth noting that differences in the morphology of metamorphosing and neotenic plethodontid *Eurycea tynerensis* salamanders were recently revealed to result from differences in the transcriptional activity of thyroid hormone receptors (Aran et al., 2014).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Severtsov Institute's Animal Ethics Committee.

AUTHOR CONTRIBUTIONS

AV performed the experiment, obtained and analyzed the data, made the photographs, prepared the figures and tables, conceptualized the hypothesis, reviewed the literature, and wrote the manuscript. SS planned and designed the study, analyzed the data, conceptualized the hypothesis, discussed the results, reviewed the literature, and wrote and edited the manuscript. Both authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors are deeply indebted to Irina Serbinova from the Moscow Zoo for breeding *Bombina variegata*, to Vitaly Trounov for technical assistance and to Alexey Tsessarsky and Fedor Shkil for their kind help with microphotography.

REFERENCES

Ajduković, M., Vučić, T., and Cvijanović, M. (2021). Effect of thiourea on the skull of *Triturus* newts during ontogeny. *PeerJ* 9:e11535. doi: 10.7717/peerj.11535

Alberch, P. (1989). "Development and the evolution of amphibian metamorphosis," in *Trends in Vertebrate Morphology (Fortschritte der Zoologie)*, eds H. Splechtna and H. Hilgers (Stuttgart: Gustav Fischer Verlag), 163–173.

Alberch, P., and Alberch, J. (1981). Heterochronic mechanisms of morphological diversification and evolutionary change in the neotropical salamander, *Bolitoglossa occidentalis* (Amphibia: Plethodontidae). *J. Morphol.* 167, 249–264. doi: 10.1002/jmor.1051670208

Alberch, P., and Gale, E. A. (1986). Pathways of cytodifferentiation during the metamorphosis of the epibranchial cartilage in the salamander *Eurycea bislineata*. *Dev. Biol.* 117, 233–244. doi: 10.1016/0012-1606(86)90366-0

Alberch, P., Gale, E. A., and Larsen, P. R. (1986). Plasma T4 and T3 levels in naturally metamorphosing *Eurycea bislineata* (Amphibia; Plethodontidae). *Gen. Comp. Endocrinol.* 61, 153–163. doi: 10.1016/0016-6480(86)90261-3

Aran, R. P., Steffen, M. A., Martin, S. D., Lopez, O. I., and Bonett, R. M. (2014). Reduced effects of thyroid hormone on gene expression and metamorphosis in a paedomorphic plethodontid salamander. *J. Exp. Zool. B. Mol. Dev. Evol.* 322B, 294–303. doi: 10.1002/jez.b.22580

Banbury, B., and Maglia, A. M. (2006). Skeletal development of the Mexican spadefoot, *Spea multiplicata* (Anura: Pelobatidae). *J. Morphol.* 267, 803–821. doi: 10.1002/jmor.10441

Bardua, C., Fabre, A. C., Clavel, J., Bon, M., Das, K., Stanley, E. L., et al. (2021). Size, microhabitat, and loss of larval feeding drive cranial diversification in frogs. *Nat. Commun.* 12:2503. doi: 10.1038/s41467-021-22792-y

Barrionuevo, J. S. (2020). Variation in the growth and development of the hind limbs in frogs of the genus *Telmatobius* (Anura: Telmatobiidae). *J. Morphol.* 281, 1534–1546. doi: 10.1002/jmor.21264

Beachy, C. K., Ryan, T. J., and Bonett, R. M. (2017). How metamorphosis is different in plethodontids: larval life history perspectives on life-cycle evolution. *Herpetologica* 73, 252–258. doi: 10.1655/herpetologica-d-16-00083.1

Bonett, R. M., Mueller, R. L., and Wake, D. B. (2005). Why should reacquisition of larval stages by desmognathine salamanders surprise us? *Herpetol. Rev.* 36, 112–113.

Bonett, R. M., Steffen, M. A., Lambert, S. M., Wiens, J. J., and Chippindale, P. T. (2014). Evolution of paedomorphosis in plethodontid salamanders: ecological correlates and re-evolution of metamorphosis. *Evolution* 68, 466–482. doi: 10.1111/evo.12274

Boy, J. A., and Sues, H.-D. (2000). "Branchiosaurs: larvae, metamorphosis and heterochrony in temnospondyls and seymouriamorphs," in *Amphibian Biology. Palaeontology*, Vol. 4, eds H. Heatwole and R. L. Carroll (Chipping Norton: Surrey Beatty & Sons), 1150–1197.

Brown, D. D. (1997). The role of thyroid hormone in zebrafish and axolotl development. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13011–13016. doi: 10.1073/pnas.94.24.13011

Brown, D. D., and Cai, L. (2007). Amphibian metamorphosis. *Dev. Biol.* 306, 20–33.

Brown, D. D., Cai, L., Das, B., Marsh-Armstrong, N., Schreiber, A. M., and Juste, R. (2005). Thyroid hormone controls multiple independent programs required for limb development in *Xenopus laevis* metamorphosis. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12455–12458. doi: 10.1073/pnas.0505989102

Callery, E. M., and Elinson, R. P. (2000). Thyroid hormone-dependent metamorphosis in a direct developing frog. *Proc. Natl. Acad. Sci. U.S.A.* 97, 2615–2620. doi: 10.1073/pnas.050501097

Chippindale, P. T., Bonett, R. M., Baldwin, A. S., and Wiens, J. J. (2004). Phylogenetic evidence for a major reversal of life history evolution in plethodontid salamanders. *Evolution* 58, 2809–2822. doi: 10.1111/j.0014-3820.2004.tb01632.x

Choi, J., Ishizuya-Oka, A., and Buchholz, D. R. (2017). Growth, development, and intestinal remodeling occurs in the absence of thyroid hormone receptor α in tadpoles of *Xenopus tropicalis*. *Endocrinology* 158, 1623–1633. doi: 10.1210/en.2016-1955

Čihák, R., Královec, K., and Roček, Z. (2003). Developmental origin of the frontoparietal bone in *Bombina variegata* (Anura: Discoglossidae). *J. Morphol.* 255, 122–129. doi: 10.1002/jmor.10055

Clemen, G., and Greven, H. (2018). Long-term effects of arrested metamorphosis on dental systems in *Salamandra salamandra* (Salamandridae: Urodela). *Vert. Zool.* 68, 143–155.

Corsin, J. (1967). "Quelques problèmes de morphogenèse du crâne chez les urodèles," in problèmes actuels de paléontologie (evolution des vertébrés). *Coll. Int. Cent. Nat. Rec. Scient.* 163, 295–299.

Cruz, J. C., and Fabrezi, M. (2020). Histology and microscopic anatomy of the thyroid gland during the larval development of *Pseudis platensis* (Anura, Hylidae). *J. Morphol.* 281, 122–134. doi: 10.1002/jmor.21085

Davies, M. (1989). Ontogeny of bone and the role of heterochrony in the myobatrachine genera *Uperoleia, Crinia*, and *Pseudophryne* (Anura: Leptodactylidae: Myobatrachinae). *J. Morphol.* 200, 269–300. doi: 10.1002/jmor.1052000305

Dent, J. N. (1942). The embryonic development of *Plethodon cinereus* as correlated with the differentiation and functioning of the thyroid gland. *J. Morphol.* 71, 577–601. doi: 10.1002/jmor.1050710307

Djorović, A., and Kalezić, M. L. (2000). Paedogenesis in European newts (*Triturus*: Salamandridae): cranial morphology during ontogeny. *J. Morphol.* 243, 127–139. doi: 10.1002/(sici)1097-4687(200002)243:2<127::aid-jmor2>3.0.co;2-0

Downie, J. R., Ramnarine, I., Sams, K., and Walsh, P. T. (2009). The paradoxical frog *Pseudys paradoxa*: larval habitat, growth and metamorphosis. *Herpetol. J.* 19, 11–19.

Duellman, W. E., and Trueb, L. (1986). *Biology of Amphibians*. New York, NY: McGraw-Hill.

Dulcey Cala, C. J., Tarazona, O. A., and Ramírez-Pinilla, M. P. (2009). The morphology and post-hatching development of the skull of *Bolitoglossa nicefori* (Caudata: Plethodontidae): developmental implications of recapitulation and repatterning. *Zoology* 112, 227–239. doi: 10.1016/j.zool.2008.09.006

Dundee, H. A. (1957). Partial metamorphosis induced in *Typhlomolge rathbuni*. *Copeia* 1957, 52–53. doi: 10.2307/1440522

Dundee, H. A. (1962). Response of the neotenic salamander *Haideotriton wallacei* to a metamorphic agent. *Science* 135, 1060–1061. doi: 10.1126/science.135.3508.1060

Eagleson, G. W., and McKeown, B. A. (1978). Changes in thyroid activity of *Ambystoma gracile* (Baird) during different larval, transforming, and postmetamorphic phases. *Can. J. Zool.* 56, 1377–1381. doi: 10.1139/z78-190

Ehmcke, J., and Clemen, G. (2000). The structure and development of the skull of costa rican plethodontid salamanders (Amphibia: Urodela). *Anna. Anat.* 182, 537–547. doi: 10.1016/s0940-9602(00)80100-4

Fabrezi, M. (2011). Heterochrony in growth and development in anurans from the Chaco of South America. *Evol. Biol.* 38, 390–411. doi: 10.1007/s11692-011-9128-5

Fabrezi, M., and Cruz, J. C. (2014). Ontogeny of the thyroid glands during larval development of South American horned frogs (Anura, Ceratophryidae). *Evol. Biol.* 41, 606–618. doi: 10.1007/s11692-014-9292-5

Fabrezi, M., and Goldberg, J. (2009). Heterochrony during skeletal development of *Pseudis platensis* (Anura, Hylidae) and the early offset of skeleton development and growth. *J. Morphol.* 270, 205–220. doi: 10.1002/jmor.10680

Fabrezi, M., and Quinzio, S. I. (2008). Morphological evolution in Ceratophryinae frogs (Anura, Neobatrachia): the effects of heterochronic changes during larval development and metamorphosis. *Zool. J. Linnean Soc.* 154, 752–780. doi: 10.1111/j.1096-3642.2008.00420.x

Fabrezi, M., Lozano, V. L., and Cruz, J. C. (2019). Differences in responsiveness and sensitivity to exogenous disruptors of the thyroid gland in three anuran species. *J. Exp. Zool. Part B.* 332, 279–293. doi: 10.1002/jez.b.22908

Fabrezi, M., Quinzio, S. I., and Goldberg, J. (2009). Giant tadpole and delayed metamorphosis of *Pseudis platensis* Gallardo, 1961 (Anura, Hylidae). *J. Herpetol.* 43, 228–243. doi: 10.1670/08-028r3.1

Fort, D. J., Degitz, S., Tietge, J., and Touart, L. W. (2007). The hypothalamic-pituitary-thyroid (HPT) axis in frogs and its role in frog development and reproduction. *Cri. Rev. Toxicol.* 37, 117–161. doi: 10.1080/10408440601123545

Fritzsch, B. (1990). The evolution of metamorphosis in amphibians. *J. Neurobiol.* 21, 1011–1021. doi: 10.1002/neu.480210707

Frost, D. R. (2021). *Amphibian Species of the World: an Online Reference. Version 6.1*. New York, NY: American Museum of Natural History.

Furlow, J. D., and Neff, E. S. (2006). A developmental switch induced by thyroid hormone: *Xenopus laevis* metamorphosis. *Trends Endocrinol. Metab.* 17, 40–47. doi: 10.1016/j.tem.2006.01.007

Galton, V. A. (1992). The role of thyroid hormone in amphibian metamorphosis. *Trends Endocrinol. Metab.* 3, 96–100. doi: 10.1016/1043-2760(92)90020-2

Gomez-Mestre, I., Pyron, R. A., and Wiens, J. J. (2012). Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes in frogs. *Evolution* 66, 3687–3700. doi: 10.1111/j.1558-5646.2012.01715.x

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae. *Herpetologica* 16, 183–190.

Grimaldi, A., Buisine, N., Miller, T., Shi, Y.-B., and Sachs, L. M. (2013). Mechanisms of thyroid hormone receptor action during development: lessons from amphibian studies. *Biochim. Biophys. Acta* 1830, 3882–3892. doi: 10.1016/j.bbagen.2012.04.020

Haas, A. (2003). Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* 19, 23–89. doi: 10.1016/s0748-3007(03)00006-9

Haas, A., and Richards, S. J. (1998). Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura: Hylidae: Pelodryadinae). *J. Morphol.* 238, 109–141. doi: 10.1002/(sici)1097-4687(199811)238:2<109::aid-jmor1>3.0.co;2-#

Hall, B. K. (1999a). *Evolutionary Developmental Biology*. Dordrecht: Springer.

Hall, B. K. (1999b). *The Neural Crest in Development and Evolution*. New York, NY: Springer-Verlag, Inc.

Hall, J. A., and Larsen, J. H. Jr. (1998). Postembryonic ontogeny of the spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae): skeletal morphology. *J. Morphol.* 238, 179–244. doi: 10.1002/(sici)1097-4687(199811)238:2<179::aid-jmor4>3.0.co;2-6

Handrigan, G. R., and Wassersug, R. J. (2007a). The anuran Bauplan: a review of the adaptive, developmental, and genetic underpinnings of frog and tadpole morphology. *Biol. Rev.* 82, 1–25. doi: 10.1111/j.1469-185x.2006.00001.x

Handrigan, G. R., and Wassersug, R. J. (2007b). The metamorphic fate of supernumerary caudal vertebrae in South Asian litter frogs (Anura: Megophryidae). *J. Anat.* 211, 271–279. doi: 10.1111/j.1469-7580.2007.00757.x

Handrigan, G. R., Haas, A., and Wassersug, R. J. (2007). Bony-tailed tadpoles: the development of supernumerary caudal vertebrae in larval megophryids (Anura). *Evol. Dev.* 9, 190–202. doi: 10.1111/j.1525-142x.2007.00149.x

Hanken, J. (1984). Miniaturization and its effects on cranial morphology in plethodontid salamanders, genus *Thorius* (Amphibia: Plethodontidae). I. osteological variation. *Biol. J. Linn. Soc. Lond.* 23, 55–75. doi: 10.1111/j.1095-8312.1984.tb00806.x

Hanken, J. (1993). Model systems versus outgroups: alternative approaches to the study of head development and evolution. *Am. Zool.* 33, 448–456. doi: 10.1093/icb/33.4.448

Hanken, J., and Hall, B. K. (1984). Variation and timing of the cranial ossification sequence of the oriental fire-bellied toad, *Bombina orientalis* (Amphibia, Discoglossidae). *J. Morphol.* 182, 245–255. doi: 10.1002/jmor.1051820302

Hanken, J., and Hall, B. K. (1988a). Skull development during anuran metamorphosis: I. Early development of the first three bones to form—the exoccipital, the parasphenoid, and the frontoparietal. *J. Morphol.* 195, 247–256. doi: 10.1002/jmor.1051950303

Hanken, J., and Hall, B. K. (1988b). Skull development during anuran metamorphosis. II. Role of thyroid hormone in osteogenesis. *Anat. Embryol.* 178, 219–227. doi: 10.1007/bf00318225

Hanken, J., and Summers, C. H. (1988). Skull development during anuran metamorphosis: III. Role of thyroid hormone in chondrogenesis. *J. Exp. Zool.* 246, 156–170. doi: 10.1002/jez.1402460208

Hanken, J., Jennings, D. H., and Olsson, L. (1997). Mechanistic basis of life-history evolution in anuran amphibians: direct development. *Am. Zool.* 37, 160–171. doi: 10.1093/icb/37.2.160

Hanken, J., Klymkowsky, M. W., Summers, C. H., Seufert, D. W., and Ingebrigtsen, N. (1992). Cranial ontogeny in the direct-developing frog, *Eleutherodactylus coqui* (Anura: Leptodactylidae), analyzed using whole-mount immunohistochemistry. *J. Morphol.* 211, 95–118. doi: 10.1002/jmor.1052110111

Hayes, T. B. (1997). Hormonal mechanisms as potential constraints on evolution: examples from the Anura. *Am. Zool.* 37, 482–490. doi: 10.1093/icb/37.6.482

Hughes, A. (1959). Studies in embryonic and larval development in *Amphibia*. I. The embryology of *Eleutherodactylus ricordii*, with special reference to the spinal cord. *J. Emhryol. Exp. Morphol* 7, 22–38.

Ivanović, A., Cvijanović, M., Denoël, M., Slijepčević, M., and Kalezić, M. L. (2014). Facultative paedomorphosis and the pattern of intra-and interspecific variation in cranial skeleton: lessons from European newts (*Ichthyosaura alpestris* and *Lissotriton vulgaris*). *Zoomorphology* 133, 99–109. doi: 10.1007/s00435-013-0202-7

Jennings, D. H., and Hanken, J. (1998). Mechanistic basis of life history evolution in anuran amphibians: thyroid gland development in the direct-developing frog, *Eleutherodactylus coqui*. *Gen. Comp. Endocrinol.* 111, 225–232. doi: 10.1006/gcen.1998.7111

Jiang, J. P., Jia, J., Zhang, M., and Gao, K. Q. (2018). Osteology of *Batrachuperus londongensis* (Urodela, Hynobiidae): study of bony anatomy of a facultatively neotenic salamander from Mount Emei, Sichuan Province, China. *PeerJ* 6:e4517. doi: 10.7717/peerj.4517

Kemp, N. E., and Hoyt, J. A. (1965b). Influence of thyroxine on ossification of the parasphenoid bone in the skull of *Rana pipiens*. *Am. Zool.* 5:710.

Kemp, N. E., and Hoyt, J. A. (1965a). Influence of thyroxine on order of ossification of bones of the skull of *Rana pipiens*. *Am. Zool.* 5:719.

Kemp, N. E., and Hoyt, J. A. (1969). Ossification of the femur in thyroxine-treated tadpoles of *Rana pipiens*. *Dev. Biol.* 20, 287–410.

Kerney, R., Meegaskumbura, M., Manamendra-Arachchi, K., and Hanken, J. (2007). Cranial ontogeny in *Philautus silus* (Anura: Ranidae: Rhacophorinae) reveals few similarities with other direct-developing anurans. *J. Morphol.* 268, 715–725. doi: 10.1002/jmor.10545

Kerney, R., Wassersug, R., and Hall, B. K. (2010). Skeletal advance and arrest in giant non-metamorphosing African clawed frog tadpoles (*Xenopus laevis*: Daudin). *J. Anat.* 216, 132–143. doi: 10.1111/j.1469-7580.2009.01176.x

Larras-Regard, E. (1985). "Hormonal determination of neoteny in facultative neotenic urodeles," in *Metamorphosis*, eds M. Balls and M. Bownes (Oxford: Clarendon Press), 294–312.

Larras-Regard, E., Taurog, A., and Dorris, M. (1981). Plasma T4 and T3 levels in *Ambystoma tigrinum* at various stages of metamorphosis. *Gen. Comp. Endocrinol.* 43, 443–450. doi: 10.1016/0016-6480(81)90228-8

Larsen, J. H. (1963). *The Cranial Osteology of Neotenic and Transformed Salamanders and its Bearing on Interfamilial Relationships.* Ph.D. Dissertation. Seattle, WA: University of Washington.

Larsen, J. H. (1968). Ultrastructure of thyroid follicle cells of three salamanders (*Ambystoma, Amphiuma*, and *Necturus*) exhibiting varying degrees of neoteny. *J. Ultrastruct. Res.* 24, 190–209. doi: 10.1016/s0022-5320(68)90058-0

Laslo, M., Denver, R. J., and Hanken, J. (2019). Evolutionary conservation of thyroid hormone receptor and deiodinase expression dynamics in ovo in a direct-developing frog, *Eleutherodactylus coqui*. *Front. Endocrinol.* 10:307. doi: 10.3389/fendo.2019.00307

Laudet, V. (2011). The origins and evolution of vertebrate metamorphosis. *Curr. Biol.* 21, R726–R737. doi: 10.1016/j.cub.2011.07.030

Lebedkina, N. S. (1979). *Evolution of Amphibian Skull*. Moscow: Nauka.

Lebedkina, N. S. (1986). "Development of correlative systems in phylogeny and ontogeny," in *Animal Morphology and Evolution*, eds E. I. Vorobyeva and N. S. Lebedkina (Moscow: Nauka), 95–101.

Lebedkina, N. S. (2004). *Evolution of Amphibian Skull (Translated and Edited by S.V. Smirnov)*. Sofia: Pensoft Publishers.

Lukas, P., and Olsson, L. (2020). Sequence of chondrocranial development in the oriental fire bellied toad *Bombina orientalis*. *J. Morphol.* 281, 688–701. doi: 10.1002/jmor.21138

Lynn, W. G. (1942). *The Embryology of Eleutherodactylus Nubicola, an Anuran Which Has no Tadpole Stage*. Washington, DC: Carnegie Institution.

Lynn, W. G. (1961). Types of amphibian metamorphosis. *Am. Zool.* 1, 151–161. doi: 10.1093/icb/1.1.151

Lynn, W. G., and Lutz, B. (1946). *The Development of Eleutherodactylus Guentheri Stdnr. 1864*. Portuguese: Boletim do Museu Nacional Zoologia.

Maglia, A. M., and Púgener, L. A. (1998). Skeletal development and adult osteology of *Bombina orientalis* (Anura: Bombinatoridae). *Herpetologica* 54, 344–363.

Marks, S. B. (2000). "Skull development in two plethodontid salamanders (genus *Desmognathus*) with different life histories," in *The Biology of Plethodontid Salamanders*, eds R. C. Bruce, R. G. Jaeger, and L. D. Houck (New York, NY: Kluwer Academic Publishers), 261–276. doi: 10.1007/978-1-4615-4255-1_12

Matsuda, R. (1987). *Animal Evolution in Changing Environments with Special Reference to Abnormal Metamorphosis*. New York, NY: Wiley-Interscience.

Medvedeva, I. M. (1975). *The Olfactory Organ in Amphibians and its Phylogenetic Significance*. Leningrad: Nauka.

Medvedeva, I. M. (1986). "On the origin of nasolacrimal duct in Tetrapoda," in *Studies in Herpetology. Third General Meeting of the Societas Europaea Herpetologica*, ed. Z. Roček (Prague: Charles University), 37–40.

Mitsui, N., Fujii, T., Miyahara, M., Oka, T., Kashiwagi, A., Kashiwagi, K., et al. (2006). Development of metamorphosis assay using Silurana tropicalis for the detection of thyroid system-disrupting chemicals. *Ecotoxicol. Environ. Saf.* 64, 281–287. doi: 10.1016/j.ecoenv.2005.07.007

Mueller, R. L., Macey, J. R., Jaekel, M., Wake, D. B., and Boore, J. L. (2004). Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proc. Natl. Acad. Sci. USA* 101, 13820–13825. doi: 10.1073/pnas.0405785101

Pugener, L. A., and Maglia, A. M. (2007). Skeletal morphology and development of the olfactory region of *Spea* (Anura: Scaphiopodidae). *J. Anat.* 211, 754–768.

Regards, E., Taurog, A., and Nakashima, T. (1978). Plasma thyroxine and triiodothyronine levels in spontaneously metamorphosing *Rana catesbeiana* tadpoles and in adult anuran amphibia. *Endocrinology* 102, 674–684. doi: 10.1210/endo-102-3-674

Reilly, S. M. (1987). Ontogeny of the hyobranchial apparatus in the salamanders *Ambystoma talpoideum* (Ambystomatidae) and *Notophthalmus viridescens* (Salamandridae): the ecological morphology of two neotenic strategies. *J. Morphol.* 191, 205–214. doi: 10.1002/jmor.1051910210

Reilly, S. M., and Altig, R. (1996). Cranial ontogeny in *Siren intermedia* (Caudata: Sirenidae): paedomorphic, metamorphic, and novel patterns of heterochrony. *Copeia* 1996:29. doi: 10.2307/1446939

Reilly, S. M., Wiley, E. O., and Meinhardt, D. J. (1997). An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biol. J. Linn. Soc. Lond.* 60, 119–143. doi: 10.1111/j.1095-8312.1997.tb01487.x

Reiss, J. (1996). Palatal metamorphosis in basal caecilians (Amphibia: Gymnophiona) as evidence for lissamphibian monophyly. *J. Herpetol.* 30, 27–39. doi: 10.2307/1564703

Reiss, J. (2002). The phylogeny of amphibian metamorphosis. *Zoology* 105, 85–96. doi: 10.1078/0944-2006-00059

Roček, Z. (2003). "Larval development and evolutionary origin of the anuran skull," in *Amphibian Biology Osteology*, Vol. Vol 5, ed. H. Heatwole (Chipping Norton: Surrey Beatty & Sons), 1875–1995.

Roček, Z., Halámková, L., and Muzzopappa, P. (2021). Developmental dynamics of two closely related species *Bombina bombina* and *B. variegata* (Amphibia: Anura) in the context of their fossil evidence. *Hist. Biol.* doi: 10.1080/08912963.2020.1867979

Rose, C. S. (1999). "Hormonal control in larval development and evolution—Amphibians," in *The Origin and Evolution of Larval Forms*, eds B. K. Hall and M. H. Wake (Cambridge, MA: Academic Press), 167–216. doi: 10.1016/b978-012730935-4/50007-9

Rose, C. S. (1995c). Skeletal morphogenesis in the urodele skull: I. Postembryonic development in the Hemidactyliini (Amphibia: Plethodontidae). *J. Morphol.* 223, 125–148. doi: 10.1002/jmor.1052230203

Rose, C. S. (1995a). Skeletal morphogenesis in the urodele skull: II. Effect of developmental stage in thyroid hormone-induced remodeling. *J. Morphol.* 223, 149–166. doi: 10.1002/jmor.1052230204

Rose, C. S. (1995b). Skeletal morphogenesis in the urodele skull: III. Effect of hormone dosage in TH-induced remodeling. *J. Morphol.* 223, 243–261. doi: 10.1002/jmor.1052230303

Rose, C. S. (1996). An endocrine-based model for developmental and morphogenetic diversification in metamorphic and paedomorphic urodeles. *J. Zool.* 239, 253–284. doi: 10.1111/j.1469-7998.1996.tb05451.x

Rose, C. S. (2003). "The developmental morphology of salamander skulls," in *Amphibian Biology Osteology*, Vol. Vol. 5, ed. H. Heatwole (Chipping Norton: Surrey Beatty & Sons Ltd), 1684–1781.

Rose, C. S., and Cahill, J. W. (2019). How thyroid hormones and their inhibitors affect cartilage growth and shape in the frog *Xenopus laevis*. *J. Anat.* 234, 89–105. doi: 10.1111/joa.12897

Rose, C. S., and Reiss, J. (1993). "Metamorphosis and the vertebrate skull: ontogenetic patterns and developmental mechanisms," in *The Skull Development*, Vol. Vol. 1, eds J. Hanken and B. K. Hall (Chicago, IL: University of Chicago Press), 289–346.

Rosenkilde, P., and Ussing, A. P. (1996). What mechanisms control neoteny and regulate induced metamorphosis in urodeles? *Int. J. Dev. Biol.* 40, 665–673.

Rosenkilde, P. (1985). "The role of hormones in the regulation of amphibian metamorphosis," in *Metamorphosis*, eds M. Balls and M. Bownes (Oxford: Clarendon Press), 221–259.

Rot-Nikcevic, I., and Wassersug, R. J. (2004). Arrested development in *Xenopus laevis* tadpoles: how size constrains metamorphosis. *J. Exp. Biol.* 207, 2133–2145. doi: 10.1242/jeb.01002

Ruibal, R., and Thomas, E. (1988). The obligate carnivorous larvae of the frog, *Lepidobatrachus laevis* (Leptodactylidae). *Copeia* 1988, 591–604. doi: 10.2307/1445377

Safi, R., Begue, A., Hänni, C., Stehelin, D., Tata, J. R., and Laudet, V. (1997a). Thyroid hormone receptor genes of neotenic amphibians. *J. Mol. Evol.* 44, 595–604. doi: 10.1007/pl00006182

Safi, R., Deprez, A., and Laudet, V. (1997b). Thyroid hormone receptors in perennibranchiate amphibians. *Int. J. Dev. Biol.* 41, 533–535.

Schlosser, G. (2001). Using heterochrony plots to detect the dissociated coevolution of characters. *J. Exp. Zool.* 291, 282–304. doi: 10.1002/jez.1104

Schoch, R. R. (1998). Homology of cranial ossifications in urodeles. *Neues Jahrb. Geol. Paläontol. Monatsh.* 1998, 1–25. doi: 10.1127/njgpm/1998/1998/1

Schoch, R. R. (2002). The evolution of metamorphosis in temnospondyls. *Lethaia* 35, 309–327. doi: 10.1080/002411602320790634

Schoch, R. R. (2009). Evolution of life cycles in early amphibians. *Annu. Rev. Earth Planet Sci.* 37, 135–162. doi: 10.1146/annurev.earth.031208.100113

Schoch, R. R., and Fröbisch, N. (2006). Metamorphosis and neoteny: alternative pathways in an extinct amphibian clade. *Evolution* 60, 1467–1475. doi: 10.1554/05-632.1

Senevirathne, G., Baumgart, S., Shubin, N., Hanken, J., and Shubin, N. H. (2020). Ontogeny of the anuran urostyle and the developmental context of evolutionary novelty. *Proc. Natl. Acad. Sci. U.S.A.* 117, 3034–3044. doi: 10.1073/pnas.1917506117

Sheil, C. A., Jorgensen, M., Tulenko, F., and Harrington, S. (2014). Variation in timing of ossification affects inferred heterochrony of cranial bones in Lissamphibia. *Evol. Dev.* 16, 292–305. doi: 10.1111/ede.12092

Smirnov, S. V. (1992). The influence of variation in larval period on adult cranial diversity in *Pelobates fuscus* (Anura: Pelobatidae). *J. Zool.* 226, 601–612. doi: 10.1111/j.1469-7998.1992.tb07503.x

Smirnov, S. V. (2006). Metamorphosis in the Urodelan amphibians: patterns, regulation mechanisms, and evolution. *Zh. Obshch. Biol.* 67, 323–334. doi: 10.1016/j.parint.2016.03.011

Smirnov, S. V., and Vasil'eva, A. B. (2002). The bony skull of the siberian salamander *Salamandrella keyserlingi* (Amphibia: Urodela: Hynobiidae) and the role of thyroid hormones in its development. *Dokl. Biol. Sci.* 385, 387–389.

Smirnov, S. V., and Vassilieva, A. B. (2003). Skeletal and dental ontogeny in the smooth newt, *Triturus vulgaris* (Urodela: Salamandridae): role of thyroid hormone in its regulation. *Rus. J. Herpetol.* 10, 93–110.

Smirnov, S. V., and Vassilieva, A. B. (2005). Skull development in normal, TH-exposed, and goitrogen-treated axolotls, *Ambystoma mexicanum*. *Russ. J. Herpetol.* 12, 113–126. doi: 10.1016/0014-4886(76)90239-9

Smirnov, S. V., and Vassilieva, A. B. (2009). Number of ossification centers in the anuran cranial bones depends upon the rate of development: experimental evidence. *Rus. J. Herpetol.* 16, 167–176.

Smirnov, S. V., and Vassilieva, A. B. (2014). Thyroid hormones in the skeletogenesis and accessory sources of endogenous hormones in *Xenopus laevis* (Amphibia; Anura) ontogeny: experimental evidence. *Dokl. Biol. Sci.* 455, 136–138. doi: 10.1134/s0012496614020185

Smirnov, S. V., Merkulova, K. M., and Vassilieva, A. B. (2020). Skull development in the Iberian newt, *Pleurodeles waltl* (Salamandridae: Caudata: Amphibia): timing, sequence, variations, and thyroid hormone mediation of bone appearance. *J. Anat.* 237, 543–555.

Smirnov, S. V., Vassilieva, A. B., and Merkulova, K. M. (2010). The nasal bone of the Iberian ribbed newt (*Pleurodeles waltl*; Salamandridae, Urodela): development and regulatory mechanism of its ontogeny. *Dokl. Biol. Sci.* 431, 113–116. doi: 10.1134/s0012496610020122

Smirnov, S. V., Vassilieva, A. B., and Merkulova, K. M. (2011). Thyroid hormone mediation in skull development of Siberian newt, *Salamandrella keyserlingi* (Urodela: Hynobiidae), with comparison to other species. *Rus. J. Herpetol.* 18, 203–209.

Tata, J. R. (1999). Amphibian metamorphosis as a model for studying the developmental actions of thyroid hormone. *Biochimie* 81, 359–366. doi: 10.1016/s0300-9084(99)80082-0

Trueb, L., and Alberch, P. (1985). Miniaturization of the anuran skull: a case study of heterochrony. *Fortschr. Zool.* 30, 113–121.

Vassilieva, A. B. (2017). Heterochronies in the cranial development of Asian tree frogs (Amphibia: Anura: Rhacophoridae) with different life histories. *Dokl. Biol. Sci.* 473, 50–52. doi: 10.1134/s001249661702003x

Vassilieva, A. B., and Smirnov, S. V. (2007). The role of thyroid hormones in the bony skull development in the common frog (*Rana temporaria*, Ranidae). *Dokl. Biol. Sci.* 413, 111–114. doi: 10.1134/s0012496607020056

Vater, M. (2007). Is the prefrontal bone in Alpine newt (*Triturus alpestris* Laurenti, 1768) of dual origin? *J. Anat.* 211, 290–295. doi: 10.1111/j.1469-7580.2007.00768.x

Vera Candioti, M. F. (2007). Anatomy of anuran tadpoles from lentic water bodies: systematic relevance and correlation with feeding habits. *Zootaxa* 1600, 1–175. doi: 10.11646/zootaxa.1600.1.1

Vera Candioti, M. F., Goldberg, J., Akmentins, M. S., Costa, P. N., Goulart Taucce, P. P., and Pombal, J. Jr. (2020). Skeleton in the closet: hidden diversity in patterns of cranial and postcranial ontogeny in Neotropical direct-developing frogs (Anura: Brachycephaloidea). *Org. Divers. Evol.* 20, 763–783. doi: 10.1007/s13127-020-00467-8

Wake, M. H. (1989). Metamorphosis of the hyobranchial apparatus in *Epicrionops* (Amphibia: Gymnophiona: Rhinatrematidae): replacement of bone by cartilage. *Ann. Sci. Nat. Zool.* 10, 171–182.

Wake, M. H. (2003). "The osteology of caecilians," in *Amphibian Biology Osteology*, Vol. Vol. 5, ed. H. Heatwole (Chipping Norton: Surrey Beatty and Sons), 1809–1876.

Wassersug, R. J. (1976). A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol.* 51, 131–134. doi: 10.3109/10520297609116684

Weisbecker, V., and Mitgutsch, C. (2010). A large-scale survey of heterochrony in anuran cranial ossification patterns. *J. Zool. Syst. Evol. Res.* 48, 332–347. doi: 10.1111/j.1439-0469.2010.00570.x

Weber, G. M., Farrar, E. S., Tom, C. K. F., and Grau, E. G. (1994). Changes in whole-body thyroxine and triiodothyronine concentrations and total content during early development and metamorphosis of the toad *Bufo marinus*. *Gen. Comp. Endocrinol.* 94, 62–71. doi: 10.1006/gcen.1994.1060

Weil, M. R. (1986). Changes in plasma thyroxine levels during and after spontaneous metamorphosis in a natural population of the green frog, *Rana clamitans*. *Gen. Comp. Endocrinol.* 62, 8–12. doi: 10.1016/0016-6480(86)90088-2

Wiens, J. J. (1989). Ontogeny of the skeleton of *Spea bombifrons* (Anura: Pelobatidae). *J. Morphol.* 202, 29–51. doi: 10.1002/jmor.1052020104

Yeatman, H. C., and Miller, H. B. (1985). A naturally metamorphosed Gyrinophilus palleucus from the type-locality. *J. Herpetol.* 19, 304–306. doi: 10.2307/1564190

Yeh, J. (2002). The effect of miniaturized body size on skeletal morphology in frogs. *Evolution* 56, 628–641. doi: 10.1554/0014-3820(2002)056[0628:teombs]2.0.co;2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 10 November 2021
doi: 10.3389/fevo.2021.715110

Thyroid-Mediated Metabolic Differences Underlie Ecological Specialization of Extremophile Salmonids in the Arctic Lake El'gygytgyn

Evgeny V. Esin[1], Grigorii N. Markevich[2], Dmitriy V. Zlenko[1,3] and Fedor N. Shkil[1,4]*

[1] A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia, [2] Kronotsky Nature Biosphere Reserve, Yelizovo, Russia, [3] The Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia, [4] Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

El'gygytgyn, the only "ancient lake" in the Arctic (3.6 MY), is a deep (176 m) and extremely cold (always $\leq$ 4°C) waterbody inhabited by unique salmonids, which colonized the ecosystem stepwise during the global fluctuations of the Quaternary climate. The descendant of the first-wave-invaders (long-finned charr) dwells in the deep waters and feeds on amphipods. The second-wave-invaders (smallmouth charr) consume copepods in the mid-waters. Recent third-wave-invaders (Boganida charr) are spread throughout the ecosystem and feed on insects when they are young shifting to piscivory at an older age. Here, we present the data on the charrs' thyroid status and metabolic characteristics, confirming their ecological specialization. The long-finned charr exhibits an extremely low thyroid content, the substitution of carbohydrates for lipids in the cellular respiration, an increased hemoglobin level and a high antioxidant blood capacity. These traits are likely to be the legacy of anaerobic survival under perennial ice cover during several Quaternary glaciations. Moderate thyroid status and reduced metabolic rate of the smallmouth charr, along with an inactive lifestyle, could be regarded as a specialization to saving energy under the low food supply in the water column. The piscivorous Boganida charr could be sub-divided into shallow-water and deep-water groups. The former demonstrates a significantly elevated thyroid status and increased metabolism. The latter is characterized by a reduced thyroid level, metabolic rate, and lipid accumulation. Thus, the endemic El'gygytgyn charrs represent a wide spectrum of contrast physiological adaptation patterns essential to survive in sympatry under extremely cold conditions.

Keywords: metabolic phenotype, evolutionary divergence, thyroid status, Arctic, salmonids, charrs

OPEN ACCESS

Edited by:
Marco António Campinho, Universidade do Algarve, Portugal

Reviewed by:
Paolo Sordino, Stazione Zoologica Anton Dohrn Napoli, Italy
Rodrigo Nunes Da Fonseca, Federal University of Rio de Janeiro, Brazil

****Correspondence:***
Evgeny V. Esin
evgesin@gmail.com

Specialty section:
This article was submitted to Evolutionary Developmental Biology, a section of the journal Frontiers in Ecology and Evolution

Received: *26 May 2021*
Accepted: *15 October 2021*
Published: *10 November 2021*

Citation:
Esin EV, Markevich GN, Zlenko DV and Shkil FN (2021) Thyroid-Mediated Metabolic Differences Underlie Ecological Specialization of Extremophile Salmonids in the Arctic Lake El'gygytgyn. Front. Ecol. Evol. 9:715110. doi: 10.3389/fevo.2021.715110

INTRODUCTION

Modern teleosts inhabit almost any kind of waterbody on the Earth, including such inhospitable habitats as hot springs in the deserts and subzero Antarctic waters, low-oxygen swamps, subterranean waterbodies, and lakes with extreme salinity (Nelson et al., 2016). The majority of fishes thriving under extreme conditions, i.e., extremophile fishes, exhibit remarkable physiological,

morphological, life-history, and behavioral adaptations, so they are attractive models for investigating the causes and consequences of adaptive evolution (Plath et al., 2015). In the Arctic, freshwater salmonids represent multiple adaptations allowing them to flourish despite the long winter seasons coupled with darkness, ice shield, and low temperatures. Among the numerous Arctic salmonid communities, the unique fauna persisting in Lake El'gygytgyn (Western Beringia, Chukotka) through several glacial periods is of particular interest. This waterbody is the only "ancient" lake in the Arctic filling a meteorite crater formed 3.6 MY ago. The 176-m deep lake of a bowl-shaped morphology and the surface area of 110 km^2 never warms up above 4°C and is covered with ice for 9–10 months per year (Nolan and Brigham-Grette, 2007; Melles et al., 2012). Given that the lake basin had escaped the continental-scale glaciation (Glushkova, 2001; Melles et al., 2012), it represents a unique model for paleoecological and evolutionary reconstructions.

Ichthyologists have discovered the endemic highly specialized El'gygytgyn charrs (g. *Salvelinus*, **Figure 1**), which stepwise colonized the lake during the global fluctuations of the Quaternary climate (Chereshnev et al., 2002; Osinov et al., 2015). The first invaders entered the lake between 3.6 and 1.7 MY ago. Their descendant, the long-finned charr, *S. svetovidovi,* dwells in the deep and demonstrates a very peculiar morphology with paedomorphic traits (Chereshnev and Skopets, 1990; Frolov, 1993; Alekseyev, 2000). The ancestors of two closely related but allopatric charrs: smallmouth, *S. elgyticus*, and Boganida, *S. boganidae*, invaded the lake much later, after the Last Glacial Maximum, 15–5 thousand years ago (Osinov et al., 2015; Oleinik et al., 2019, 2021; Osinov and Volkov, 2020). The former species is a gracile-bodied charr possessing planktivorous traits (Chereshnev et al., 2002). The latter, being the representatives of the Boganida charr, are found throughout the ecosystem and characterized by the phenotype typical of piscivorous salmonids (Chereshnev et al., 2002).

Surviving in the extremely cold, deep, and low productive waters of El'gygytgyn, is an energy-balancing task requiring various physiological adaptations, foremost the consistent metabolic changes. We suggest that El'gygytgyn charrs possess common traits essential for life in the cold, such as enriching the cell membranes by the polyunsaturated fatty acids, changing the enzymes' activity, respiration rate and glycogen metabolism (Cossins and Prosser, 1978; Guderley, 2004; White et al., 2012; DeLong et al., 2018). However, the endemic charrs varying in lifestyle should develop some specific metabolic features related to their ecological peculiarities.

To test this hypothesis, we compared the El'gygytgyn charrs' metabolic phenotypes defined by a set of biochemical traits regulating the intensity of life processes. In particular, we assessed the blood glucose and liver glycogen levels as the circulating and stored source of the immediate energetic reserve (Masanori and Shizunori, 1971; Polakof et al., 2012). We evaluated the lipids in plasma lipoproteins and muscles, and especially triacylglycerides, as the circulating and stored source of deferred energetic reserve (Jobling, 1994b). As the indicator of the biochemical transport activity, we assessed plasma proteins (Rehulka et al., 2005) and phospholipids, which determine the cell membrane permeability, and outspread with lipoproteins throughout the body to intensify metabolism (Freeman et al., 1963). The oxidative balance was assessed via measuring the intensity of oxygen transport by hemoglobin and lipid oxidation. The latter was analyzed by the concentration of thiobarbituric acid active compounds in plasma (Debevec et al., 2017).

Finally, we compared the content of thyroid hormones as the primary endocrine factors regulating the energy balance, contributing to the thermal acclimatization, and acting as a mediator of adaptation to the changing environment in fish (Blanton and Specker, 2007; Little et al., 2013; McMenamin and Parichy, 2013; Campinho, 2019; Deal and Volkoff, 2020; Lema, 2020; Esin et al., 2021).

MATERIALS AND METHODS

The lake was fished evenly at all depths using multipanel gillnets (18–40 mm mesh size) in August 2020. All the catches were carried out in accordance with the permission No. 412020032162 issued 07/21/2020 by the North-Eastern Territorial Administration of the Federal Agency for Fishery. The procedures with fish were approved by the Bioethics commission of the A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences. The catch turned out to be represented by four and not three contrasting phenotypes separated on the spot by eye (**Figure 1**). Only the adult individuals of the deepwater slender-bodied Boganida charrs (BD) and long-finned charrs (LF) overlapped in size. Nevertheless, even these individuals were easily distinguished from each other, as well as from the large robust-bodied Boganida charrs (BL) and small-sized smallmouth charrs (SM) (**Supplementary Figure 1**) in a mixed catch.

To standardize the data on the physiological performance of the charrs for further comparison, only adult fish skipping the spawning of the appropriate year were subsampled for the analysis. The final set comprised 23 BL, 21 BD, 19 SM, and 26 LF individuals. All these fish were processed freshly caught following the standard protocol. The blood was collected from the caudal vessel with Vacuette serum tubes. The samples of muscle (cut behind the dorsal fin) and liver (distal lobe) were weighted on a balance AND HR-AZ with an accuracy of ±0.001 g. The set of biochemical parameters (see **Supplementary Figure 2**) was measured spectrophotometrically using the photometers Hatch DR2400 and StatFax 303 Plus. Centrifugation procedures were performed at 2,900 *g* (Velocity 6u, Dynamica).

Carbohydrate Analysis

The level of stored and circulated carbohydrates was analyzed in terms of liver glycogen and blood glucose content. The caudal fragment of the liver of 2 mg in weight was digested in 500 μl 30% NaOH at 100°C for 30 min. Then, 100 μl of 60% H_2SO_4 was added to the sample, and the volume was brought up to 200 μl with Milli-Q water. The sample was mixed with 1.5 mg of 0.2% anthrone reagent (Sigma) and incubated at 100°C for 20 min (Templeton, 1961). The optical density was read at 630 nm against glycogen standards. The glucose concentration was determined in blood by an automatic glucose-meter Contour TS (Harrison et al., 2011).

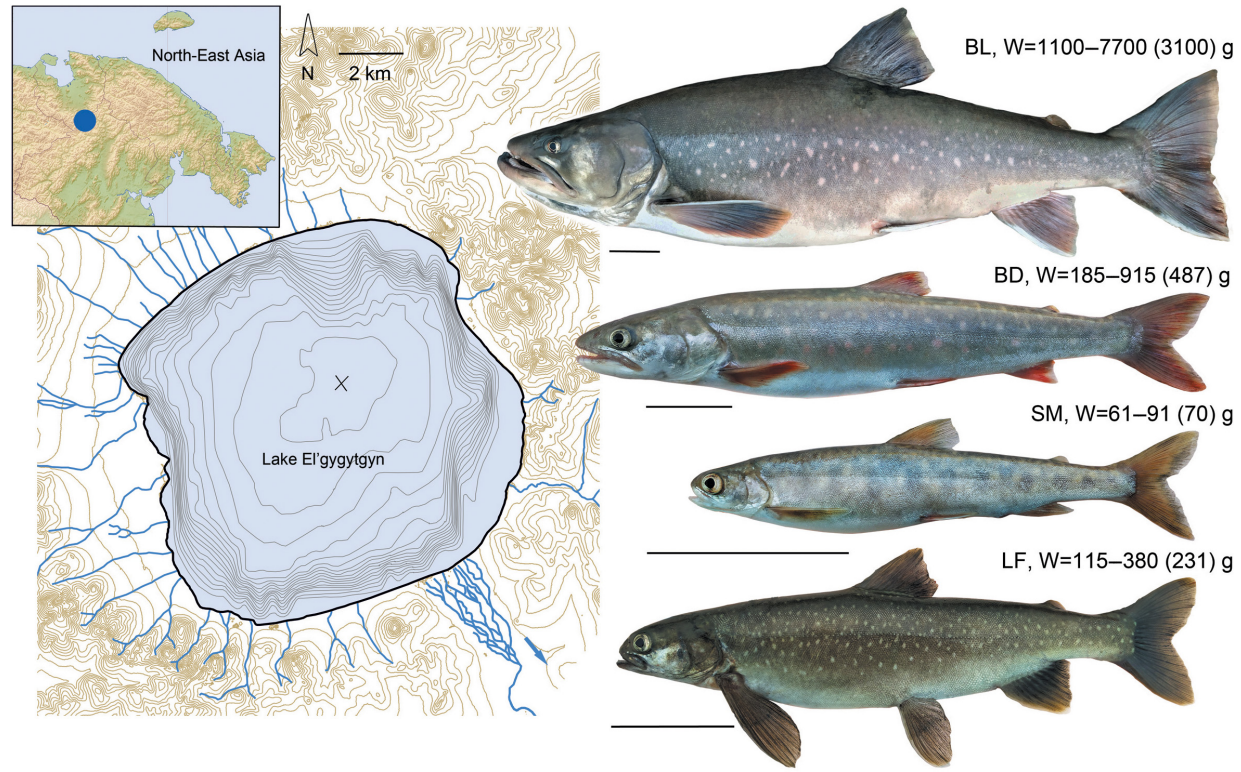


FIGURE 1 | Lake El'gygytgyn map (on the *left*), its location (incut), and charr fauna discovered (on the *right*): BL, Boganida charr, large-sized epilimnetic; BD, Boganida charr, deepwater; SM, smallmouth charr; LF, long-finned charr. The weights (the range and mean value) of adult fish used for the analysis are presented. The lines under fish images correspond to 5 cm.

Blood Protein Analysis

The hemoglobin concentration was measured by the cyanmethemoglobin method (Gammon and Baker, 1977) with 5 ml of transforming reagent (Renam kit) added to 20 μl of whole blood. The plasma protein content was identified in 10 μl samples by the Biuret test (Smith et al., 1985) using the assembled Agat-Med kit. The absorbance of both components was measured at 540 nm against standard solutions.

Lipid Analysis

The level of circulated lipids was analyzed in terms of plasma lipoproteins' content (excluding high-density fraction), which were precipitated using BioSystems kit. The samples of 20 μl plasma dissolved in 0.5 ml phosphovolframate-$MgCl_2$ reactive were incubated at 100°C for 15 min followed by 1-h centrifugation (Burstein et al., 1980; Regerand et al., 1990). Then, following Folch et al. (1957) procedure, lipids were extracted from the resuspended precipitate and purified with 1% KCl solution employed as the aqueous phase. The Folch method was also used to extract lipids from 200-mg samples of shredded muscle.

The total lipids' concentration was measured via sulfo-phospho-vanillin spectrophotometric approach, absorbance was measured at 520 nm (Knicht et al., 1972). The enzymatic hydrolysis reactions (Spinreact kits) provided triglyceride (TAG) and phospholipid (PHL) values (Trinder, 1969; Takeyama et al., 1977). The indicator substance kinonimin was measured at 505 nm. The lipid concentration was recalculated to the weight of muscle samples dried to constant weight.

Thiobarbituric Acid Reactive Substances

The intensity of lipid (per)oxidation in plasma was assessed by the content of thiobarbituric acid reactive substances (TBARS). Malondialdehyde was obtained from 0.2 ml plasma by adding 1 ml of thiobarbituric acid in phosphate buffer, subsequent incubation at 100°C for 45 min and 10-min centrifuging with 4 ml of butanol. The supernatant concentration was determined at 570 and 535 nm (Gavrilov et al., 1987).

Thyroid Hormone Content

The total triiodothyronine (T_3, bioactive form) and thyroxine (T_4, prohormone) concentrations in plasma were evaluated by enzyme-linked immunosorbent assay. We used commercially available Monobind kits and measured the hormones at 450 nm with a sensitivity of 0.04 pg ml^{-1} (tT3) and 3.2 pg ml^{-1} (T4).

Data Processing

The obtained data were statistically checked for the distribution matching in different charr morphs using the Kolmogorov-Smirnov test ($p > 0.05$ in all cases). To investigate the differences

among the morphs, we applied ANOVA complemented with *post hoc* Tukey HSD. Then, the biochemical parameters were tested for the correlation with each over and with the thyroid level. To elucidate the metabolic phenotypes of the charrs based on all eleven parameters, we used canonical variate analysis based on Wilks' Lambda statistics launched in Statsoft v.10 (Hill and Lewicki, 2006).

RESULTS

Distribution and Feeding

The charrs exhibited spatial segregation and different food preferences. BL was mostly caught in the epilimnetic zone (∼70% of catch). Its stomach contained fish and insects regardless of the fish size. BD was collected in the profundal zone (>90%), where it consumed fish. SM was represented mainly on the lake slope (∼70%), and their stomachs contained planktonic copepods. LF was found in the profundal zone and on the slope approximately in equal proportion. This species fed on deepwater amphipods and planktonic copepods.

Carbohydrate Characteristics

The content of blood glucose was interrelated with that of liver glycogen ($r = 0.67$; $p = 0.0001$) and morph-specific, ANOVA for the glucose/glycogen concentrations: $F_{3;86} = 9.70$; $p = 0.0002/F_{3;86} = 187.42$; $p = 0.0001$; $p < 0.0001$. LF exhibited the lowest content of both carbohydrates. SM showed a significantly reduced glycogen reserve and a slightly reduced glucose level relative to Boganida charrs. Among the latter, BL had a slightly increased glycogen reserve and a more stable glucose level than BD (**Figures 2A,B** and **Supplementary Table 1** for pairwise *p*-values).

The liver glycogen reserve correlated with the plasma protein concentration ($r = 0.53$; $p = 0.0005$), as well as TAG reserve in the plasma ($r^2 = 0.51$; $p = 0.0006$) and muscles ($r = 0.86$; $p < 0.0001$).

Blood Protein Characteristics

The morphs were defined by the specific ratio of hemoglobins and plasma proteins, ANOVA for the hemoglobin/plasma protein concentrations: $F_{3;86} = 26.86$; $p < 0.0001/F_{3;86} = 10.31$; $p = 0.0001$. SM possessed a reduced level of both parameters. LF demonstrated an increased level of hemoglobin but a decreased level of plasma proteins. A slight elevation in the blood protein content was revealed in BL compared to BD (**Figures 2C,D** and **Supplementary Table 2** for pairwise *p*-values).

Lipid Characteristics

Lipoproteins

The charrs differed in the content of lipoproteins (ANOVA for the total lipid concentration in the plasma: $F_{3;86} = 18.66$; $p < 0.0001$). LF stood apart by the maximum lipid reserve, whereas SM demonstrated the lowest plasma lipid concentration (**Figure 2E** and **Supplementary Table 3** for pairwise *p*-values). The plasma lipid composition also differed (ANOVA for% of PHL/TAG in plasma lipids: $F_{3;86} = 23.34$; $p < 0.0001/F_{3;86} = 64.02$; $p < 0.0001$). The PHL and TAG plasma contents displayed a significant inverse correlation ($r = 0.56$; $p = 0.0003$). The PHL level was significantly higher in BL (**Figure 2F**), TAG level – in LF; whereas SM showed a very low TAG plasma reserve (**Figure 2G** and **Supplementary Table 3**).

Muscle Lipids

The total lipid concentration in muscles correlated with the lipoprotein content in blood ($r = 0.71$; $p = 0.0001$). SM displayed a very low muscle lipid reserve, and LF had an extremely high one, ANOVA $F_{3;86} = 98.25$; $p < 0.0001$ (**Figure 2H** and **Supplementary Table 4** for pairwise *p*-values). The ratio of negatively interrelated PHL and TAG ($r = 0.95$; $p < 0.0001$) differed between the charrs, ANOVA for% of PHL/TAG: $F_{3;86} = 37.76$; $p < 0.0001/F_{3;86} = 22.35$; $p < 0.0001$. The PHL level significantly declined, while the TAG level significantly increased in the row: Boganida charrs – SM – LF (**Figures 2I,J** and **Supplementary Table 4**). The PHL and TAG levels in the muscles and blood were correlated ($r = 0.54$; $p = 0.0003$ for both).

Plasma Thiobarbituric Acid Reactive Substances

The Boganida charrs demonstrated a plasma thiobarbituric acid reactive substances (TBARS) level equal to 1.33 ± 0.11 (from 0.8 to 2.8) nmol ml^{-1}. A slight elevation of the parameter was revealed for SM, 1.61 ± 0.16 (1.0–2.8) nmol ml^{-1}. At the same time, LF displayed significantly reduced TBARS levels (ANOVA $F_{3;70} = 8.13$; $p = 0.0206$) indicating a higher antioxidant capacity of the blood.

Thyroid Status

The charrs demonstrated morph-specific hormonal status, ANOVA for the T_3/T_4 concentrations: $F_{3;77} = 49.32$; $p < 0.0001/F_{3;38} = 14.15$; $p < 0.0001$. LF had a significantly reduced hormonal content, while BL demonstrated an increased hormonal level. BD and SM were characterized by a moderate T_3 supply. However, SM differed by an extremely low T_4 concentration (**Figure 3** and **Supplementary Table 5** for *post hoc* pairwise *p*-values).

The T_3 content manifested a significant correlation with the blood glucose ($r = 0.58$; $p = 0.0002$), liver glycogen ($r^2 = 0.95$; $p < 0.0001$), TAG in the plasma ($r = 0.85$; $p < 0.0001$) and muscles ($r^2 = 0.68$; $p < 0.0001$), plasma proteins ($r = 0.52$; $p = 0.0010$), as well as the PHL in the plasma ($r = 0.60$; $p = 0.0001$) and muscles ($r^2 = 0.72$; $p < 0.0001$). Thus, the T_3 level was associated with the biochemical parameters responsible for the energy storage and transport, but not with the levels of hemoglobin and TBARS ($r^2 = 0.09$; $p > 0.05$).

Charr Metabolic Phenotypes

Using CV analysis for the complex of biochemical parameters, we identified four morph-specific physiological phenotypes (niches), $F_{33;231} = 43.12$; $p < 0.0001$, Wilks' Lambda = 0.0027, canonical $R = 0.9813$. In the CV space, LF occupied a remote position separating by the main root mainly due to the specific glycogen and muscle lipid levels (**Figure 4** and **Supplementary Table 6**). BL, BD, and SM were ordinated mainly along the second root following their discrete slowdown in the metabolic rate. The liver

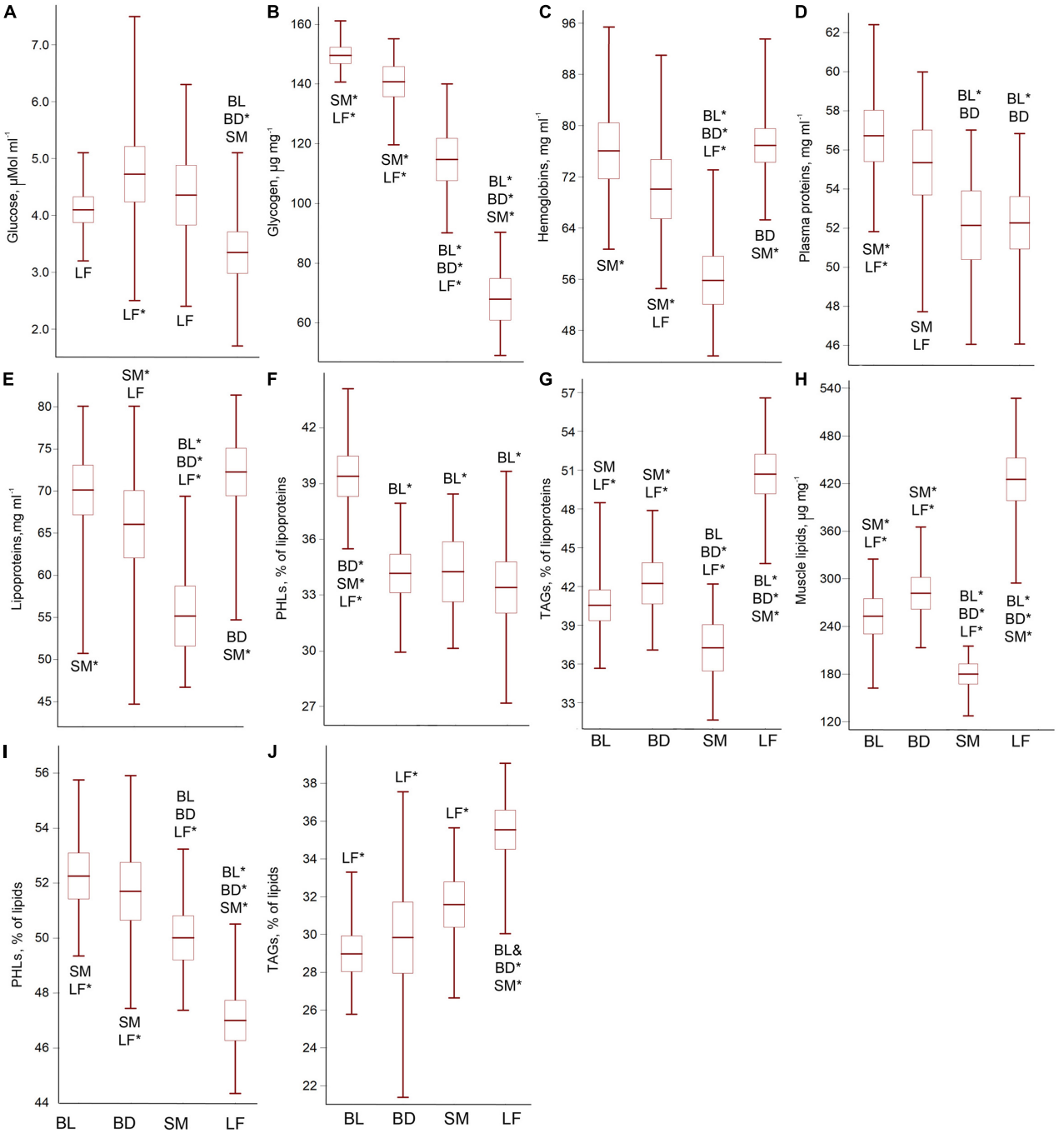


FIGURE 2 | The blood glucose **(A)**, liver glycogen **(B)**, blood hemoglobin **(C)** and plasma protein **(D)** contents, as well as total plasma lipid content **(E)**,% of PHL **(F)**, and TAG **(G)** in the plasma lipids, total muscle lipid content **(H)**, and% of PHL **(I)** and TAG **(J)** in the muscle lipids of the Lake El'gygytgyn charrs. Mean ± 95% confidence interval and min-max values are shown. Abbreviations with whisker boxes denote significantly differed groups (*$p < 0.001$; for the significance level of differences see **Supplementary Tables 1–4**). No correlation between the biochemical parameters and the fish weight (size) was found ($r < 0.30$; $p > 0.05$), except for the lipoproteins content in BD ($r = 0.78$; $p = 0.0163$) and muscle lipids' content in BL ($r = 0.96$; $p = 0.0111$). In both cases, the fish accumulated lipids with the growth.

glycogen, blood hemoglobin and plasma lipid content were the most different parameters among these charrs. An additional multivariate comparison of BL and BD confirmed their distinct metabolic phenotypes (**Figure 4**, incut).

DISCUSSION

The Lake El'gygytgyn charrs survive at the edge of ecological tolerance for non-migratory freshwater fishes. The lake was

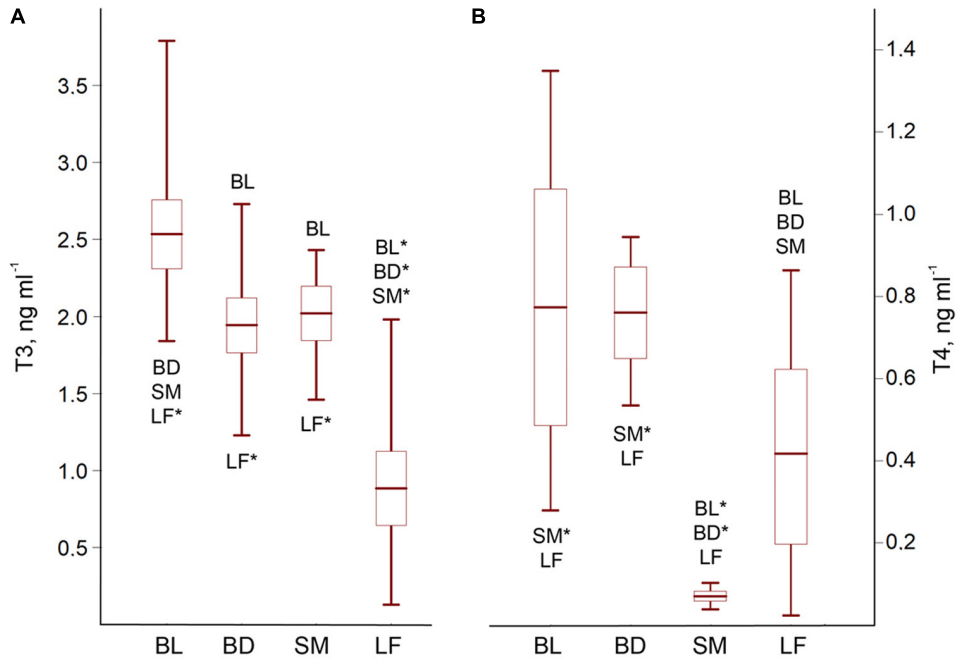


FIGURE 3 | The T_3 **(A)** and T_4 **(B)** blood content of the El'gygytgyn charrs. Mean ± 95% confidence interval and min-max values are shown. Abbreviations with whisker boxes denote significantly differed groups (*$p < 0.001$; for the significance level of differences see **Supplementary Table 5**).

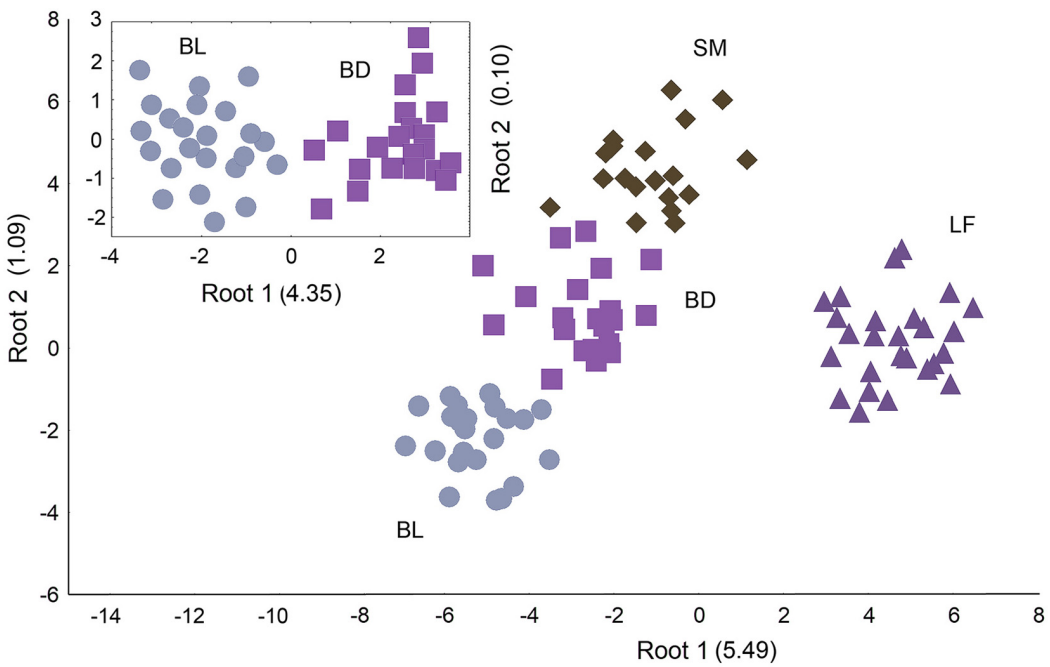


FIGURE 4 | Results of physiological niche identification in the El'gygytgyn charrs via CV analysis of 11 biochemical parameters (the blood glucose, liver glycogen, blood hemoglobin, plasma protein, plasma lipid content,% of PHL and TAG in the plasma lipids, muscle lipid content,% of PHL and TAG in the muscle lipids, plasma TBARS). Incut figure represents the result of comparing two Boganida charrs separately from other species. The charr groups and the root eigen values are labeled.

covered with ice many times over the decades during the late Pleistocene – early Holocene (Melles et al., 2012). To date, the average annual air temperature over the lake surface is close to −9°C, which is lower than over Lake Hazen, the northernmost resident fish-inhabited lake, with the average surface temperature of approximately −5°C (France, 1993).

Further to the north, non-annual ice breaking results in a sharp drop in the ecosystem productivity (Keatley et al., 2007), and the inability of lacustrine fishes to survive in these harsh conditions could be assumed.

A good indicator of fish fitness and performance in food acquisition is the proximate tissue composition (Beamish et al., 1989; Shearer, 1994; Heath, 1995; Speranza and Colombo, 2009). We found that all the El'gygytgyn charrs possess a specific common trait. They are very fatty: up to 37–53% (dry weight) and, therefore, adapted to the extremely cold conditions (White et al., 2012; DeLong et al., 2018). Lipids act as a resource store in ultra-oligotrophic ecosystems and also provide for the buoyancy essential for energy saving while swimming through the water column (Muir et al., 2014).

At the same time, the El'gygytgyn charrs differ in the ratio of biochemical parameters defining the dynamics of energy processing and storage, and biopolymer transport activity, which are related to the ecological differences in fish (Alexander, 1993). Following the authors describing the physiological discrepancies for the ecomorphs of various charr species (Proulx and Magnan, 2002; Goetz et al., 2014), we considered the revealed peculiarities of the El'gygytgyn charrs as the metabolic phenotypes corresponding to their ecological niches.

Long-finned charr (LF) occupies the unique niche – a deepwater amphipod-consumer capable of dwelling in anaerobic conditions. The increased hemoglobin level and decreased intensity of transporting lipid oxidation are inherent in this species. The last characteristic could be interpreted as the enhanced defense against oxidative stress, a likely adaptation to the low-oxygen conditions (Pollock et al., 2007; Debevec et al., 2017). Long-finned charr is also characterized by the substitution of carbohydrates for lipids in cellular respiration. The body fatness and TAG level in muscles and blood of this charr were out of scale.

The reduced metabolic rate and inactive lifestyle enable the energy saving necessary under the conditions of low food supply. Both features were characteristic of smallmouth charr (SM). Among the El'gygytgyn fish, this species possesses the lowest lipid level due to the planktivorous diet. In one respect, zooplankton contains approximately 21% (dry weight) of lipids as compared with about 14% in benthic insect larvae (Jobling, 1994b) and 15–20% in amphipods (Greze, 1977). Meanwhile, the main lipid storage product in many zooplankters is wax esters (Cavaletto et al., 1989; Brett et al., 2009), which are harder to digest than TAGs and PHLs from insects, amphipods, and fish prey. Thus, following Jobling (1994a), we suggest that the low lipid content found in the SM muscles is related to the presence of wax esters in its diet.

The piscivorous Boganida charrs are physiologically heterogeneous, but demonstrate an accelerated metabolism as compared with SM and LF. We found that Boganida charrs split into two discrete phenotypes related to the specific lifestyles: epilimnetic (BL) and deepwater (BD). The former displays high glycogen and PHL contents which allows considering it as more metabolically advanced than the latter. This finding goes along with the suggestion that metabolic level should be higher in fish that has to chase prey (Childress et al., 1990). At depth, where the visibility is reduced, the predator's throw to prey is shorter, so the metabolism may be slower. Probably, BD hunts in an "ambush way," and BL - in a "chasing way." Moreover, the metabolic differences between the Boganida phenotypes were supported by the heterogeneity in the rate of their somatic growth (Chereshnev et al., 2002). Fast-growing BL is characterized by a higher plasma protein content, which can be associated with effective muscle anabolism (Houlihan et al., 1993).

The ecologically determined metabolic phenotypes of the El'gygytgyn charrs are related to their specific hormonal phenotypes. The metabolically advanced BL displays the highest level of thyroid hormones. The piscivorous but metabolically retarded BD has a significantly lower concentration of T_3. The planktivorous SM characterized by slow metabolism exhibits a moderate level of T_3 and a very low level of T_4. The metabolically unique LF is the most hypothyroid El'gygytgyn fish. We also found sharp differences in the blood plasma biochemistry among the charrs, which is known to strongly correlate with the thyroid hormone status (Abdollahpour et al., 2019).

Given that the thyroid hormones are the key regulators of metabolism (Blanton and Specker, 2007; Lanni et al., 2016; Lema, 2020), the assumption that this endocrine axis determines the metabolic phenotypes of the El'gygytgyn charrs seems plausible. Moreover, the knowledge that the thyroid signaling links the ontogeny and metabolism to the environmental variables (Holzer and Laudet, 2015) allows proposing that this endocrine axis played a decisive role during the lake colonization and subsequent specialization of the charrs. Above all, a life-history shift from anadromy to fresh-water residency is associated with the changes in endocrinology. The salt-water migration decline leads to a number of consequences. In particular, it results in the loss of a parr-smolt transformation (Ferguson et al., 2019), which is regarded as a "pan-hyperendocrine state" [*sensu* Bern (1978) in McCormick (2013)], when the hormone synthesis consistently increases. In resident salmonid populations, the activity of some of the pivotal smolting endocrine axes (growth hormone and hypothalamic-pituitary-interrenal hormones) significantly decreases. In contrast, the thyroid axis, which is also regarded as a crucial smolting endocrine axis, does not change the activity and enhances the influence on development and physiology (McCormick et al., 2019). This finding allows us to speculate that the thyroid axis took on the role of the main endocrine factor orchestrating the developmental and physiological changes in the El'gygytgyn charrs. Initially, the thyroid hormones could act as the mediators providing for the development of thermal acclimation (Little et al., 2013) and specific lipid metabolism (Plisetskaya et al., 1983; Lanni et al., 2016; Deal and Volkoff, 2020) common for all El'gygytgyn charrs. Further, the thyroid axis most likely participated in the evolution of the species-specific traits.

Particularly, after the lake colonization, LF have been surviving and specializing under the long-term ice cover during at least four global climatic coolings of Pleistocene (Osinov et al., 2015). Stepwise "evolutionary shifts" (sensu Szappanos et al., 2016) led to the formation of the unique phenotype under

the pressure of sequential cooling and low-oxygen conditions, each time pushing the population toward a deeper ecological specialization. As a result, LF evolved into a hypothyroid fish with a low metabolic rate, which is regarded as an adaptation to the low-oxygen and poor food environment (Childress and Seibel, 1998; Kitano et al., 2010; Weidner et al., 2020). Low hormonal level, which often leads to paedomorphosis in low vertebrates (Laudet, 2011), also affected morphology. LF possesses neotenic morphological traits (Alekseyev, 2000).

Following Lema (2020) considering the endocrine regulation as a crucial proximate mechanism initiating evolutionary adaptations, we presume the thyroid status differences to be one of the key factors underpinning the radiation of the Boganida charrs. We suggest that BD is an evolutionary young ecological morph radiating from BL, a morph strongly resembling ancestral anadromous *Salvelinus taranetzi* (Chereshnev et al., 2002; Osinov et al., 2015). Occupying the new ecological niche (deep-water predator) required the life-style and physiological transformations, which seem to be associated with the decrease of the thyroid axis activity. Thus, we consider the development of the El'gygytgyn charr diversity as a stepwise adaptive evolution of metabolic phenotypes driven by the thyroid axis in response to the environmental challenges.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics committee of A.N. Severtsov Institute of Ecology and Evolution RAS.

AUTHOR CONTRIBUTIONS

EE and FS conceived the manuscript idea. All authors collected the materials, developed the theory, discussed the results, performed the computations, wrote, and revised the manuscript.

FUNDING

This work was supported by Russian Science Foundation, Grant No. 18-74-10085.

ACKNOWLEDGMENTS

We would like to thanks G. A. Kroshkin for the helicopter ride to the lake.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.715110/full#supplementary-material

REFERENCES

Abdollahpour, H., Falahatkar, B., Efatpanah, I., Meknatkhah, B., and Van Der Kraak, G. (2019). Hormonal and physiological changes in Sterlet sturgeon Acipenser ruthenus treated with thyroxine. *Aquaculture* 507, 293–300. doi: 10.1016/j.aquaculture.2019.03.063

Alekseyev, S. S. (2000). Reduction of skeletal elements in Arctic charr *Salvelinus alpinus* from Transbaikalia and the origin of some morphological features of Svetovidov's long-finned charr *Salvethymus svetovidovi*. *J. Ichthyol.* 40, 495–504.

Alexander, R. M. (1993). "Buoyancy," in *The Physiology of Fishes*, 2nd Edn, ed. D. H. Evans (Boca Raton, FL: CRC Press), 75–97.

Beamish, F. W. H., Howlett, J. C., and Medland, T. E. (1989). Impact of diet on metabolism and swimming performance in juvenile lake trout, *Salvelinus namaycush*. *Can. J. Fish. Aquat. Sci.* 46, 384–388. doi: 10.1139/f89-050

Bern, H. A. (1978). "Endocrinological studies on normal and abnormal salmon smoltification," in *Comp. Endocrinol.* eds P. J. Gaillard and H. H. Boer (Amsterdam: Elsevier/North Holland Biomedical Press), 77–100.

Blanton, M. L., and Specker, J. L. (2007). The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. *Crit. Rev. Toxicol.* 37, 97–115. doi: 10.1080/10408440601123529

Brett, M. T., Müller-Navarra, D. C., and Persson, J. (2009). "Crustacean zooplankton fatty acid composition," in *Lipids in Aquatic Ecosystems*, eds M. Kainz, M. T. Brett, and M. T. Arts (New York, NY: Springer), 115–146. doi: 10.1007/978-0-387-89366-2_6

Burstein, M., Scholnick, H. R., and Morfin, R. (1980). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *Scand. J. Clin. Lab. Invest.* 40, 583–595. doi: 10.1016/s0022-2275(20)42943-8

Campinho, M. A. (2019). Teleost metamorphosis: the role of thyroid hormone. *Front. Endocrinol.* 10:383. doi: 10.3389/fendo.2019.00383

Cavaletto, J. F., Vanderploeg, H. A., and Gardner, W. S. (1989). Wax esters in two species of freshwater zooplankton. *Limnol. Oceanogr.* 34, 785–789. doi: 10.4319/lo.1989.34.4.0785

Chereshnev, I. A., and Skopets, M. B. (1990). *Salvethymus svetovidovi* gen. et sp. nova – a new endemic fish of the subfamily Salmoninae from Lake El'gygytgyn (Central Chukotka). *Voprosy Ikhtiologii* 30, 201–213.

Chereshnev, I. A., Volobuev, V. V., Shestakov, A. V., and Frolov, S. V. (2002). *Salmonid fishes in Russian North-East*. Vladivostok: Dal'nauka Press.

Childress, J. J., Price, M. H., Favuzzi, J., and Cowles, D. (1990). Chemical composition of midwater fishes as a function of depth of occurrence off the Hawaiian Islands: food availability as a selective factor? *Mar. Biol.* 105, 235–246. doi: 10.1007/bf01344292

Childress, J. J., and Seibel, B. A. (1998). Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J. Exp Biol.* 201(Pt 8), 1223–1232. doi: 10.1242/jeb.201.8.1223

Cossins, A. R., and Prosser, C. L. (1978). Evolutionary adaptation of membranes to temperature. *Proc. Natl. Acad. Sci. U.S.A.* 75, 2040–2043. doi: 10.1073/pnas.75.4.2040

Deal, C. K., and Volkoff, H. (2020). The role of the thyroid axis in fish. *Front. Endocrinol.* 11:596585. doi: 10.3389/fendo.2020.596585

Debevec, T., Grégoire, P. M., and Pialoux, V. (2017). Hypoxia-induced oxidative stress modulation with physical activity. *Front. Physiol.* 8:84. doi: 10.3389/fphys.2017.00084

DeLong, J. P., Bachman, G., Gibert, J. P., Luhring, T. M., Montooth, K. L., Neyer, A., et al. (2018). Habitat, latitude and body mass influence the temperature dependence of metabolic rate. *Biol. Lett.* 14:0442. doi: 10.1098/rsbl.2018.0442

Esin, E. V., Markevich, G. N., Melnik, N. O., Kapitanova, D. V., and Shkil, F. N. (2021). Natural toxic impact and thyroid signaling interplay orchestrates riverine adaptive divergence of salmonid fish. *J. Anim. Ecol.* 90, 1004–1019. doi: 10.21203/rs.3.rs-15718

Ferguson, A., Reed, T. E., Cross, T. F., McGinnity, P., and Prodöhl, P. A. (2019). Anadromy, potamodromy and residency in brown trout *Salmo trutta*: the role of genes and the environment. *J. Fish. Biol.* 95, 692–718. doi: 10.1111/jfb.14005

Folch, J., Lees, M., and Sloan-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226, 497–509. doi: 10.1016/s0021-9258(18)64849-5

France, R. L. (1993). The Lake Hazen trough: a late winter oasis in a polar desert. *Biol. Conserv.* 63, 149–151. doi: 10.1016/0006-3207(93)90503-s

Freeman, N. K., Lindgren, F. T., and Nichols, A. V. (1963). "The chemistry of serum lipoproteins," in *Progress in the Chemistry of Fats and Other Lipids*, eds R. T. Holman, W. O. Lundberg, and T. Malkin (NewYork, NY: The MacMillan Comp.), 215–250.

Frolov, S. V. (1993). Unique karyotype and constitutive heterochromatin in the endemic Salmoninae fish *Salvethymus svetovidovi*. *J. Ichthyol.* 33, 42–49.

Gammon, A., and Baker, S. J. (1977). Studies in methods of hemoglobin estimation suitable for use in public health programmes. *Ind. J. Med. Res.* 65, 150–156.

Gavrilov, V. B., Gavrilova, A. R., and Mazhul', L. M. (1987). Analysis of methods for determination of lipid peroxidation products in blood serum in the thiobarbituric acid test. *Vopr. Med. Khim.* 1, 118–121.

Glushkova, O. Y. (2001). Geomorphological correlation of Late Pleistocene glacial complexes of Western and Eastern Beringia. *Quat. Sci. Rev.* 20, 405–417. doi: 10.1016/S0277-3791(00)00108-6

Goetz, F., Jasonowicz, A., Johnson, R., Biga, P., Fischer, G., and Sitar, S. (2014). Physiological differences between lean and siscowet Lake trout morphotypes: are these metabolotypes? *Can. J. Fish. Aquat. Sci.* 71, 427–435. doi: 10.1139/cjfas-2013-0463

Greze, I. I. (1977). *Amfipody Chernogo Morya Ikh Biologiya [Amphipods of the Black Sea and Their Biology]*. Kiev: Noukova Dumka.

Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biol. Rev.* 79, 409–427. doi: 10.1017/s1464793103006328

Harrison, B., Leazenby, C., and Halldorsdottir, S. (2011). Accuracy of the CONTOUR® blood glucose monitoring system. *J. Diabetes Sci. Technol.* 5, 1009–1013. doi: 10.1177/193229681100500425

Heath, A. G. (1995). *Water Pollution and Fish Physiology*. Boca Raton, FL: CRS press.

Hill, T., and Lewicki, P. (2006). *STATISTICS Methods and Applications*. Tulsa: StatSoft.

Holzer, G., and Laudet, V. (2015). Thyroid hormones: a triple-edged sword for life history transitions. *Curr. Biol.* 25, R344–R347. doi: 10.1016/j.cub.2015.02.026

Houlihan, D. F., Mathers, E. M., and Foster, A. (1993). "Biochemical correlates of growth rate in fish," in *Fish Ecophysiology*, eds J. C. Rankin and F. B. Jensen (London: Chapman & Hall), 45–71. doi: 10.1007/978-94-011-2304-4_2

Jobling, M. (1994b). *Fish Bioenergetics*. London: Chapman & Hall.

Jobling, M. (1994a). *Environmental Biology of Fishes*. London: Chapman & Hall.

Keatley, B. E., Douglas, M. S. V., and Smol, J. P. (2007). Limnological characteristics of a High Arctic oasis and comparisons across northern Ellesmere Island. *Arctic* 60, 294–308.

Kitano, J., Lema, S. C., Luckenbach, J. A., Mori, S., Kawagishi, Y., Kusakabe, M., et al. (2010). Adaptive divergence in the thyroid hormone signaling pathway in the stickleback radiation. *Curr. Biol.* 20, 2124–2130. doi: 10.1016/j.cub.2010.10.050

Knicht, J. A., Anderson, S., and Rawle, J. M. (1972). Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. *Clin. Chem.* 18, 199–202. doi: 10.1093/clinchem/18.3.199

Lanni, A., Moreno, M., and Goglia, F. (2016). Mitochondrial actions of thyroid hormone. *Compr. Physiol.* 6, 1591–1607. doi: 10.1002/cphy.c150019

Laudet, V. (2011). The origins and evolution of vertebrate metamorphosis. *Curr. Biol.* 21, R726–R737.

Lema, S. C. (2020). Hormones, developmental plasticity, and adaptive evolution: endocrine flexibility as a catalyst for 'plasticity-first' phenotypic divergence. *Mol. Cell. Endocrinol.* 502:110678. doi: 10.1016/j.mce.2019.110678

Little, A. G., Kunisue, T., Kannan, K., and Seebacher, F. (2013). Thyroid hormone actions are temperature-specific and regulate thermal acclimation in zebrafish (*Danio rerio*). *BMC Biol.* 11:26. doi: 10.1186/1741-7007-11-26

Masanori, N., and Shizunori, I. (1971). Effects of sarvation and dietary composition on the blood glucose level and the hepatopancreatic glycogen and lipid contents in carp. *Nippon Suisan Gakkaishi.* 37, 404–409.

McCormick, S. D. (2013). "Smolt physiology and endocrinology," in *Euryhaline Fishes*, eds S. D. McCormick, A. P. Farrell, and C. J. Brauner (Cambridge, MA: Academic Press), 199–251. doi: 10.1016/b978-0-12-396951-4.00005-0

McCormick, S. D., Regish, A. M., Ardren, W. R., Björnsson, B. T., and Bernier, N. J. (2019). The evolutionary consequences for seawater performance and its hormonal control when anadromous Atlantic salmon become landlocked. *Sci. Rep.* 9:968. doi: 10.1038/s41598-018-37608-1

McMenamin, S. K., and Parichy, D. M. (2013). Metamorphosis in teleosts. *Curr. Top. Dev. Biol.* 103, 127–165. doi: 10.1016/b978-0-12-385979-2.00005-8

Melles, M., Brigham-Grette, J., Minyuk, P. S., Nowaczyk, N. R., Wennrich, V., DeConto, R. M., et al. (2012). 2.8 Million years of Arctic climate change from Lake El'gygytgyn, NE Russia. *Science* 337, 315–320. doi: 10.1126/science.1222135

Muir, A. M., Bronte, C. R., Zimmerman, M. S., Quinlan, H. R., Glase, J. D., and Krueger, C. C. (2014). Ecomorphological diversity of Lake charr *Salvelinus namaycush* at Isle Royale, Lake Superior. *Trans. Am. Fish. Soc.* 143, 972–987.

Nelson, J. S., Grande, T. C., and Wilson, M. V. (2016). *Fishes of the World*, 5th Edn. London: John Wiley & Sons.

Nolan, M., and Brigham-Grette, J. (2007). Basic hydrology, limnology, and meterology of modern Lake El'gygytgyn, Siberia. *J. Paleolimnol.* 37, 17–35. doi: 10.1007/s10933-006-9020-y

Oleinik, A. G., Skurikhina, L. A., Kukhlevsky, A. D., and Bondar, E. I. (2019). Genetic differentiation of the Arctic phylogenetic group of charr from Northeast Asia and North America. *Rus. J. Genet.* 55, 349–359. doi: 10.1134/s1022795419030116

Oleinik, A. G., Skurikhina, L. A., Kukhlevsky, A. D., and Semenchenko, A. A. (2021). The first complete mitochondrial genomes of two species of charr, *Salvelinus boganidae* and *Salvelinus elgyticus*, from Lake El'gygytgyn (Chukotka). *Polar Biol.* 44, 1209–1217. doi: 10.1007/s00300-021-02861-0

Osinov, A. G., Senchukova, A. L., Mugue, N. S., Pavlov, S. D., and Chereshnev, I. A. (2015). Speciation and genetic divergence of three species of charr from ancient Lake El'gygytgyn (Chukotka) and their phylogenetic relationships with other representatives of the genus *Salvelinus*. *Biol. J. Linn. Soc.* 116, 63–85. doi: 10.1111/bij.12559

Osinov, A. G., and Volkov, A. A. (2020). On the origin of two species of charrs (*Salvelinus*, Salmonidae) in Lake El'gygytgyn: data on the ATPase6-NADH4L region of mitochondrial DNA. *J. Ichthyol.* 60, 618–625. doi: 10.1134/S003294522004013X

Plath, M., Tobler, M., and Rüdiger, R. (2015). "Extremophile fishes: an introduction," in *Extremophile Fishes. Ecology, Evolution, and Physiology of Teleosts in Extreme Environments*, eds R. Riesch, M. Tobler, and P. Martin (Switzerland: Springer International Publishing), 1–7. doi: 10.1007/978-3-319-13362-1_1

Plisetskaya, E., Woo, N. Y. S., and Murat, J.-C. (1983). Thyroid hormones in cyclostomes and fish and their role in regulation of intermediary metabolism. *Comp. Biochem. Physiol. A Physiol.* 74, 179–187. doi: 10.1016/0300-9629(83)90586-8

Polakof, S., Panserat, S., Soengas, J. L., and Moon, T. W. (2012). Glucose metabolism in fish: a review. *J. Compar. Physiol. Part B* 182, 1015–1045. doi: 10.1007/s00360-012-0658-7

Pollock, M. S., Clarke, L. M. J., and Dube, M. G. (2007). The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environ. Rev.* 15:6. doi: 10.1139/a06-006

Proulx, R., and Magnan, P. (2002). Physiological performance of two forms of lacustrine brook charr, *Salvelinus fontinalis*, in the open-water habitat. *Environ. Biol. Fish.* 64, 127–136. doi: 10.1023/a:1016049820846

Regerand, T. I., Lizenko, E. I., Petrovsky, V. I., and Sidorov, V. S. (1990). Isolation of serum lipoproteins by precipitation and determination of their lipid composition. *Lab. Delo.* 4, 48–52.

Rehulka, J., Minarik, B., Adamec, V., and Rehulkova, E. (2005). Investigations of physiological levels of total plasma protein in Rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.* 36, 22–32.

Shearer, K. D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture* 119, 63–88. doi: 10.1016/0044-8486(94)90444-8

Smith, P. K., Krohn, R. I, Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., et al. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150, 76–85. doi: 10.1016/0003-2697(85)90442-7

Speranza, E. D., and Colombo, J. C. (2009). Biochemical composition of a dominant detritivorous fish *Prochilodus lineatus* along pollution gradients in the Paraná-Río de la Plata Basin. *J. Fish. Biol.* 74, 1226–1244. doi: 10.1111/j.1095-8649.2009.02191.x

Szappanos, B., Fritzemeier, J., Csörgő, B., Lázár, V., Lu, X., Fekete, G., et al. (2016). Adaptive evolution of complex innovations through stepwise metabolic niche expansion. *Nat. Commun.* 7:11607. doi: 10.1038/ncomms/11607

Takeyama, M., Itoh, S., Nagasaki, T., and Tanimizu, I. (1977). A new enzymatic method for determination of serum choline-containing phospholipids. *Clin. Chem. Acta* 79, 93–98. doi: 10.1016/0009-8981(77)90465-x

Templeton, M. C. (1961). *Microdetermination of Glycogen with Anthrone Reagent*. Evanston, IL: Northwestern University, Dept. Medical Anatomy, 670–672.

Trinder, P. (1969). Estimation of triglyceride in blood GPO-PAP enzymatic method. *Am. Clin. Biochem.* 6, 24–27.

Weidner, J., Håkonsrud, J. C., Giske, J., Eliassen, S., and Jørgensen, C. (2020). Hormones as adaptive control systems in juvenile fish. *Biol. Open* 9:bio046144. doi: 10.1242/bio.046144

White, C. R., Alton, L. A., and Frappell, P. B. (2012). Metabolic cold adaptation in fishes occurs at the level of whole animal, mitochondria and enzyme. *Proc. Biol. Sci.* 279, 1740–1747. doi: 10.1098/rspb.2011.2060

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership