Research Topics

Insect conservation behavior

Edited by
Philip Thomas Starks, Rachael E. Bonoan and
Helen McCreery

Published in
Frontiers in Ecology and Evolution

ISSN 1664-8714
ISBN 978-2-8325-5330-5
DOI 10.3389/978-2-8325-5330-5

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

Insect conservation behavior

Topic editors

Philip Thomas Starks — Tufts University, United States
Rachael E. Bonoan — Providence College, United States
Helen McCreery — Tufts University, United States

Citation

Starks, P. T., Bonoan, R. E., McCreery, H., eds. (2024). *Insect conservation behavior*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5330-5

Table of contents

Frontiers in Ecology and Evolution

TYPE Editorial
PUBLISHED 06 August 2024
DOI 10.3389/fevo.2024.1468337

EDITED AND REVIEWED BY
Jordi Figuerola,
Spanish National Research Council (CSIC),
Spain

*CORRESPONDENCE
Philip T. Starks
philip.starks@tufts.edu

RECEIVED 21 July 2024
ACCEPTED 23 July 2024
PUBLISHED 06 August 2024

CITATION
Starks PT, Bonoan RE and McCreery HF (2024) Editorial: Insect conservation behavior. *Front. Ecol. Evol.* 12:1468337. doi: 10.3389/fevo.2024.1468337

Editorial: Insect conservation behavior

Philip T. Starks[1]*, Rachael E. Bonoan[2] and Helen F. McCreery[1]

[1]Biology Department, Tufts University, Medford, MA, United States, [2]Biology Department, Providence College, Providence, RI, United States

KEYWORDS

insect, conservation, behavior, global change, climate change

Editorial on the Research Topic
Insect conservation behavior

Introduction

In today's era of rapid global climate change, conservation biology has become an essential field (Sage, 2020). Its primary goal is to protect ecosystems and preserve species diversity. At its core, conservation biology seeks to understand and safeguard the intricate web of life that sustains our planet. Achieving these objectives requires a thorough understanding of the behavior of organisms within their ecological contexts (Buchholz, 2007).

The articles in this Research Topic take us on an insightful journey through the world of conservation biology, advocating for a detailed exploration of behavior as a key strategy to achieve conservation goals. Specifically, we argue that insects, often overlooked (Miličić et al., 2021), provide exceptional insights into the mechanisms driving biodiversity dynamics.

Focusing on insects is crucial because they occupy a unique position in ecology, significantly influencing ecosystem functioning and species interactions (Verma et al., 2023). As primary pollinators, decomposers, herbivores, and predators, their roles are diverse and irreplaceable. Additionally, insects display a wide variety of behavior, from intricate communication systems to complex social interactions (Cordoba-Aguilar et al., 2018).

Insects also offer an attractive opportunity for scientific research due to their relative ease of study. Their abundance, rapid reproductive rates, and often observable behavior facilitate empirical investigations into fundamental ecological and evolutionary questions. Insects frequently serve as model organisms, providing insights with broad relevance across various taxa. By studying insect behavior, we can gain valuable lessons applicable to diverse conservation scenarios.

Behavior is the means through which animals interact with their environment and with each other. As such, behavior must be central to conservation efforts. Despite this, conservation behavior is often under-discussed, even in situations where the natural history of a species is well understood (see, e.g., Berger-Tal et al., 2011, 2016). Although critical to ecosystems, insects have lagged behind other groups within the conservation literature.

This Research Topic aims to begin to address this gap and emphasize the importance of insect behavior in conservation. By highlighting the significant roles insects play and the insights gained from studying their behavior, we hope to inspire further research and action in this vital area of conservation biology.

Objectives of the research topic

Our goals for this Research Topic were twofold. First, we aimed to present the latest in insect behavior research and how such research could be applied to conservation. Second, we hoped to promote a diverse set of authors from diverse geographic locations. The target audience for this Research Topic includes conservation biologists, behavioral ecologists, and nature enthusiasts.

In this Research Topic, we present a collection of 10 papers, comprising two reviews and eight original research papers, that collectively offer valuable insights into the conservation of insects through the lens of behavior.

Review articles

1. Productivity-Richness Relationships Framework
   Wang et al. introduce a new framework for understanding productivity-richness relationships (PRR) in ecosystems. Their review examines the diverse shapes of PRR and the processes underlying these patterns, offering a predictive tool for ecosystem functions.
2. Bioluminescent Insects and Light Pollution
   Owens et al. explore the threats to bioluminescent insects from artificial light pollution. Their comprehensive review provides insights into the ecological impacts of light pollution and offers recommendations for mitigating these threats to preserve these fascinating creatures.

Original research papers

1. Thermal Tolerance and Foraging Behavior in Stingless Bees
   Robinson & Baudier explore how age and size differences among *Tetragonisca angustula* bees influence their thermal tolerance and foraging behavior amidst climate change. Their study reveals that foragers experience greater thermal stress, which may affect bee activity and pollination efficiency, underscoring the broader implications of climate change on these crucial pollinators.
2. Nutrient Enrichment and Bumble Bee Queens
   Thuma et al. review the impact of nutrient enrichment and rainfall changes on plant phenology and resource availability for bumble bee queens. Their study highlights the interconnectedness of plant and pollinator conservation and the need for integrated approaches to manage these systems effectively.
3. Honey Bee Thermoregulation Behavior
   Weinberg et al. explore honey bee thermoregulation behavior, such as altering comb arrangement under heat stress. Their findings support the idea that such behavior are crucial for colony survival amidst climate change, offering insights into the resilience of honey bee colonies and the future of our agricultural system in the face of climate change.
4. Roosting Behavior of Endangered Damselflies
   Mahdjoub et al. investigate the roosting behavior and microhabitat selection of the endangered damselfly *Calopteryx exul* in Algeria. The study highlights the critical importance of bank vegetation for roosting, emphasizing habitat protection for effective conservation strategies and showing how roosting behavior influences lifespan.
5. Impact of Human Activities on Odonata
   Rocha et al. evaluate how human impacts on streams in the eastern Amazon affect the functional traits and reproductive behavior of Odonata. Their research indicates that altered environments favor larger, heliothermic, exophytic species, while preserved areas support smaller, specialized species, highlighting the resilience and adaptability of these insects to changing habitats.
6. Butterfly Behavior and Grazing Strategies
   Bussan & Schultz examine the effects of different cattle grazing strategies on butterfly behavior. They find that conservation grazing via rotating stock and deferment periods can support butterfly habitats by promoting diverse plant communities, suggesting that such strategies can be beneficial for butterfly conservation.
7. Bat Predation and Moth Development
   Zhang et al. investigate how bat predation risks influence the growth, development, reproduction, and hormone levels of *Spodoptera litura* moths. This study highlights adaptive responses that could be leveraged for biological control strategies.
8. Cannibalism in Stink Bugs
   Wu et al. discover that female *Arma custos* stink bugs, a common biocontrol agent, cannibalize more eggs than males, a behavior that does not impact their biocontrol efficacy. This research provides a nuanced understanding of biocontrol agents' behavior and their implications for pest management.

Conclusion

Working on this Research Topic has been a delightful experience. We hope this Research Topic underscores the significance of insects in conservation-based behavioral research and acts as a catalyst for future studies. The intricate connections between behavior, environment, and conservation strategies are highlighted throughout these articles, demonstrating the crucial role of behavior in conservation efforts.

Understanding insect conservation behavior is essential for understanding the resilience of ecosystems in a rapidly changing global environment. We urge the scientific community and nature enthusiasts alike to explore these studies and apply the insights gained to broader conservation initiatives. Through collaborative and informed action, we can strive to preserve the biodiversity that is vital to the health of our planet.

Author contributions

PS: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. RB: Conceptualization, Writing – original draft, Writing – review & editing. HM: Writing – original draft, Writing – review & editing.

Acknowledgments

We extend our gratitude to the authors, reviewers, and editors who played significant roles in the creation of this Research Topic. Their dedication and expertise have been instrumental in bringing this Research Topic to fruition.

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

References

Berger-Tal, O., Blumstein, D. T., Carroll, S., Fisher, R. N., Mesnick, S. L., Owen, M. A., et al. (2016). A systematic survey of the integration of animal behavior into conservation. *Conserv. Biol.* 30, 744–753. doi: 10.1111/cobi.12654

Berger-Tal, O., Polak, T., Oron, A., Lubin, Y., Kotler, B. P., and Saltz, D. (2011). Integrating animal behavior and conservation biology: a conceptual framework. *Behav. Ecol.* 22, 236–239. doi: 10.1093/beheco/arq224

Buchholz, R. (2007). Behavioural biology: an effective and relevant conservation tool. *Trends Ecol. Evol.* 22, 401–407. doi: 10.1016/j.tree.2007.06.002

Cordoba-Aguilar, A., Gonzalez-Tokman, D., and Gonzalez-Santoyo, I. (Eds.) (2018). *Insect Behavior: From Mechanisms to Ecological and Evolutionary Consequences* (United Kingdom: Oxford University Press).

Miličić, M., Popov, S., Branco, V. V., and Pedro Cardoso, P. (2021). Insect threats and conservation through the lens of global experts. *Conserv. Lett.* 14. doi: 10.1111/conl.12814

Sage, R. F. (2020). Global change biology: A primer. *Global Change Biol.* 26, 3–30. doi: 10.1111/gcb.14893

Verma, R. C., Waseem, M. A., Sharma, N., Bharathi, K., Singh, S., Rashwin, A., et al. (2023). The role of insects in ecosystems, an in-depth review of entomological research. *Int. J. Environ. Climate Change* 13, 4340–4348. doi: 10.9734/ijecc/2023/v13i103110

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 10 March 2022
doi: 10.3389/fevo.2022.758587

Parental Sex and Not Kinship Determines Egg Cannibalism in *Arma custos* Fallou (Hemiptera: Pentatomidae: Asopinae)

Shaolong Wu[1,2†], Weiai Zeng[3†], Wan Deng[4], Jiaying Li[5], Mi Li[2], Lin Tan[1], Hailin Cai[3], Xiaohong Li[6], Youzhi Li[1]* and Zhicheng Zhou[2]*

[1] College of Plant Protection, Hunan Agricultural University, Changsha, China, [2] Hunan Province Tobacco Company, Changsha, China, [3] Hunan Changsha Municipal Tobacco Company, Changsha, China, [4] Hunan Forestry Academy, Changsha, China, [5] Hunan Yongzhou Municipal Tobacco Company, Yongzhou, China, [6] College of Shaoyang Urban and Rural Construction, Shaoyang, China

Edited by:
Sasha Raoul Xola Dall,
University of Exeter, United Kingdom

Reviewed by:
Anindita Bhadra,
Indian Institute of Science Education and Research Kolkata, India
Bram Kuijper,
University of Exeter, United Kingdom

***Correspondence:**
Youzhi Li
liyouzhi@hunau.edu.cn
Zhicheng Zhou
zhouzchnyc@126.com

†These authors have contributed equally to this work

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

Received: *14 August 2021*
Accepted: *12 January 2022*
Published: *10 March 2022*

Citation:
Wu S, Zeng W, Deng W, Li J, Li M, Tan L, Cai H, Li X, Li Y and Zhou Z (2022) Parental Sex and Not Kinship Determines Egg Cannibalism in Arma custos Fallou (Hemiptera: Pentatomidae: Asopinae). Front. Ecol. Evol. 10:758587. doi: 10.3389/fevo.2022.758587

Background: *Arma custos* Fallou (Hemiptera: Asopinae) is an important predatory insect native to China, South Korea, and Mongolia. It is important to understand the evolution of egg cannibalism in *A. custos* to evaluate the biocontrol potential of this species. However, few reports have suggested egg cannibalism in *A. custos*, and whether hungry adult *A. custos* males and females prey on their eggs remains unknown. Here, we investigated the effects of the parental sex of *A. custos* adults on egg cannibalism of parental and non-parental eggs (kinship) under no-choice and free-choice conditions, along with the effects of predator and egg density on egg cannibalism under starvation conditions.

Results: Females frequently visited and cannibalized a higher proportion of eggs, whereas males almost did not participate in egg cannibalism (less than 17% males showed egg cannibalism behavior). Moreover, regardless of their relationship with the egg, neither male nor female adults consumed all available eggs even in the absence of an alternative food source, and >70% of eggs remained unconsumed. In contrast, cannibalistic males and females did not discriminate between parental and non-parental egg types. Meanwhile, cannibalism rates were similar when adults were offered 30 eggs or more. However, when offered fewer than 30 eggs, cannibalism rates declined disproportionally, suggesting that limited egg availability reduced cannibalism. Additionally, the lifespan of *A. custos* adult females increased significantly with increasing number of consumed eggs ($p < 0.05$).

Conclusion: *Arma custos* females exhibit a higher tendency for egg cannibalism than males. Neither male or female *A. custos* discriminated between parental and non-parental egg types. Cannibalism enhances survival in that a starved individual who predates on eggs survives similarly to a well-fed individual. These findings provide a model to study the evolution and biological significance of egg cannibalism in *A. custos* and also contribute to the efficient mass rearing and realization of *A. custos* for biological control.

Keywords: *Arma custos* Fallou, cannibalistic behavior, predatory, egg cannibalism, kinship difference

INTRODUCTION

Cannibalism, the consumption of conspecifics, is a behavioral trait observed in several animal species (Fox, 1975; Elgar and Crespi, 1992). Egg cannibalism, an important mechanism for self-regulating population size (Fox, 1975; Polis, 1981), is widespread in insects (Dobler and Kölliker, 2010; Parsons et al., 2013; Schultner et al., 2013; Bayoumy and Michaud, 2015; Jacobs and Stigall, 2019), including *Hippodamia convergens* (Guerin-Meneville) (Bayoumy and Michaud, 2015) and *Tribolium confusum* (du Val) (Parsons et al., 2013) of the order Coleoptera and *Forficula auricularia* L. (Dermaptera) (Dobler and Kölliker, 2010), *Formica aquilonia* Yarr. (Hymenoptera) (Schultner et al., 2013), *Rhinocoris tristis* Stal (Thomas and Manica, 2003), and *Callicorixa producta* Reut. of the order Hemiptera (Zalom, 1978). Although egg cannibalism reduces population size in insect species (Hamilton, 1964; Pfennig, 1997), it can be beneficial in the following ways: (1) by serving as the source of nutrients under starvation conditions (Pizzatto and Shine, 2008; Dobler and Kölliker, 2010); (2) by improving fitness, as predation on unrelated eggs decreases intraspecific competition (Fox, 1975; Polis, 1981; Vickery et al., 1988; Ichikawa, 1991); (3) by eliminating parasitized and infected eggs to improve offspring survival and development (Rohwer, 1978); (4) by increasing lifespan and boosting reproductive rate (Rohwer, 1978; Manica, 2002a).

Sex of the predator (Revynthi et al., 2018a,b) and kinship (Samu et al., 1999; Hoffman, 2012; Parsons et al., 2013) affect egg cannibalism in insects. The effect of the gender of the preying adult on egg cannibalism is species-specific. For instance, adult females of *H. convergens* (Bayoumy and Michaud, 2015), *Coccinella undecimpunctata* L. (Bayoumy et al., 2016), and *Adalia bipunctata* L. (Agarwala and Dixon, 1992) are more cannibalistic than conspecific males, whereas adult males of *F. aquilonia* (Schultner et al., 2013) are more cannibalistic than conspecific females. Moreover, some species avoid consuming their own eggs when non-parental eggs are available (Dobler and Kölliker, 2010, 2011; Parsons et al., 2013). For example, female red flour beetles (*T. confusum*) are more likely to consume non-parental eggs than their own eggs (Parsons et al., 2013), and European earwigs (*F. auricularia*) delay preying upon their juvenile offspring when unrelated juveniles are available (Dobler and Kölliker, 2010, 2011).

Arma custos (Fallou) (Hemiptera: Pentatomidae: Asopinae) (Zhao et al., 2018) is an important predaceous insect species native to China, Korea, and Mongolia (Rider and Zheng, 2002), which preys upon lepidopterans, coleopterans, hymenopterans, and hemipterans (Zheng and Chen, 1992; Gao et al., 2011; Zou et al., 2012, 2015). There are three stages in the lifecycle of *A. custos*, including egg, nymph (with five instars), and the adult (Zou et al., 2012). The developmental time of eggs, instar nymphs, and male and female adults are 6.43, 20.66, 37.25, and 44.18 days, respectively, and adult *A. custos* females oviposit approximately 20–30 eggs at one time with 90% egg hatchability (Zou et al., 2012). The first-instar nymphs of *A. custos* require only water, while other instar nymphs and adults require live insect larvae or pupae for growth and development and suck the body fluids of their prey using their proboscis (Wu et al., 2019, 2020). Notably, several organisms also feed upon the same prey item (Zou et al., 2012).

Many hemipteran insects, such as *Arizona backswimmers* (Frank) (Zalom, 1978), *Triatoma sordida* (Stal) and *Triatoma infestans* (Klug) (Ryckman, 1951), *Arctocorisa carinata* and *Callicorixa producta* (Pajunen and Pajunen, 1991), *Xylocoris flavipes* (Reuter) (Arbogast, 1979), *Macrolophus pygmaeus* (Ranmbur), and *Dicyphus cerastii* (Wagner), are known for their cannibalistic behavior, and adults prey on immature offspring (Duarte et al., 2021). Most previous studies have focused on the predation ability and predation behavior of *A. custos* (Gao et al., 2019; Li et al., 2020; Wang et al., 2020). Furthermore, *A. custos* could efficiently control *Ambrostoma quadriimpressum* (Motschulsky), *Cnidocampa flavescens* (Walk.) and *Spodoptera exigua* (Hübner) with an *A. custos*-to-pest ratio of 1:15 (Gao et al., 2019; Li et al., 2020; Wang et al., 2020). However, to the best of our knowledge, there have been no studies on egg cannibalism in *A. custos*. Moreover, whether the species exhibits significant egg cannibalistic behavior remains unknown.

In the laboratory, some *A. custos* adults, mostly females, prey on the eggs of other insects, suggesting that this species exhibits egg cannibalism with significant differences between males and females that may be shaped by some selection pressure. Furthermore, it may be difficult for the insect to find a suitable prey to survive in the wild. Thus, it is important to understand how male and female adults of this species select eggs (parental versus non-parental) for feeding and what proportion of eggs is consumed under starvation conditions. Additionally, the effects of gender and kinship on egg cannibalism have not been studied in this species. Therefore, further investigations will not only allow us to elucidate the mechanism of egg cannibalism in this species but also help to evaluate its suitability as a long-term pest biocontrol agent. It would also provide a model to study the evolution and biological significance of egg cannibalism in insects.

In view of the above observations, we hypothesized the following: (1) starved *A. custos* adults would cannibalize a significant proportion of the eggs provided; (2) adult females would exhibit significant egg cannibalism, whereas males would not; (3) *A. custos* would distinguish between parental and non-parental eggs; and (4) egg cannibalism would increase the survival rate of *A. custos*. To verify these hypotheses, we performed the following experiments: (1) Evaluation of the probability of cannibalism exhibited by *A. custos* male and female adults on parental and non-parental eggs under selection and non-selective conditions; (2) Evaluation of the probability of egg cannibalism in adult males and females under different prey and predator densities; (3) Comparison of lifespan of cannibalistic and non-cannibalistic adults.

MATERIALS AND METHODS

Insect Rearing and Experimental Conditions

Arma custos adults were collected from the wild in Langfang, Hebei Province, China, in 2018 and reared in artificial climate boxes in the laboratory, as previously described by Pan et al. (2019). The insects were fed with Chinese oak silk moth, *Antherea pernyi* (Guérin-Méneville) (Lepidoptera: Saturniidae) pupae purchased from a supermarket in Liaoning, China. After hatching, first-instar *A. custos* nymphs from individual egg mass were placed in transparent plastic dishes (10×1.5 cm^2) and fed only with water, using a piece of moist absorbent cotton. Chinese oak silk moth pupae were provided to second-instar nymphs, which were replenished every 4–5 days (Pan et al., 2019).

Newly emerged adults (<6 h old) were paired, placed in 6×10 cm^2 Petri dishes containing one Chinese oak silk moth pupa and lined with a paper tube (diameter: 1 cm; height: 6 cm), and allowed to mate. After 4–5 days, the eggs laid in the paper tube were used for the experiments within 24 h, and the remnants of the moth pupa were removed.

We assessed the cannibalistic behavior of adult *A. custos* under laboratory conditions by placing freshly laid eggs (<24 h old) in small plastic dishes (10×1.5 cm^2) covered with an insect-proof screen (80-μm mesh) for ventilation. All adults used in the experiments were 8 days old and starved for 24 h. The females used in the experiments had laid eggs, and the males had mated. All experiments were performed at 25 ± 2°C, 60 ± 10% RH, under a photoperiod of 16:8 h (L:D). No additional food was supplied to the adults during the observation period.

Adult females of *A. custos* typically lay batches of 20–40 eggs, and the findings of a preliminary test indicated that the adults of this species consume a daily average of 1 s-instar beet armyworm larva, the weight of which is equivalent to that of 30 eggs. We accordingly conducted free-choice and no-choice experiments using a predator-to-egg ratio of 1:30 and used the same proportions to analyze the effects of different egg and predator densities on egg cannibalism in *A. custos*.

Observation of Egg Cannibalism in *Arma custos*

To establish whether *A. custos* adults simply break eggshells or break eggshells and consume the egg contents, we monitored the duration of male and female cannibalism on parental and non-parental eggs based on 30 replicate video observations (Sony, FDR-AX700, Japan). Eggs with broken shells were considered to have been cannibalized, and the number of replicates with cannibalized eggs was used to determine the extent of cannibalism.

Egg Cannibalism in *Arma custos* Under No-Choice and Free-Choice Conditions

For the food-choice experiments, eggs were monitored under a 40× magnifying glass for 5 min post-release and every 15 min thereafter, during the first 48 h. After 48 h, the eggs were monitored every 3 h for 7 days. The number of consumed and unconsumed eggs (broken eggshells) was counted. The number of replicates with evident egg cannibalism were used to determine the probability of egg-residual behavior in male and female adults, and the number of eggs consumed by *A. custos* under each food choice condition were used to estimate the strength of female and male egg cannibalism.

In addition, the number of replicates with cannibalized eggs and the number of consumed eggs after every 6 h were used to assess the effects of the incubation period on egg cannibalism in *A. custos*. Subsequently, the remaining eggs not eaten by male or female adults were recorded.

No-Choice Test

To determine whether *A. custos* adults feed on eggs regardless of kinship (parental or non-parental) with the eggs, 30 eggs were placed in each plastic dish, and no-choice tests were conducted using the following solitary adults: parental female, parental male, non-parental female, and non-parental male. In the parental treatments, the eggs were the parental offspring of the predatory adults, whereas in the non-parental treatments, the eggs were non-parental to the predatory adults.

Free-Choice test

At the beginning of the free-choice experiments, we drew six flabellate grids on the Petri dishes (**Figure 1**), which divided the dishes into six equal parts. To test whether *A. custos* preferentially preyed on its parental eggs when allowed to freely choose between them and non-parental eggs, either a male or a female adult was placed in a plastic dish containing 15 parental and 15 non-parental eggs. The eggs from the same parent were evenly divided into three groups of five eggs each and then placed on non-adjacent sections of the grid. Similarly, 15 eggs from a different set of parents were placed on non-adjacent grid sections in groups of five, as shown in **Figure 1**. Each treatment combination was replicated 30 times and consisted of one adult, male or female, exposed to 30 conspecific eggs of mixed kinship.

Density-Dependent Egg Cannibalism in *Arma custos*

We previously observed that *A. custos* adults do not consume all available eggs and always cannibalize the same number of eggs irrespective of the number of eggs available to feed on. Therefore, we tested the following two hypotheses. (1) Egg cannibalism in *A. custos* adults does not change with varying number of eggs; (2) Egg cannibalism in *A. custos* improves with increasing number of adults.

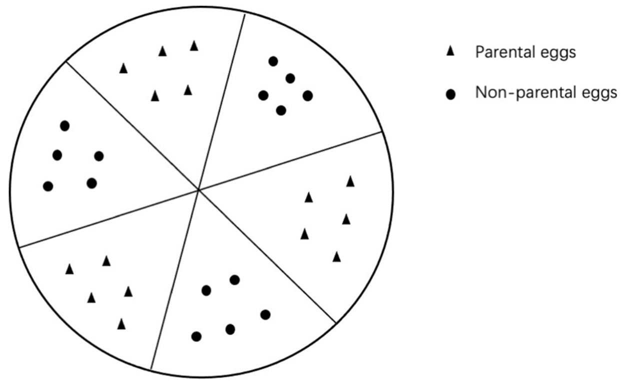


FIGURE 1 | Set-up and distribution of the two kinds of eggs in the free-choice experiments.

Varying Number of Eggs Test

Each female *A. custos* lays 10–60 eggs at one time; therefore, the egg-density experiments comprised 14 treatments (i.e., a 2 × 7 factorial design), wherein one female or one male was placed in the experimental Petri dish arena and exposed to either 1, 10, 20, 30, 40, 50, or 60 non-parental eggs. All the adults used in these experiments were used only once and observed for 7 days.

Varying Number of Adult *Arma custos* Test

In nature, multiple adults may randomly attack the eggs at one place at the same time. Hence, in the predator-density experiments, 1, 2, 4, 6, 8, or 10 *A. custos* females or males were placed in plastic dishes containing 30 non-parental eggs, and egg cannibalism was observed under artificial conditions. Each density treatment consisted of 30 replicates, and each dish was considered an experimental replicate. All the adults used in these experiments were used only once and observed for 7 days.

For both the density experiments, the number of eggs cannibalized by *A. custos* adults in each replicate were used to evaluate the effect of egg or predator density on egg cannibalism in *A. custos*. Moreover, the number of eggs consumed by *A. custos* were recorded to reveal whether the number of eggs consumed by the adults increases linearly with the increase in egg or predator density. Subsequently, the remaining eggs from the male and female adult tests were recorded.

Effect of Egg Cannibalism on the Lifespan of *Arma custos*

We assumed that egg cannibalism would supply energy for *A. custos* to understand its effect on the lifespan of *A. custos.* Furthermore, we assumed that the lifespan of *A. custos* who cannibalized eggs would be longer than those that did not. Therefore, we observed the lifespan of female and male *A. custos* under the following conditions: (1) Supplying 1-day food for female or male *A. custos*; (2) Female or male *A. custos* does not cannibalize eggs; and (3) Female or male *A. custos* cannibalize eggs. Each treatment involved 30 replicates with 30 different adults.

Supplying One-Day Food Test

One female or one male was placed in the experimental Petri dish arena after being starved for 24 h. Chinese oak silk moth was provided as food for 1 day. Then, food was not supplied to female or male *A. custos* until they died. The lifespan of female or male *A. custos* was recorded. Each treatment involved 30 replicates with 30 different adults.

Non-cannibalized and Cannibalized Test

One female or one male was placed in the experimental Petri dish arena after being starved for 24 h. Then, 30 normal eggs were provided as food until female or male *A. custos* died. If yes, it becomes part of the cannibalized group; If no, it becomes part of the non-cannibalized group. The lifespan of female or male *A. custos* were recorded. Each treatment involved 30 replicates with 30 different adults. Meanwhile, most female or male *A. custos* did not cannibalize eggs, but we want to know the lifespan of female or male *A. custos*, respectively, under the cannibalized group and the non-cannibalized group in 30 replicates. So, we only observed 30 times for each treatment. Although, more female or male *A. custos* did not cannibalize eggs when we observed the lifespan of the cannibalized group, we no longer record their lifespans, because we have got 30 replicates.

Statistical Analyses

Chi-square analyses were performed to compare the cannibalistic behavior of adult males and females and test whether kinship affected egg cannibalism in the two genders under different conditions. An analysis of variance was performed to assess the differences in egg consumption, the ratio of remaining eggs, and the lifespan of male and female *A. custos.* Bartlett's test was used to test the homogeneity of variances, and the square root transformation (sqrt) was calculated to analyze datasets when the variance was heterogeneous, with $p < 0.05$ being significant. Multiple comparisons of the lifespan of cannibalistic and non-cannibalistic male and female *A. custos* adults were performed using Tukey's honest significant difference (HSD) tests between male and female *A. custos* egg consumption and egg emergence ratio. Benjamini–Hochberg adjustment was used to control false discovery rates (FDRs) for such multiple comparisons, with $q < 0.05$ being significant (Lee, 2016; Lee and Lee, 2018). Furthermore, a generalized linear model (GLM) using Poisson's distribution was used to evaluate the relationships of male and female *A. custos* lifespans with the number of consumed eggs. All statistical analyses were performed using R v.3.3.3 (R Development Core Team, 2017).

RESULTS

Egg Cannibalism in *Arma custos*

We observed that the egg-puncturing behavior exhibited by *A. custos* adults was followed by egg cannibalism, which continued for 65 s (**Figures 2, 3**). The adults used their proboscis and not their legs or other body parts to puncture the eggshell (**Figure 2**). After puncturing, eggs protein were eaten and a silver-white eggshell was left out (**Figure 2**). The normal eggs

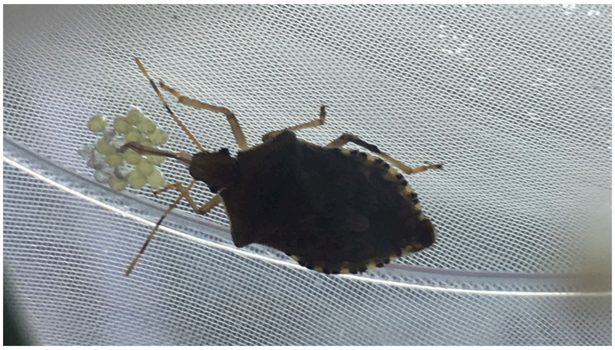


FIGURE 2 | *Arma custos* adult consuming eggs. After being cannibalized by the proboscis of *A. custos*, eggs protein were eaten and a silver-white eggshell was left out.

were integral, round, smooth, and yellow (**Figure 3A**) and the cannibalized eggs were tattered, dim, shrunken, and silver-white (**Figure 3B**). Moreover, egg cannibalism in the adults was neither affected by kinship nor by the sex of the predator (Tukey's HSD test, $p > 0.4$; **Figure 4**).

Egg Cannibalism in *Arma custos* Under No-Choice and Free-Choice Conditions

Kinship had no effect on egg cannibalism exhibited by *A. custos* adults (**Figure 5**). Of the 30 no-choice experimental replicates, adult females preyed on kin eggs in 16 replicates and on non-parental eggs in 18 replicates (**Figure 5A**), whereas, adult males preyed upon kin eggs in four experimental replicates and on non-kin eggs in four replicates (**Figure 5A**). Chi-square analysis showed that the differences observed for kin and non-kin eggs were not significant in both males ($c^2 = 0$, df = 1, $p = 1$) and females ($c^2 = 0.07$, df = 1, $p = 0.79$). Similarly, in the 30 free-choice experimental replicates, the number of replicates in which female adults preyed upon kin eggs (15) was not significantly different from the number of replicates in which the females preyed upon the non-kin eggs (16) ($c^2 = 0$, df = 1, $p = 1$; **Figure 5B**). Moreover, there was no significant difference between the number of replicates wherein adult females preyed upon kin eggs (5) and those in which adult males preyed on non-parental eggs (4) ($c^2 = 0$, df = 1, $p = 1$; **Figure 5B**).

Chi-square analysis revealed that sex-based differences in predator behavior were significant between parental and non-parental egg types under both the food choices (**Figures 5A,B**), wherein *A. custos* females more frequently preyed upon eggs than conspecific males under no-choice (parental eggs: $c^2 = 5.81$, df = 1, $p < 0.05$; non-parental eggs: $c^2 = 4.31$, df = 1, $p < 0.05$) and free-choice (parental eggs: $c^2 = 4.69$, df = 1, $p < 0.05$; non-parental eggs: $c^2 = 7.33$, df = 1, $p < 0.05$) conditions (**Figures 5A,B**).

Moreover, out of 30 males, only four exhibited cannibalism (13.33%) of either parental or non-parental origin (**Figures 5A,B**); out of 30 females, only 16 in the parental (53%) test and 18 (60%) in the non-parental test exhibited cannibalism (**Figures 5A,B**). Egg cannibalism by neither sex was altered by the origin of the eggs (**Figure 5**). In addition to frequent predation, adult females consumed significantly more eggs (4.23–5.13) than adult males (0.34–0.37) under no-choice conditions, irrespective of egg origin (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figures 5C,D**). Similarly, under free-choice conditions, adult females consumed a significantly larger number of eggs (2.33–2.86) than adult male (0.30–0.33), irrespective of origin (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figure 5B**). However, <20% of the available eggs were consumed by both sexes, and >80% of the available eggs remained under both no-choice and free-choice conditions with female and male adults (**Figures 5E,F**). Furthermore, the ratios of remaining eggs with males were significantly higher than those with females under both no-choice and free-choice conditions (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figures 5E,F**).

Under both the no-choice and free-choice conditions, no differential preference for parental and non-parental eggs was observed over the study period at any moment (Chi-square: All, $p > 0.05$; **Figures 6A,B**). Meanwhile, the number of consumed parental eggs was also not significantly different from that of non-parental eggs under the experimental conditions at any moment (Tukey's HSD test: $p > 0.05$; **Figures 6C,D**).

Furthermore, under both no-choice and free-choice conditions, egg cannibalism in adults was observed to be more prominent within the initial 48 h (2 day) of incubation with eggs. No adult preyed eggs in the first 12 h (0.5 day) of all tests. Both female and male adults preyed on eggs during 12–36 h (0.5–1.5 day) of no-choice tests [Chi-square of 12 vs. 36 h (0.5 vs. 1.5 days): Parental female, $c^2 = 19.18$, $df = 1$, $p < 0.05$; Nonparental female, $c^2 = 22.94$, $df = 1$, $p < 0.05$; Parental male, $c^2 = 2.41$, $df = 1$, $p = 0.12$; Nonparental male, $c^2 = 2.41$, $df = 1$, $p = 0.12$; **Figures 7A,B**] and free-choice tests [Chi-square of 12 vs. 36 h (0.5 vs. 1.5 days): Parental female, $c^2 = 15.75$, $df = 1$, $p < 0.05$; Nonparental female, $c^2 = 19.18$, $df = 1$, $p < 0.05$; Parental male, $c^2 = 3.49$, $df = 1$, $p = 0.06$; Nonparental male, $c^2 = 2.41$, $df = 1$, $p = 0.12$; **Figures 7A,B**]. None of the adults consumed eggs after 48 h (2 day) under both no-choice and free choice conditions (Tukey's HSD test: All, $p > 0.05$; **Figures 7C,D**).

Density-Dependent Egg Cannibalism in *Arma custos*

We also studied the effects of different densities of non-parental eggs and predators on egg cannibalism in *A. custos* (**Figure 8**). We observed no significant differences in the number of replicates in which females exhibited egg cannibalism ($c^2 = 0$, df = 1, $p = 1$) and the number of consumed eggs (Tukey's HSD test: $p > 0.05$) between the replicates with 30 and >30 non-parental eggs (**Figures 8A,C**). Similar results were obtained when adult males were incubated with >30 non-parental eggs (**Figures 8A,C**). However, when the number of eggs was reduced, egg cannibalism exhibited by *A. custos* adults decreased significantly, especially when the number of eggs was reduced from 30 to 10 (for all 30 replicates), wherein only eight females showed egg cannibalism ($c^2 = 4.39$, df = 1, $p < 0.05$) and the average number of eggs

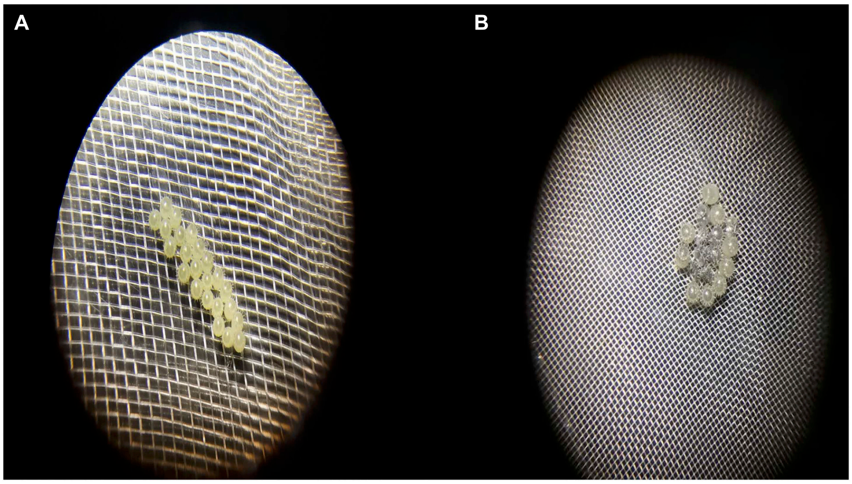


FIGURE 3 | Normal eggs **(A)** and cannibalized eggs **(B)**. The normal eggs were integral, round, smooth, and yellow, the cannibalized eggs were tattered, dim, shrunken, and silver-white.

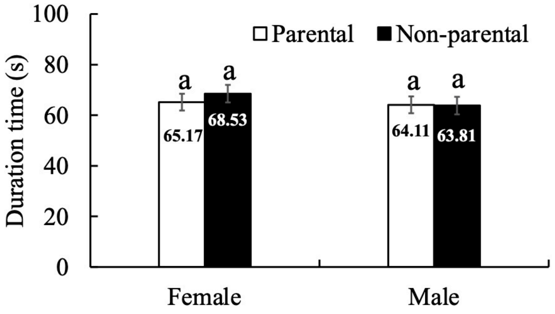


FIGURE 4 | Duration times of egg cannibalism in *A. custos* adults upon incubation with parental and non-parental eggs. Lowercase letters indicate significant differences ($p < 0.05$). Thirty replicates were used for each experimental condition.

consumed was 1.4 (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figures 8A,C**).

Similarly, when only one egg was supplied, neither the adult males nor females showed egg cannibalism throughout the study period (7 days), the eggs remained intact, and 93% of them hatched into first-instar nymphs (**Figures 8A,C**), suggesting that limited egg availability reduces egg cannibalism in this species. In contrast, despite the greater availability of eggs, no more than 14.0 and 2.6% of the available eggs were consumed by *A. custos* females and males, respectively, and more than 85% of the available eggs remained (**Figure 8E**). Moreover, the numbers of eggs consumed by adult predators was significantly lower than the number of unconsumed eggs ($p < 0.05$), irrespective of the number of available eggs (**Figure 8E**).

When the number of predators was increased, the number of replicates with egg cannibalism also increased (**Figure 8B**). Chi-square analysis revealed that when the number of predators increased four times, there were significant differences in egg cannibalism in both males (c^2 = 3.89, df = 1, $p < 0.05$) and females (c^2 = 6.43, df = 1, $p < 0.05$; **Figure 8B**). Meanwhile, when the number of predators increased four times, the number of consumed eggs was also significantly different in both males (Tukey's HSD test: 1 vs. 4 adults, $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$) and females (Tukey's HSD test: 1 vs. 4 adults, $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figure 8D**). However, when the number of predators increased six, eight, and 10 times, there were no significant differences in egg cannibalism in both males (Chi-square of 6, 8, and 10 adults, respectively, with four adults: All, $p < 0.05$) and females (Chi-square of 6, 8, and 10 adults, respectively, with four adults: All, $p < 0.05$; **Figure 8D**). Furthermore, the residual food intake in females at densities of 4, 5, 6, 8, and 10 was significantly higher than that of a single *A. custos* female (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figure 8D**). Similarly, the residual food intake by males at densities of 4, 5, 6, 8, and 10 was also significantly higher than that of a single *A. custos* male (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figure 8D**).

In addition, despite the increasing number of predators and the subsequent increasing chances of egg cannibalism, the number of consumed eggs was consistently low for both males (9.5%) and females (28.7%), and more than 70% of the available eggs remained (**Figure 8F**). These findings suggest that high-density groups decrease egg cannibalism exhibited by a solitary *A. custos* adult.

Effect of Egg Cannibalism on the Lifespan of *Arma custos*

Egg cannibalistic behavior significantly affected the lifespan of male and female *A. custos* adults (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figure 9A**). Linear regression analysis revealed that the number of consumed eggs positively correlated with the lifespan of adult *A. custos*

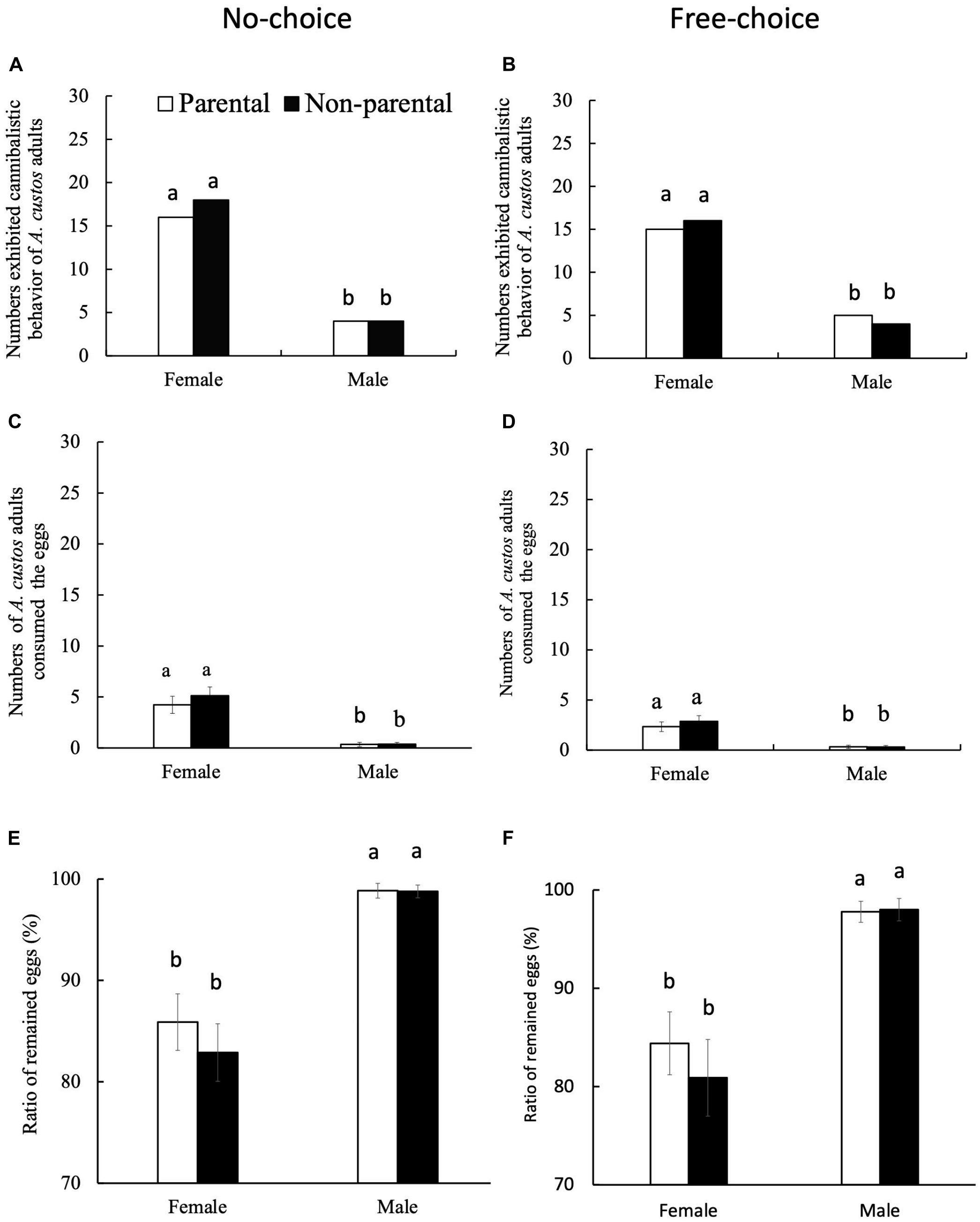


FIGURE 5 | Egg cannibalism exhibited by female and male *A. custos* adults incubated under no-choice and free-choice conditions for 7 days. Under the no-choice condition, each *A. custos* adult was either incubated with 30 parental or 30 non-parental eggs, whereas under the free-choice condition, each adult was simultaneously incubated with 15 parental and 15 non-parental eggs. Different lowercase letters on pillars indicate significant differences ($p < 0.05$). A total of 30 replicates were used for each food-choice experiment. Numbers exhibited cannibalistic behavior of *A. custos* adults in No-choice **(A)** and Free-choice **(B)** conditions. Numbers of *A. custos* adults consumed the eggs in No-choice **(C)** and Free-choice **(D)** conditions. Ratio of remained eggs in No-choice **(E)** and Free-choice **(F)** conditions.

(males: $F = 215.73$, $p < 0.05$; females: $F = 394.60$, $p < 0.05$; **Figure 9B**). However, there was no significant difference in the lifespan of non-cannibalistic adult males (10.08 days) and females (10.59 days) (Tukey's HSD test: $p = 0.99$; **Figure 9A**), whereas the lifespan of cannibalistic *A. custos* adults were significantly increased (**Figure 9B**) and females' lifespan were much longer than that of males (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figure 9A**). Furthermore, the lifespan of cannibalistic males and females were not significantly different from those of the adults provided with 1-day food (Tukey's HSD test: $p < 0.05$; Benjamini–Hochberg adjustment of FDR: $q < 0.05$; **Figure 9A**), indicating that egg

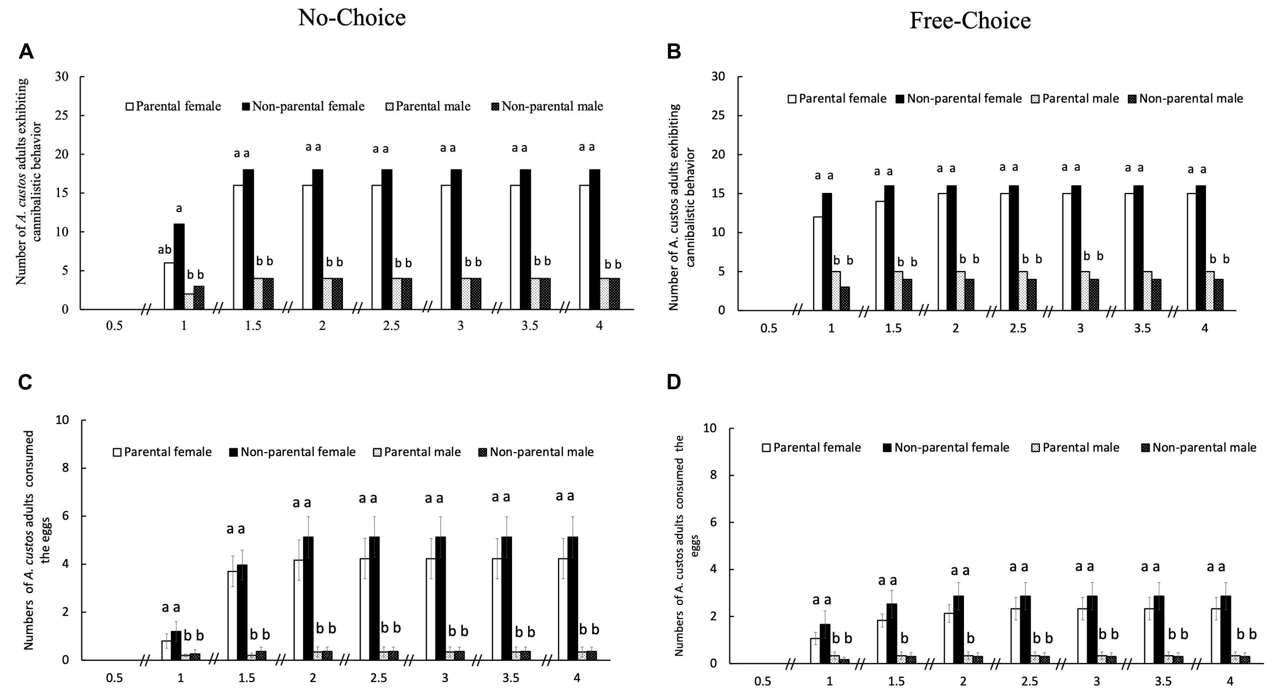


FIGURE 6 | Real-time egg cannibalistic behavior observed in male and female *A. custos* adults for 4 days at different times. Cannibalistic behavior did not change after 4 days of incubation with eggs. Under the no-choice condition, each *A. custos* adult was incubated with 30 parental or 30 non-parental eggs, whereas under the free-choice condition, each adult was simultaneously incubated with 15 parental and 15 non-parental eggs. A total of 30 replicates were used for each food choice. Different lowercase letters on pillars indicate significant differences ($p < 0.05$). A total of 30 replicates were used for each food-choice experiment. Numbers of *A. custos* adults exhibiting cannibalistic behavior in no-choice **(A)** and free-choice **(B)** condition. Numbers of *A. custos* adults consumed the eggs in no-choice **(C)** and free-choice **(D)** condition.

availability contributed to the longevity of male and female *A. custos* adults.

DISCUSSION

Although *A. custos* is widely used for biocontrol of pests, egg cannibalistic behavior of this species has not yet been investigated. Therefore, we comprehensively and systematically studied the effects of gender of the predator and kinship on egg cannibalism in *A. custos* and showed that kinship does not affect egg cannibalism, while egg cannibalism is significantly affected by the gender of the predatory adult. We also showed that egg cannibalism in *A. custos* is markedly affected by the number of eggs and predators present at one location.

We observed that only a small proportion of *A. custos* adults exhibited egg cannibalism, which was not significant; 86.77% of male adults did not consume eggs of either parental or non-parental origin, 40% and 47% of female adults did not consume eggs of parental and non-parental origin, respectively, and the number of eggs consumed by *A. custos* was insignificant. This may be because the eggs release hormones or substances that inhibit insect feeding (Narasimha et al., 2019) or because adults regulate their diets (Polis, 1981; Smith and Reay, 1991; Manica, 2002b). The small number of adults exhibiting egg cannibalism also indicates the existence of such a regulatory mechanism.

Thomas and Manica (2003) reported that although *R. tristis* cannibalizes its own eggs, it can selectively prey on parasitized eggs. However, in the present study, no parasitized eggs were used. Moreover, Okada et al. (2015) reported that hungry *Andrias japonicus* preys on unfertilized and dead eggs; however, in our study, only two-third of the females exhibited egg cannibalism under similar conditions. Therefore, these studies could not explain the results of the present study. It is unclear whether *A. custos* adults only prey on unfertilized eggs. Zou et al. (2012) observed that virgin insect females also lay eggs, indicating that the eggs eaten by the adults may either be vegetative, which cannot develop into adults (Perry and Roitberg, 2006) or unfertilized eggs (Mrowka, 1987). For example, mouthbrooding females of *Pseudocrenilabrus multicolor* consume unfertilized eggs for the first few days after spawning (Mrowka, 1987). However, we do not know whether the eggs consumed by the female and male *A. custos* were unfertilized. In future, unfertilized and fertilized eggs should be used to test the egg cannibalism behavior of female and male *A. custos* toward fertilized eggs, which would help us to further identify whether *A. custos* adults only prey on unfertilized eggs.

Rohwer (1978) showed that males prey on eggs for nutrition and longer lifespan, thereby, taking care of the subsequent generation, and providing their offspring with better conditions for growth and development. Rohwer (1978) has suggested that insects selectively consume a small number of eggs to obtain

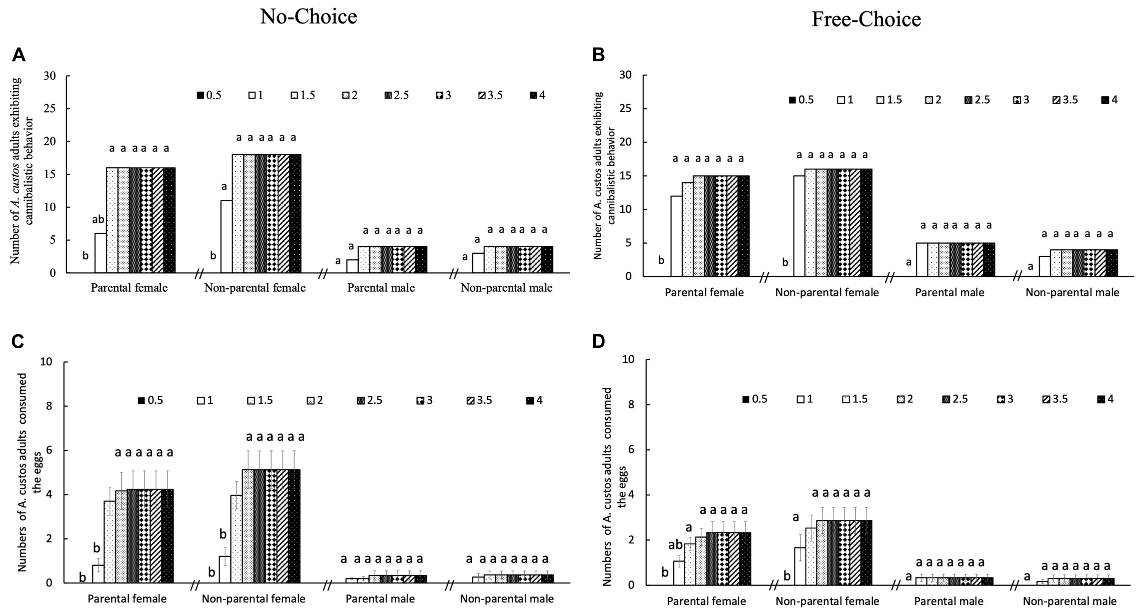


FIGURE 7 | Egg cannibalistic behavior of female and male *A. custos* adults under no-choice and free-choice conditions at different times for 4 days. Cannibalistic behavior did not change after 4 days of incubation with eggs. Under the no-choice condition, each *A. custos* adult was incubated with 30 parental or 30 non-parental eggs, whereas under the free-choice condition, each adult was simultaneously incubated with 15 parental and 15 non-parental eggs. A total of 30 replicates were used for each food choice. Different lowercase letters on pillars indicate significant differences ($p < 0.05$). A total of 30 replicates were used for each food choice experiment. Numbers of *A. custos* adults exhibiting cannibalistic behavior in no-choice **(A)** and free-choice **(B)** condition. Numbers of *A. custos* adults consumed the eggs in no-choice **(C)** and free-choice **(D)** condition.

sufficient nutrition and better opportunities for reproduction, which tends to be consistent with the findings of the present study, wherein we observed that adults cannibalized their own eggs by using their mouthparts, and that females consuming a larger number of eggs are generally characterized by a longer lifespan, thereby enhancing reproductive opportunities. Furthermore, *A. custos* females lay eggs multiple times during their lifetime (Zou et al., 2012), and we observed that cannibalistic females of this species lived longer than their non-cannibalistic counterparts.

The evolution of cannibalism is driven by the balance between its benefits and costs (Hamilton, 1964; Rudolf et al., 2010). One evident benefit of egg cannibalism is starvation avoidance (Pizzatto and Shine, 2008; Dobler and Kölliker, 2010). Moreover, selective cannibalism provides an alternative food source for adult insects, while ensuring the survival of most of their offspring and maintaining the population size (Polis, 1981; Smith and Reay, 1991; Manica, 2002b). Our study revealed that hungry *A. custos* adults do not prey on all the available eggs (>70% of the eggs were unconsumed under high predator density) irrespective of the predator or egg density, and adult males exhibit minimal egg cannibalism. Moreover, when the egg density is extremely low, the egg cannibalistic behavior exhibited by *A. custos* reduces significantly; none of the adults exhibited egg cannibalism when only one egg was available.

Although more *A. custos* adults participated in egg cannibalism under high predator density, the percentage of eggs consumed by *A. custos* adults was less than 30%. Previous studies suggest that a solitary male or female *A. custos* adult can consume 1 s-instar beet armyworm larva per day, and adult *A. custos* remain alive for at least 7 days. In the present study, although *A. custos* adults were hungry for the first 48 h, all adults did not consume eggs after 48 h, suggesting that *A. custos* exhibits a cannibalism-regulating mechanism irrespective of the selection pressure to ensure the survival of most of its eggs and maintain the population size (Hamilton, 1964; Pfennig, 1997). Meanwhile, it is also possible that adults interfere with each other as predator density increases, and this may limit cannibalism in some way. In future, we will study the relationship between density and cannibalism in *A. custos* adults.

Kin-killing behavior is observed when the nutritional profits outweigh the costs of decreased inclusive fitness (Pfennig, 1997). Although strategies for avoiding cannibalism have been studied in many taxa (Samu et al., 1999; Siegel et al., 2007; Widdig, 2007; Edenbrow and Croft, 2012; Parsons et al., 2013; Bayoumy and Michaud, 2015; Garza and Waldman, 2015; Ringler et al., 2017), little is known about the mechanisms regulating the predatory behavior of hemipterans (Arbogast, 1979; Pajunen and Pajunen, 1991; Agarwala and Dixon, 1992). Most arthropods avoid consuming their own kin when given the choice (Dobler and Kölliker, 2010, 2011; Parsons et al., 2013). Female *C. undecimpunctata* avoided cannibalizing their own eggs and preferentially consumed non-filial eggs in a choice-based study (Bayoumy and Michaud, 2015). Female *A. bipunctata* recognize and avoid eating their own eggs (Dumont et al., 2020), and female *H. convergens* recognize their own egg clusters,

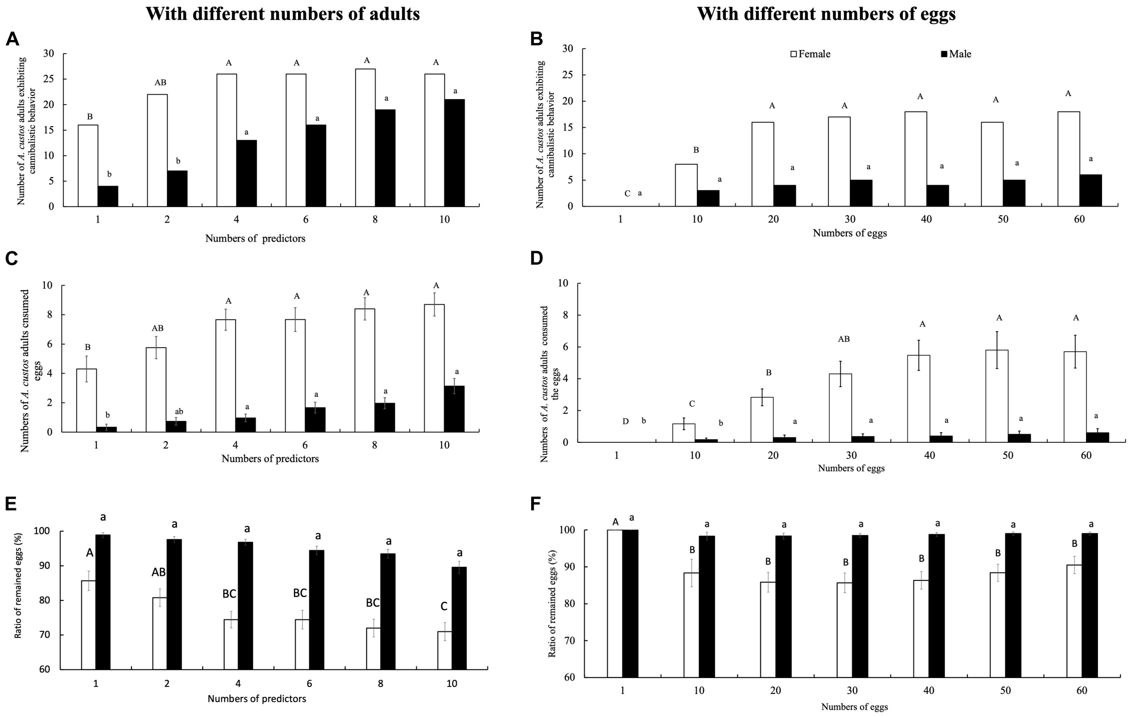


FIGURE 8 | Egg cannibalistic behavior exhibited by *A. custos* adults incubated at different egg or predator densities. Different uppercase letters indicate significant differences among females ($p < 0.05$), and different lowercase letters indicate significant differences among males ($p < 0.05$). **(C,D)** The number of consumed eggs overall individual. A total of 30 replicates were used for each experiment. Numbers of *A. custos* adults exhibiting cannibalistic behavior in no-choice **(A)** and free-choice **(B)** condition. Numbers of *A. custos* adults consumed eggs in no-choice **(C)** and free-choice **(D)** condition. Ratio of remained eggs in no-choice **(E)** and free-choice **(F)** condition..

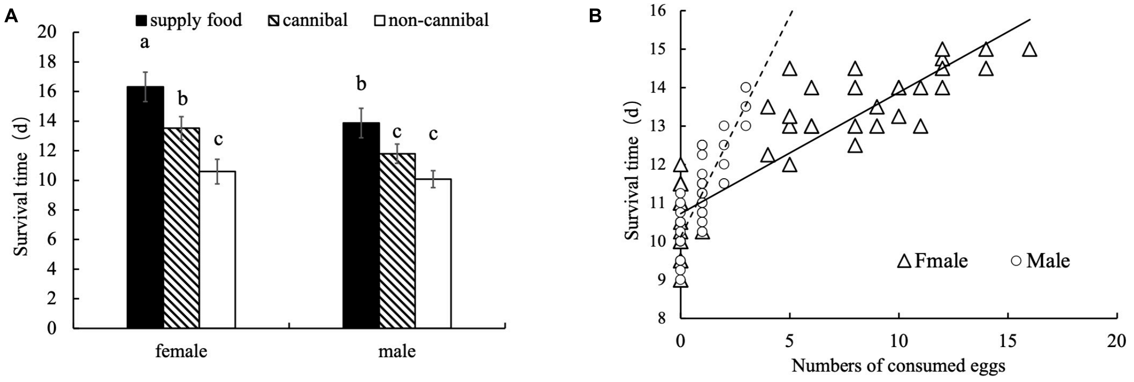


FIGURE 9 | Effect of egg cannibalism on the lifespan of male and female *A. custos* adults provided with sufficient eggs (number of eggs = 30). Different lowercase letters indicate significant differences ($p < 0.05$). Survival time of female and male *A. custos* adults in supply food, cannibal and non-cannibal conditions **(A)**. Survival time of female and male *A. custos* adults as numbers of consumed eggs **(B)**.

sometimes even non-kin eggs added to them, and preferentially cannibalize non-filial clusters (Bayoumy and Michaud, 2015). However, the present study showed that kinship did not play a significant role in egg cannibalism exhibited by *A. custos* under free-choice or no-choice conditions, which coincides with the observations in *Phytoseiulus persimilis* Athias-Henriot (a phytoseiid mite) (Revynthi et al., 2018b). However, we could not determine whether *A. custos* recognized parental and non-parental eggs.

In the present study, adult females exhibited a higher predation frequency than adult males. This is concordant with the observations in *H. convergens* (Bayoumy and Michaud, 2015), *C. undecimpunctata* (Bayoumy et al., 2016), and *A. bipunctata* (Agarwala and Dixon, 1992) but is in contrast to the observations in *F. aquilonia*, wherein males reduce mating competition using egg cannibalism (Schultner et al., 2013). The difference between the predation frequencies of males and females may be attributed to the high food demand of females during spawning (Neff, 2003; Harshman and Zera, 2007; Miller and Zink, 2012).

Previous studies have shown that developmental stage, including the ages of the adults, nymph and eggs, and food quality (King and Dawson, 1972) also influence egg cannibalistic behavior (Ho and Dawson, 1966; Pajunen and Pajunen, 1991; Parsons et al., 2013; Schultner et al., 2013; Bayoumy and Michaud, 2015). King and Dawson (1972) showed that improving food quality reduced cannibalism rates in *Tribolium*, while Pajunen and Pajunen (1991) showed that female rock-pool corixids cannibalized new eggs at a greater frequency than 1-day old eggs. Moreover, in some species, such as *T. confusum* (Parsons et al., 2013), *C. undecimpunctata* (Bayoumy et al., 2016), and male *H. convergens* (Bayoumy and Michaud, 2015), virgin adults are more cannibalistic than mated adults, whereas mated females of *H. convergens* (Bayoumy and Michaud, 2015) and some mite species are more cannibalistic than virgin females (Schausberger, 2003).

In the present study, we observed that adults exhibited egg cannibalism during the first 48 h of incubation, but its cause could not be determined. It would be noteworthy to determine the effect on egg cannibalism when older eggs are replaced with fresh eggs (<24 h old) every day or when *A. custos* adults have a choice between fresh and older eggs. In addition, it remains unknown whether virgin males and females cannibalize eggs, and whether different aged nymph cannibalize eggs. Hence, further studies are required to determine whether the egg cannibalistic behavior of *A. custos* depends on developmental stage, including the ages of the eggs, nymphs, and adults. The lifespan of insects with higher appetite was longer in the present study than those with lower appetite, and the lifespan of male and female predators increased with the increasing number of cannibalized eggs. These findings are similar to the observations made in the ant *F. aquilonia* (Schultner et al., 2013).

Egg cannibalism offers insect species a means to avoid starvation and prolong lifespan by providing an alternative source of nutrition and energy (Polis, 1981; Smith and Reay, 1991; Pizzatto and Shine, 2008; Okada et al., 2015). However, under field conditions, whether *A. custos* would exhibit the same behavior, possibly avoiding egg cannibalism by protecting at least some of its eggs and instead searching for other prey, is still unknown (Revynthi et al., 2018b). Furthermore, egg cannibalistic behavior has also been reported in the nymphs of *F. aquilonia* (Schultner et al., 2013) and *T. castaneum* (Ho and Dawson, 1966). Given that compared with laboratory conditions, nymphs generally have little difficulty in locating eggs in the wild, it will be instructive to investigate egg cannibalism among *A. custos* nymphs, as well as egg cannibalism as a whole, under natural conditions. However, our observations were conducted under confined conditions, and it remains to be determined whether *A. custos* adult would exhibit the same behavior at more extensive spatial scales.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SW, WZ, JL, YL, XL, LT, HC, and ZZ conceived and designed the experiments. SW, WZ, JL, ML, XL, WD, and ZZ performed the experiments. SW, WZ, YL, and ZZ analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript for submission.

FUNDING

This study was funded by the Natural Science Foundation of Hunan Province (Nos. 2020JJ5290 and 2018JJ2364), Natural Enemies Breeding Project supported by the Science Foundation of Hunan (Nos. 18–21 Aa06, HN2020KJ12, and 110201901040), Major Program for Science and Technology of Hunan Province (No. 2020NK2034), and Double First-Class Construction Project of Hunan Agricultural University (No. SYL 2019029), and Hunan Forestry Science and Technology Innovation Funding Project (No. XLK202104-1).

ACKNOWLEDGMENTS

We thank Changhua Zhang and Fangzhao Jia for providing the insects and Anfang Zhu for feeding the insects.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.758587/full#supplementary-material

REFERENCES

Agarwala, B. K., and Dixon, A. F. G. (1992). Laboratory study of cannibalism and interspecific predation in ladybirds. *Ecol. Entomol.* 17, 303–309. doi: 10.1111/j.1365-2311.1992.tb01062.x

Arbogast, R. T. (1979). Cannibalism in *Xylocoris flavipes* (Hemiptera: Anthocoridae), a predator of stored-product insects. *Entomol. Exp. Appl.* 25, 128–135. doi: 10.1111/j.1570-7458.1979.tb02862.x

Bayoumy, M. H., Abou-Elnaga, A. M., Ghanim, A. A., and Mashhoot, G. A. (2016). Egg cannibalism potential benefits for adult reproductive performance and offspring fitness of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae). *Egypt. J. Biol. Pest Control* 26, 35–42.

Bayoumy, M. H., and Michaud, J. P. (2015). Egg cannibalism and its life history consequences vary with life stage, sex, and reproductive status in *Hippodamia convergens* (Coleoptera: Coccinellidae). *J. Econ. Entomol.* 108, 1665–1674. doi: 10.1093/jee/tov148

Dobler, R., and Kölliker, M. (2010). Kin-selected siblicide and cannibalism in the European earwig. *Behav. Ecol.* 21, 257–263. doi: 10.1093/beheco/arp184

Dobler, R., and Kölliker, M. (2011). Influence of weight asymmetry and kinship on siblicidal and cannibalistic behaviour in earwigs. *Anim. Behav.* 82, 667–672. doi: 10.1016/j.anbehav.2011.06.017

Duarte, A. G., Caldas, F., Pechirra, A., Borges da Silva, E., and Figueiredo, E. (2021). Intraguild predation and cannibalism 1190 among Dicyphini: Dicyphus cerastii vs. two commercialized 1191 species. *Entomol. Exp. Appl.* 169, 90–96. doi: 10.1111/eea.12943

Dumont, F., Lucas, É., and Alomar, O. (2020). Oviposition behaviour of the mirid Macrolophus pygmaeus under risk of intraguild predation and cannibalism. *Insect Sci.* 28, 224–230. doi: 10.1111/1744-7917.12752

Edenbrow, M., and Croft, D. P. (2012). Kin and familiarity influence association preferences and aggression in the mangrove killifish *Kryptolebias marmoratus*. *J. Fish Biol.* 80, 503–518. doi: 10.1111/j.1095-8649.2011.03181.x

Elgar, M. A., and Crespi, B. J. (1992). *Cannibalism: Ecology and Evolution Among Diverse Taxa*. Oxford: Oxford University Press.

Fox, L. R. (1975). Factors influencing cannibalism, a mechanism of population limitation in the predator *Notonecta hoffmanni*. *Ecology* 56, 933–941. doi: 10.2307/1936303

Gao, Q., Wang, D., Zhang, W. H., Liu, Y. D., Meng, F. C., Wu, B., et al. (2019). Study on predatory function of *Arma chinensis* on *Spodoptera litura* (Fabricius). *Chin. Tob. Sci.* 40, 55–59.

Gao, Z., Wang, X., Zhang, L., Sun, Y., Fan, J., and Wang, G. (2011). Biological characteristic of *Arma chinensis*. *J. Eng.* 2, 72–77.

Garza, S., and Waldman, B. (2015). Kin discrimination in polyphenic salamander larvae: trade-offs between inclusive fitness and pathogen transmission. *Behav. Ecol. Sociobiol.* 69, 1473–1481. doi: 10.1007/s00265-015-1959-0

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *J. Theor. Biol.* 7, 17–52. doi: 10.1016/0022-5193(64)90039-6

Harshman, L. G., and Zera, A. J. (2007). The cost of reproduction: the devil in the details. *Trends Ecol. Evol.* 22, 80–86. doi: 10.1016/j.tree.2006.10.008

Ho, F. K., and Dawson, P. S. (1966). Egg cannibalism by *Tribolium* larvae. *Ecology* 47, 318–322. doi: 10.2307/1933784

Hoffman, C. R. (2012). *Kinship and Familiarity Affect Recognition and Foraging in the Wolf Spider, Pardosa milvina (Araneae: Lycosidae)*. Honors thesis. Oxford, OH: Miyami University.

Ichikawa, N. (1991). Egg mass destroying and guarding behaviour of the giant water bug, *Lethocerus deyrollei* Vuillefroy (Heteroptera: Belostomatidae). *J. Ethol.* 9, 25–29. doi: 10.1007/BF02350293

Jacobs, A. C., and Stigall, T. (2019). Paternity and egg cannibalism in the ringlegged earwig *Euborellia annulipes* (Dermaptera: Anisolabididae). *Entomol. Sci.* 22, 250–257. doi: 10.1111/ens.12363

King, C. E., and Dawson, P. S. (1972). Population biology and the *Tribolium* model. *Evol. Biol.* 5, 133–227. doi: 10.1007/978-1-4757-0256-9-5

Lee, S. (2016). Avoiding negative reviewer comments: common statistical errors in anesthesia journals. *Korean J. Anesthesiol.* 69, 219–226. doi: 10.4097/kjae.2016.69.3.219

Lee, S., and Lee, D. K. (2018). What is the proper way to apply the multiple comparison test? *Korean J. Anesthesiol.* 71, 353–360. doi: 10.4097/kja.d.18.00242

Li, F. Q., Li, B. P., and Meng, L. (2020). Effects of plant structure on searching and predation behaviors on *Spodoptera litura* caterpillars by *Arma chinensis* (Hemiptera: Pentatomidae) across life stages. *Chin. J. Biol. Control* 37, 464–471. doi: 10.16409/j.cnki.2095-039x.2021.01.006

Manica, A. (2002a). Filial cannibalism in teleost fish. *Biol. Rev. Camb. Philos. Soc.* 77, 261–277. doi: 10.1017/s1464793101005905

Manica, A. (2002b). Alternative strategies for a father with a small brood: mate, cannibalise or care. *Behav. Ecol. Sociobiol.* 51, 319–323. doi: 10.1007/s00265-001-0444-0

Miller, J. S., and Zink, A. G. (2012). Parental care trade-offs and the role of filial cannibalism in the maritime earwig, *Anisolabis maritima*. *Anim. Behav.* 83, 1387–1394. doi: 10.1016/j.anbehav.2012.03.006

Mrowka, W. (1987). Filial cannibalism and reproductive success in the maternal mouthbrooding cichlid fish *Pseudocrenilabrus multicolor*. *Behav. Ecol. Sociobiol.* 21, 257–265. doi: 10.1007/BF00292507

Narasimha, S., Nagornov, K. O., Menin, L., Mucciolo, A., Rohwedder, A., Humbel, B. M., et al. (2019). *Drosophila melanogaster* cloak their eggs with pheromones, which prevents cannibalism. *PLoS Biol.* 17:e2006012. doi: 10.1371/journal.pbio.2006012

Neff, B. D. (2003). Paternity and condition affect cannibalistic behavior in nest-tending bluegill sunfish. *Behav. Ecol. Sociobiol.* 54, 377–384. doi: 10.1007/s00265-003-0645-9

Okada, S., Fukuda, Y., and Takahashi, M. K. (2015). Paternal care behaviors of Japanese giant salamander *Andrias japonicus* in natural populations. *J. Ethol.* 33, 1–7. doi: 10.1007/s10164-014-0413-5

Pajunen, V. I., and Pajunen, I. (1991). Oviposition and egg cannibalism in rock-pool corixids (Hemiptera: Corixidae). *Oikos* 60, 83–90. doi: 10.2307/3544996

Pan, M. Z., Zhang, H., Zhang, L., and Chen, H. (2019). Effects of starvation and prey availability on predation and dispersal of an omnivorous predator *Arma chinensis* Fallou. *J. Insect Behav.* 32, 134–144. doi: 10.1007/s10905-019-09718-9

Parsons, W., Zhong, W., and Rudolf, V. H. W. (2013). Mating status and kin recognition influence the strength of cannibalism. *Anim. Behav.* 85, 365–369. doi: 10.1016/j.anbehav.2012.11.006

Perry, J. C., and Roitberg, B. D. (2006). Trophic egg laying: hypotheses and tests. *Oikos* 112, 706–714. doi: 10.1111/j.0030-1299.2006.14498.x

Pfennig, D. W. (1997). Kinship and cannibalism. *Bioscience* 47, 667–675. doi: 10.2307/1313207

Pizzatto, L., and Shine, R. (2008). The behavioral ecology of cannibalism in cane toads (*Bufo marinus*). *Behav. Ecol. Sociobiol.* 63, 123–133. doi: 10.1007/s00265-008-0642-0

Polis, G. A. (1981). The evolution and dynamics of intraspecific predation. *Annu. Rev. Ecol. Syst.* 12, 225–251. doi: 10.1146/annurev.es.12.110181.001301

R Development Core Team (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Revynthi, A. M., Egas, M., Janssen, A., and Sabelis, M. W. (2018a). Prey exploitation and dispersal strategies vary among natural populations of a predatory mite. *Ecol. Evol.* 8, 10384–10394. doi: 10.1002/ece3.4446

Revynthi, A. M., Janssen, A., and Egas, M. E. (2018b). Gender-specific differences in cannibalism between a laboratory strain and a field strain of a predatory mite. *Exp. Appl. Acarol.* 74, 239–247. doi: 10.1007/s10493-018-0232-4

Rider, D. A., and Zheng, L. Y. (2002). Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera) I. Asopinae. *Entomotaxonomia* 24, 107–115. doi: 10.1520/STP11985S

Ringler, E., Barbara Beck, K., Weinlein, S., Huber, L., and Ringler, M. (2017). Adopt, ignore, or kill? Male poison frogs adjust parental decisions according to their territorial status. *Sci. Rep.* 7:43544. doi: 10.1038/srep43544

Rohwer, S. (1978). Parent cannibalism of offspring and egg raiding as a courtship strategy. *Am. Nat.* 112, 429–440. doi: 10.1086/283284

Rudolf, V. H. W., Kamo, M., and Boots, M. (2010). Cannibals in space: the coevolution of cannibalism and dispersal in spatially structured populations. *Am. Nat.* 175, 513–524. doi: 10.1086/651616

Ryckman, R. E. (1951). Recent observations of cannibalism in *Triatoma* (Hemiptera: Reduviidae). *J. Parasitol.* 37, 433–434. doi: 10.2307/3273249

Samu, F., Toft, S., and Kiss, B. (1999). Factors influencing cannibalism in the wolf spider *Pardosa agrestis* (Araneae, Lycosidae). *Behav. Ecol. Sociobiol.* 45, 349–354. doi: 10.1007/s002650050570

Schausberger, P. (2003). Cannibalism among phytoseiid mites: a review. *Exp. Appl. Acarol.* 29, 173–191. doi: 10.1023/A:1025839206394

Schultner, E., d'Ettorre, P., and Helanterä, H. (2013). Social conflict in ant larvae: egg cannibalism occurs mainly in males and larvae prefer alien eggs. *Behav. Ecol.* 24, 1306–1311. doi: 10.1093/beheco/art067

Siegel, S. M., Lee, J. W., and Oaklander, A. L. (2007). Needlestick distal nerve injury in rats models symptoms of complex regional pain syndrome. *Anesth. Analg.* 105, 1820–1829. doi: 10.1213/01.ane.0000295234.21892.bc

Smith, C., and Reay, P. (1991). Cannibalism in teleost fish. *Rev. Fish Biol. Fish.* 1, 41–64. doi: 10.1007/BF00042661

Thomas, L. K., and Manica, A. (2003). Filial cannibalism in an assassin bug. *Anim. Behav.* 66, 205–210. doi: 10.1006/anbe.2003.2202

Vickery, W. L., Whoriskey, F. G., and FitzGerald, G. J. (1988). On the evolution of nest raiding and male defensive behaviour in sticklebacks (Pisces: Gasterosteidae). *Behav. Ecol. Sociobiol.* 22, 185–193. doi: 10.1007/BF00300568

Wang, L., Meng, L., and Li, B. P. (2020). Effects of diets with *Harmonia axyridis* pupae on growth and development performance of predatory stinkbug *Arma chinensis*. *J. Nanjing Agric. Univ.* 43, 645–649.

Widdig, A. (2007). Paternal kin discrimination: the evidence and likely mechanisms. *Biol. Rev. Camb. Philos. Soc.* 82, 319–334. doi: 10.1111/j.1469-185X.2007.00011.x

Wu, H., Coudron, T. A., Zhang, L., Aldrich, J. R., Xu, W., Xu, J., et al. (2019). Identification and field verification of aggregation-sex pheromone from the predaceous bug, *Arma chinensis*. *Chemoecology* 29, 235–245.doi: 10.1007/s00049-019-00292-2

Wu, S. L., Deng, W., Cai, H. L., Yang, J. S., Zeng, W. A., Zhou, Z. C., et al. (2020). The occurrence period and effect of intraspecific cannibalism behavior of *Arma chinensis* under starvation. *Chin. J. Biol.* 36, 169–174. doi: 10.16409/j.cnki.2095-039x.2020.3.001

Zalom, F. G. (1978). Backswimmer prey selection with observations on cannibalism (Hemiptera: Notonectidae). *Southwest. Nat.* 23, 617–622. doi: 10.2307/3671183

Zhao, Q., Wei, J., Bu, W., Liu, G., and Zhang, H. (2018). Synonymize *Arma chinensis* as *Arma custos* based on morphological, molecular and geographical data. *Zootaxa* 4455, 161–176. doi: 10.11646/zootaxa.4455.1.7

Zheng, Z. Y., and Chen, Y. W. (1992). Experiments on the use of *Arma custos* (Fabricius) [Hem.: Pentatomidae] to control forest pests. *Chin. J. Biol. Control* 8, 155–156.

Zou, D., Wang, M., Zhang, L., Zhang, Y., Zhang, X., and Chen, H. (2012). Taxonomic and bionomic notes on *Arma chinensis* (Fallou). *Zootaxa* 3382, 41–52. doi: 10.11646/zootaxa.3382.1.4

Zou, D. Y., Coudron, T. A., Wu, H. H., Gu, X. S., Xu, W. H., Zhang, L. S., et al. (2015). Performance and cost comparisons for continuous rearing of *Arma chinensis* (Hemiptera: Pentatomidae: Asopinae) on a zoophytogenous artificial diet and a secondary prey. *J. Econ. Entomol.* 108, 454–461. doi: 10.1093/jee/tov024

Conflict of Interest: WZ and HC were employed by the Hunan Changsha Municipal Tobacco Company. SW, ML and ZZ were employed by the Hunan Province Tobacco Company. JL was employed by the Hunan Yongzhou Municipal Tobacco Company.

The remaining 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 | Frontiers in Ecology and Evolution

TYPE Review
PUBLISHED 27 September 2022
DOI 10.3389/fevo.2022.946640

OPEN ACCESS

EDITED BY
Rachael E. Bonoan,
Providence College, United States

REVIEWED BY
Maria Albo,
Universidad de la República, Uruguay
Arja Kaitala,
University of Oulu, Finland

*CORRESPONDENCE
Mira Van den Broeck
Mira.VandenBroeck@uantwerpen.be

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 17 May 2022
ACCEPTED 06 September 2022
PUBLISHED 27 September 2022

CITATION
Owens ACS, Van den Broeck M, De Cock R and Lewis SM (2022) Behavioral responses of bioluminescent fireflies to artificial light at night. *Front. Ecol. Evol.* 10:946640. doi: 10.3389/fevo.2022.946640

Behavioral responses of bioluminescent fireflies to artificial light at night

Avalon C. S. Owens[1]†, Mira Van den Broeck[2]*†, Raphaël De Cock[2] and Sara M. Lewis[1]

[1]Department of Biology, Tufts University, Medford, MA, United States, [2]Evolutionary Ecology Group, Department of Biology, University of Antwerp, Antwerp, Belgium

Bioluminescent insects have been the subject of scientific interest and popular wonder for millennia. But in the 21st century, the fireflies, click beetles, and cave glow-worms that brighten our nights are threatened by an unprecedented competitor: anthropogenic light pollution. Artificial lights can obscure the light-based signals on which these and other bioluminescent organisms rely to court mates, deter predators, and attract prey. In the following review we summarize a recent influx of research into the behavioral consequences of artificial light at night for firefly beetles (Coleoptera: Lampyridae), which we organize into four distinct courtship signaling systems. We conclude by highlighting several opportunities for further research to advance this emerging field and by offering a set of up-to-date lighting recommendations that can help land managers and other stakeholders balance public safety and ecological sustainability.

KEYWORDS

Lampyridae, bioluminescence, firefly, light pollution, artificial light at night (ALAN)

Introduction

Advances in lighting technology have rapidly increased the scale and intensity of anthropogenic light pollution over the past century. Recent estimates suggest that at least 22.5% of the habitable land surface of the earth currently experiences artificially bright night skies (Falchi et al., 2016), and that night skies have become 49% brighter on average within the last 25 years (Sánchez de Miguel et al., 2021). Artificial light sources responsible include streetlights, car headlights, security lights, facade lighting, stadium lighting, illuminated advertisements, and sources associated with agriculture and industry, e.g., greenhouse lighting or uncurtained office windows (Luginbuhl et al., 2009). Atmospheric scattering of upwelling light produces skyglow, a bright haze visible many kilometers beyond its source that further contributes to light pollution (Kyba et al., 2015; **Figures 1A–E**).

Unprecedented increases in night sky brightness threaten all taxa that have evolved under predictable cycles of light and dark (Gaston et al., 2017). However, nocturnal insects may be especially vulnerable to the encroachment of artificial light at night (ALAN) into their habitats due to their high visual sensitivity (Warrant, 2017), small body size relative to artificial light sources, and tendency toward positive phototaxis or "flight-to-light" behavior (Owens and Lewis, 2018; Owens et al., 2020). Certain charismatic nocturnal insect taxa capable of bioluminescent communication, fireflies the most successful and species among them (Oba and Schultz, 2014), are likely to be both particularly at risk and particularly able to inspire public interest in dark sky conservation.

Worldwide, over 2200 species of firefly and glow-worm beetles (family Lampyridae, hereafter referred to collectively as fireflies), are currently described (Martin et al., 2019). All firefly species bioluminesce in their larval stage, an adaptation which presumably originated to warn predators of their unpalatable chemical defenses (Branham and Wenzel, 2003; Powell et al., 2022; but note Kok et al., 2019). Most species subsequently co-opted this ability to produce both aposematic signals and sexual advertisements during their brief adult stage (Leavell et al., 2018). Firefly adults typically restrict their courtship activity to a characteristic crepuscular or nocturnal temporal niche, with flight periods lasting from only 20 min up to several hours. Certain species in the genus *Photuris* have further adapted their signals into foraging lures (Lloyd, 2017).

All types of bioluminescent signal are susceptible to environmental masking (Johnsen et al., 2004). Natural light from the sun or moon can be a predictable obstacle to visibility (Gunn and Gunn, 2012; see also Branham and Faust, 2019), but artificial light from local sources is not only significantly brighter but also spatially, temporally, and spectrally novel. Consequently, ALAN likely acts as a strong selective pressure (Hopkins et al., 2018) that has rapidly intensified in recent years following the development of increasingly efficient and broad-spectrum lighting technologies such as LEDs (Elvidge et al., 2010). If artificial light masks bioluminescent signals, increases in predation rates and decreases in mate success could cause firefly populations to rapidly decline.

In this review, we update an earlier synthesis (Owens and Lewis, 2018) to summarize a flurry of recent studies examining the influence of ALAN on the bioluminescent behaviors of fireflies. Throughout we discuss relevant risk factors that are likely to make certain species especially vulnerable to artificial light. We conclude by pointing out persistent gaps in our understanding of firefly conservation behavior, highlighting several future research directions that we believe will advance this emerging field, and offering a set of informed guidelines to help conservationists develop and maintain "firefly friendly" light environments for at-risk species. The studies reviewed below (**Table 1**) employed artificial lights with widely varying intensities as well as different temporal, spatial, and spectral distributions; to simplify comparisons, where possible we report intensities in lux, an incomplete but relatively accessible measure of the brightness of broad-spectrum light as viewed by humans.

Firefly visual systems

To understand the effects of ALAN on fireflies, it is crucial to first understand how they perceive light. Fireflies are red-green colorblind (**Figure 2**; Buck, 1937; Booth et al., 2004; Owens et al., 2018). The common ancestor of all beetles lacked a blue photoreceptor, presumably sacrificing spectral sensitivity to increase total sensitivity (Sharkey et al., 2017). Fireflies appear to have maintained this inherited configuration, with only one unique copy each of UV-sensitive (UVS) and long-wavelength-sensitive (LWS) photoreceptors detected in all species investigated to date (Oba and Kainuma, 2009; Sander and Hall, 2015). Firefly LWS photoreceptors can be tuned to absorb conspecific bioluminescence through the addition of overlying filter pigments (Cronin et al., 2000). Neural comparisons of input from LWS and UVS photoreceptors may permit limited color discrimination in the UV-blue-green range (e.g., Booth et al., 2004).

The bioluminescence emission spectrum and corresponding long-wavelength spectral sensitivity of a given species are usually characteristic of its particular temporal or photic niche. Nocturnal fireflies tend to produce green bioluminescence and to be broadly sensitive to long wavelengths (**Figure 2A**). In contrast, the compound eyes of crepuscular fireflies often contain red or pink filter pigments that screen out the blue-green ambient light of twilight, increasing the visibility of their signals against surrounding foliage (Cronin et al., 2000; Lall et al., 2009); many of these species produce yellow bioluminescence to maximize visibility within this visual system (**Figure 2B**; Lall et al., 1980b). Notably some nocturnal fireflies with green bioluminescence, including *Photuris versicolor* (Cronin et al., 2000) and *Lampyris noctiluca* (Booth et al., 2004) have yellow filter pigments in the frontal-dorsal regions of their compound eyes, which have been posited to screen out downwelling skylight.

In contrast to the large and complex compound eyes of adults, most firefly larvae possess only a single pair of bilateral stemmata (Murphy and Moiseff, 2019). Despite structural differences, however, the simple eyes of *Photuris* larvae are functionally similar to the compound eyes of *Photuris* adults: they are most sensitive to light in the blue-to-green region of the visible light spectrum and appear capable of discriminating colors in this region as well (Murphy and Moiseff, 2019). *Photuris* larvae move away from artificial light even after their optic nerve has been severed, suggesting that an alternative sensory pathway transmits information on ambient light intensity to the brain (Murphy and Moiseff, 2020). Intrinsically

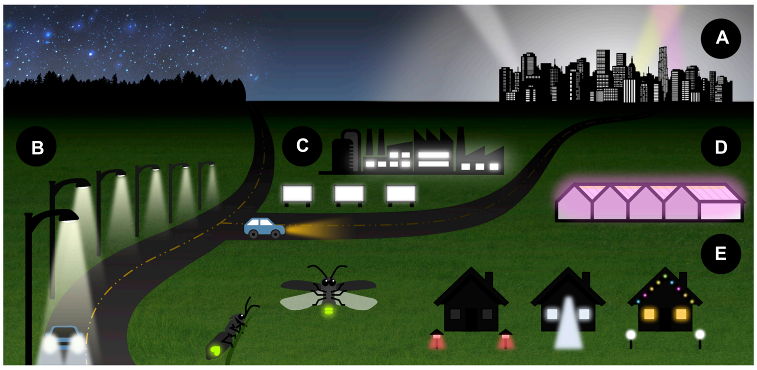


FIGURE 1
Artificial light at night (ALAN) emanates from diverse sources that vary widely in intensity as well as temporal, spatial, and spectral distribution. Major sources of ALAN include **(A)** diffuse skyglow, sometimes visible at a great distance from urban centers, **(B)** artificial lights used in transportation such as streetlights and car headlights, **(C)** commercial lighting, including billboards and the lit windows of commercial buildings, **(D)** agricultural lighting, including grow lights used in greenhouses, and **(E)** private lighting such as security lights, decorative lights, and lit windows.

photosensitive areas of the brain, previously documented in *Luciola lateralis* and *Luciola cruciata* adults (Hariyama, 2000), may be responsible.

Firefly courtship signaling systems

Fireflies employ diverse visual and chemical signals as sexual advertisements (Ohba, 2004; Lloyd, 2008; da Silveira and Mermudes, 2014), but most species can be categorized into one of four primary signaling systems [**Figure 3**; adapted from Lewis (2009)]. The impact of artificial light is likely to be contingent upon these different courtship behaviors, briefly described below.

Dark fireflies (**Figure 3A**) are species with diurnal adults that do not employ bioluminescent courtship signals. Instead, females are known or presumed to use pheromones to attract flying males, which tend to have elongated and elaborated antennae but limited vision (da Silveira and Mermudes, 2014; Stanger-Hall et al., 2018). Male attraction to female pheromones has been documented in *Lucidota atra* (Lloyd, 1972), *Lucidina biplagiata* (Ohba, 2004), and *Phosphaenus hemipterus* (De Cock and Matthysen, 2005).

Glow-worm fireflies or glow-worms (**Figure 3B**) are sexually dimorphic species with brachypterous or apterous females that are incapable of flight, and which initiate courtship by emitting continuous glows while perched near the ground. In some species such as *Pyrocoelia rufa* (Ohba, 2004) females attract males with pheromones in addition to continuous glows. The European glow-worm *L. noctiluca* is widespread throughout Europe and the UK, where glow-worm fireflies predominate (De Cock, 2009). Glow-worm males are fully winged and search for female glow signals, and possibly pheromones, from the air. In some species such as the blue ghost *Phausis reticulata* (De Cock et al., 2014) and the tracker ghost *Amydetes fastigiata* (Vaz et al., 2021) males also emit prolonged glows. Due to the presumably limited dispersal ability of flightless females, glow-worm fireflies may be highly vulnerable to habitat degradation and fragmentation resulting from ALAN.

Roving flashing fireflies (**Figure 3C**) are species where both sexes signal using discrete bursts of light. Females of these species are often sedentary, though usually fully winged and capable of flight. Flying males advertise by repeating a species-specific flash pattern at regular intervals, and receptive females answer with response flashes (Ohba, 2004; Lewis and Cratsley, 2008). This flash exchange – known as a courtship dialog – continues until the male locates and mounts the female, and is characteristic of many firefly genera including *Luciola*, *Aquatica*, *Pyractomena*, and *Photinus* (Stanger-Hall and Lloyd, 2015). Females in the genus *Photuris* (Souto et al., 2019) are specialist predators of other flashing fireflies, luring patrolling males by mimicking the response flashes of conspecific females (Lloyd, 1980), hawking them from the air (Lloyd and Wing, 1983), and stealing them from spider webs (Faust, 2012). In a few species, such as *Photinus carolinus*, *Photinus knulli*, and *Photuris frontalis* in the United States (Sarfati et al., 2021, 2022), *Photinus palaciosi* in Mexico, and *Luciola* sp. in eastern Europe (Baldaccini et al., 1969), flying males synchronize their courtship advertisements when they reach sufficient densities.

Stationary synchronous fireflies (**Figure 3D**) have in recent decades become popular tourist attractions due to the visual spectacle of their courtship displays (Cheng et al., 2021; Lewis et al., 2021). Males of these species form conspicuous leks, congregating *en masse* each evening within particular visually prominent display trees (Cratsley et al., 2012; Prasertkul, 2018; Jaikla et al., 2020b). Stationary males perch on leaves and collectively signal by flashing together in tight synchrony (Cratsley et al., 2012; Prasertkul, 2018; Jaikla et al., 2020b).

TABLE 1 Conclusively documented behavioral and developmental effects of artificial light on fireflies. Where appropriate, the intensity of each artificial light treatment has been provided in lux.

Group	Species	Behavioral response	Light treatment	Reference
Glow-worms	*Lampyris noctiluca* (nocturnal)	Males in the field less likely to approach artificially illuminated imitation females	≤0.35 lux	Stewart et al. (2020)
			7 lux	Elgert et al. (2020)
			≤0.025 lux	Van den Broeck et al. (2021b)
		Females in the field glow over more evenings under artificial illumination	0.1–8.5 lux existing streetlights (low-pressure sodium)	Van den Broeck et al. (2021a)
		Females in the laboratory glow for fewer minutes under artificial illumination	15–20 lux	Elgert et al. (2021b)
			2.5 lux (incandescent)	Dreisig (1978)
		Females in the laboratory never glow under artificial illumination	10 lux (incandescent)	Dreisig (1978)
Roving Flashing Fireflies	*Aquatica ficta*	Males in the laboratory emit more conspicuous alarm flashes less often under artificial illumination	N/A Short/mid-wavelength monochromatic LEDs	Owens et al. (2018)
	Photinus pyralis (crepuscular)	Equal male courtship flash activity observed near artificial lights in the field but tethered females respond less often	300 lux	Firebaugh and Haynes (2016)
		Males in the field emit fewer courtship flashes under artificial illumination	175 lux	Firebaugh and Haynes (2019)
		Marked females in the field move independently and mate successfully under artificial illumination	>20 lux existing floodlights (white LED)	Owens and Lewis (2022)
	Photinus marginellus (crepuscular)	Marked females in the field move independently and mate successfully under artificial illumination	>20 lux existing floodlights (white LED)	Owens and Lewis (2022)
		Less male courtship flash activity observed near artificial lights in the field	1.2 lux (mercury vapor)	Costin and Boulton (2016)
	Photinus greeni (crepuscular)	Males in the field less likely to approach artificially illuminated imitation females	5 lux	Owens and Lewis (2022)
	Photinus obscurellus (semi-nocturnal)	Males in the laboratory emit more conspicuous courtship flashes less often under artificial illumination, while females nearly entirely cease responding	Amber: 24–240 lux monochromatic LEDs	Owens and Lewis (2021b)
		Pairs in the laboratory mate under dim but not bright artificial illumination	Dim: 3 lux, Bright: 30 lux	Owens and Lewis (2022)
		Females in the field less likely to mate successfully under artificial illumination	5 lux	Owens and Lewis (2022)
	Photinus carolinus (nocturnal)	Less male courtship flash activity observed near artificial lights in the field	Amber: 150 lux monochromatic LEDs	Owens et al. (2022)

(Continued)

TABLE 1 (Continued)

Group	Species	Behavioral response	Light treatment	Reference
	Photuris sp.	Less courtship/foraging flash activity observed near artificial lights in the field	1.2 lux (mercury vapor)	Costin and Boulton (2016)
	Photinus sp1 (nocturnal)	Fewer flashing males observed along artificially illuminated transects	0.5–4.5 lux existing floodlights (metal halide)	Hagen et al. (2015)
	Photuris versicolor (nocturnal)	Less courtship/foraging flash activity observed near artificial lights in the field	300 lux	Firebaugh and Haynes (2016)
	Sclerotia aquatilis	Pairs in the laboratory eventually mate successfully under artificial illumination	0.05–0.3 lux (fluorescent)	Thancharoen (2007)
Stationary synchronous fireflies	*Pteroptyx valida*	Males in the field repeatedly congregate (lek) within artificially illuminated display trees	7–14 lux existing streetlights (fluorescent)	Prasertkul (2018)
	Pteroptyx malaccae	Males in the laboratory flash less often, and with less synchrony, in response to camera flashes	N/A	Thancharoen and Masoh (2019)
Larvae	*Aquatica ficta*	Aquatic larvae in the laboratory show high mortality following chronic exposure to artificial illumination	<0.01 lux	Chen et al. (2021)
	Lampyris noctiluca	Larvae in the field less likely to glow under moonlight and/or urban skyglow	N/A	De Cock (2004)
	Lamprigera sp.	Larvae in the field less likely to forage under artificial illumination, more likely to move away or appear immobilized	3–4.5 lux existing streetlights (white LED)	Wanjiru Mbugua et al. (2020)
	Phosphaenus hemipterus	Larvae in the field less likely to glow under moonlight and/or urban skyglow	N/A	De Cock (2004)
	Photinus obscurellus	Larvae in the laboratory unaffected by chronic exposure to artificial illumination	50 lux	Owens and Lewis (2021a)
	Photuris sp.	Larvae in the laboratory gain weight more quickly during chronic exposure to artificial illumination	50 lux	Owens and Lewis (2021a)
		Larvae in the laboratory less likely to forage near artificial lights, more likely to move away horizontally or vertically	800 lux	Owens and Lewis (2021a)
		Larvae in the laboratory reduce surface activity under artificial illumination	915 lux	Murphy and Moiseff (2020)

Because lux is a measurement of the brightness of downwelling light as perceived by humans, it is strongly skewed by the position of the lux meter sensor with respect to the target light source as well as differences in the spectral distribution of said source. We note the bulb type employed (where available) when it differs from the current experimental standard, broad-spectrum white LED.

Flying and flashing females orient toward display trees from surrounding areas. Once females reach the lek, complex courtship interactions precede mating (Case, 1980). Example include southeast Asian mangrove species *Pteroptyx tener*, *Pteroptyx malaccae* (Jusoh et al., 2018), and *Pygoluciola qingyu* (Fu and Ballantyne, 2008).

In the following section we review what is currently known about how ALAN impacts adult courtship in each of these

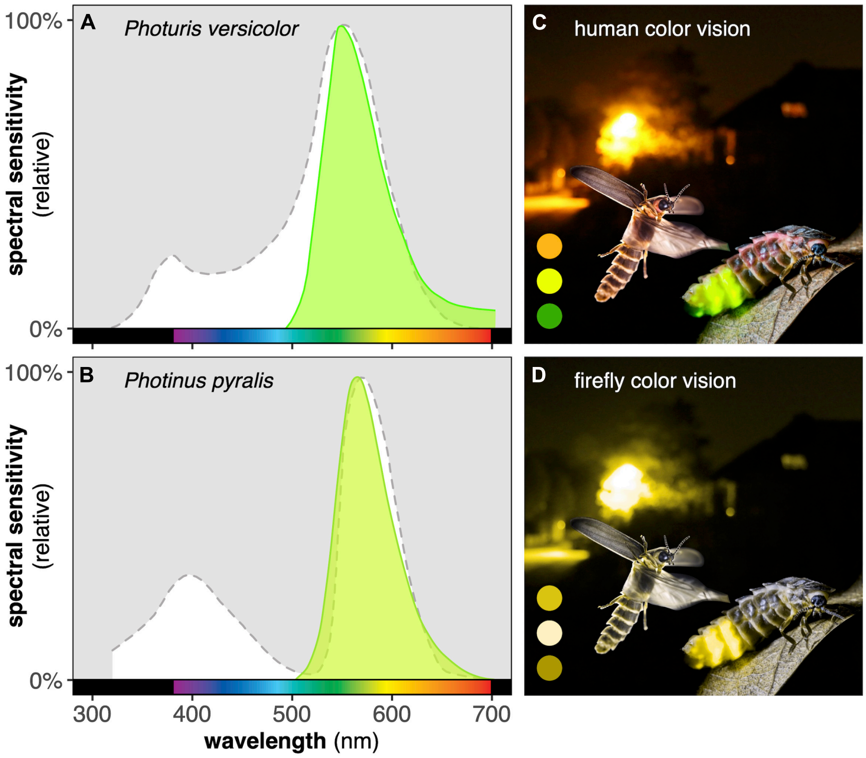


FIGURE 2

Firefly eyes have been optimized to absorb conspecific signals. The spectral sensitivities of nocturnal *Photuris versicolor* **(A)** and crepuscular *Photinus pyralis* **(B)** fireflies both peak near the peak wavelength of conspecific bioluminescence (emission spectra shown in green). Fireflies only possess one LWS photoreceptor and are therefore fully red-green colorblind. This means that an image containing a range of green, yellow, and orange regions, as viewed by most humans **(C)**, will appear nearly monochromatic to fireflies **(D)**, with orange and green regions indistinguishable from dim yellow regions. Spectral sensitivities of both species and spectral distribution of *P. versicolor* bioluminescence modified from Lall et al. (1980a) and Lall (1981); spectral distribution of *P. pyralis* bioluminescence modified from Hall et al. (2016). Image credits: background by MVDB, stationary female glow-worm by Robert Canis (robertcanis.com), flying male glow-worm by Hubert Polacek; protanopia color vision simulation from pilestone.com.

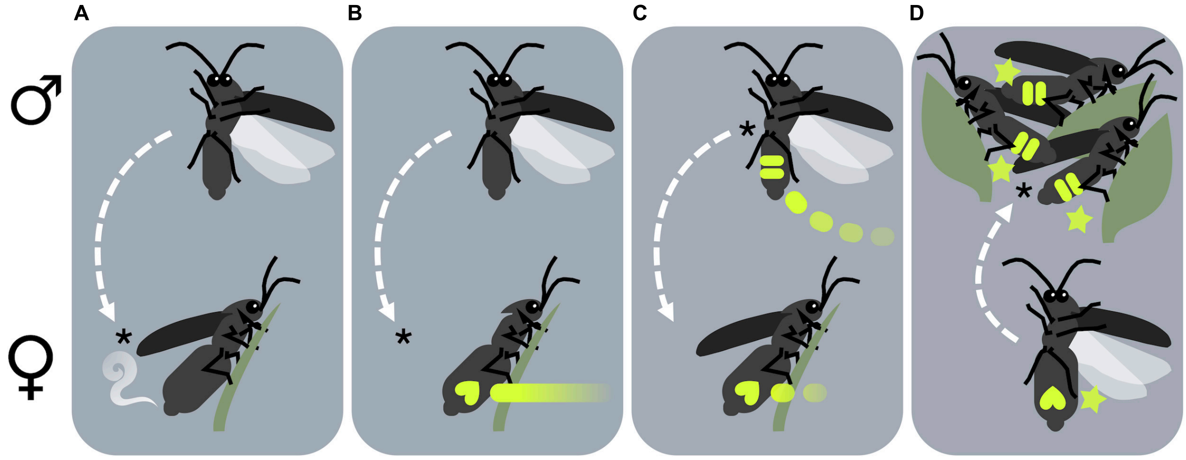


FIGURE 3

Fireflies may be grouped according to their courtship behavior into one of four signaling systems: either **(A)** dark, **(B)** glow-worm, **(C)** roving flashing, or **(D)** stationary synchronous fireflies (see main text for explanations of each signaling system). Each panel depicts males (above) and females (below), with asterisks (*) denoting the primary signaler (the sex which initiates courtship), and dashed arrows indicating the direction of movement toward the mating location.

distinct signaling systems, then consider how ALAN impacts adult survivorship and larval development.

Effects of artificial light at night on firefly behavior

Courtship

Dark fireflies

Dark firefly adults do not rely on light signals for courtship (**Figure 3A**). The impact of artificial light on courtship and mating success in these species is therefore likely to depend upon how novel external light cues interact with the internal timekeeping mechanisms ("clocks") that regulate daily and seasonal rhythms of activity in most terrestrial taxa (Gaston et al., 2017), and whether this results in temporal disorientation (*sensu* Owens and Lewis, 2018). All fireflies, including species with non-bioluminescent adults, pupate and eclose at certain times of year, and partake in behaviors such as courtship, mating, and oviposition at certain times of day. Artificial light exposure late in the day or summer may prolong daily and seasonal activity, delaying dormancy, while artificial light exposure early in the day or spring may advance activity. Artificial light rich in blue wavelengths may be especially disorienting, as these wavelengths have been shown to most strongly suppress production of melatonin in *Drosophila* and other taxa (Cashmore et al., 1999).

Glow-worm fireflies

Because patrolling male glow-worms are attracted to continuous glows (**Figure 3B**), they may confuse continuously operating artificial lights for large receptive females and thereby fall into a potent evolutionary trap (Haynes and Robertson, 2021). Glow-worm flight-to-light behavior has not been documented on a large scale (but see Bek, 2015; Kivelä, 2022). However, small points of artificial light (e.g., from LEDs) have been effectively used to trap males both as a part of surveys (Pacheco et al., 2016) and studies of glow-worm courtship (De Cock et al., 2014). Bespoke "imitation females" have been used to elucidate several aspects of glow-worm physiology and behavior (e.g., Booth et al., 2004).

The European glow-worm *L. noctiluca* enjoys a wide distribution (De Cock, 2009), and has served as a model organism for the majority of studies investigating the impact of ALAN on the courtship and mate success of bioluminescent fireflies (**Table 1**). Stewart et al. (2020), Elgert et al. (2021a), and Van den Broeck et al. (2021b) all found that when imitation female glow signals were illuminated by broad-spectrum white LEDs, they attracted fewer *L. noctiluca* males compared to unilluminated controls (see also Ineichen and Rüttimann, 2012; Bird and Parker, 2014; Kivelä, 2022), and that this difference was magnified under brighter illumination. ALAN spectra, intensity, and directionality have become a focus of recent research as they might be adjusted to minimize detrimental effects. Elgert et al. (2020) found that 7 lux of downwelling ALAN, which is dimmer than the light cast by most streetlights, significantly decreased the attractiveness of imitation females to males. Stewart et al. (2020) found effects of horizontally-directed ALAN up to 40 m away from the source, where it measured approximately 0.35 lux. Using a similar experimental setup with upwelling ALAN, Van den Broeck et al. (2021b) saw significantly decreased attractiveness of imitation females under only 0.025 lux of ALAN, similar in intensity to moonlight (Kyba et al., 2017). The authors also compared the impact of illumination by cool vs. warm white LEDs, which emit different ratios of blue to longer wavelengths, and found that both treatments interfered equally with the ability of males to locate imitation females.

Limiting the duration of artificial light could be another way to minimize detrimental effects. Van den Broeck et al. (2021a) observed the effects of long-term exposure to ALAN on the mate success of live females in the field by repeatedly checking whether or not they were glowing (*L. noctiluca* females cease glowing once they have mated; Tyler, 2002). While females in dark sites (< 0.1 lux) glowed for only a single evening, females in areas lit by low-pressure sodium street lights (0.1–8.5 lux) glowed for a median of six and as many as 24 evenings, indicating that these artificially illuminated females experienced significantly decreased mate success. However, in a recent laboratory experiment Elgert et al. (2021b) observed that females were significantly more likely to cease glowing when artificially illuminated: on average, females went dark after only 26 min of continuous ALAN exposure. This behavioral difference could be explained by the brighter (15–20 lux) and more direct artificial light used in the lab study, or by the relatively short timescale. In an early laboratory experiment, Dreisig (1978) found that 2.5 lux of artificial light reduced the duration of glow activity from an average of 139 min to only 51 min and that females never began to glow under 10 lux of artificial light. Between 0.001 and 1.0 lux the duration of glow activity was similar to that under natural conditions. The suppressive effect of ALAN on the production of glow signals by female glow-worms may therefore be weaker in the field, where artificial illumination is comparatively dim and indirect, and may also abate over multiple evenings.

Both abbreviated and prolonged glowing periods due to ALAN could be associated with fitness costs. *L. noctiluca* females that never glow are significantly less likely to attract nearby males (De Cock et al., 2014), while those that glow over multiple evenings likely do so only because they remain unmated (Tyler, 2002). Even if these females do eventually mate, delays are energetically costly: glow-worms are capital breeders that rarely feed as adults (Wing, 1989; Tyler, 2002), and the energy they require for self-maintenance and bioluminescence depletes over time (Baudry et al., 2021). Some of this energy comes from metabolized eggs (Wing, 1989), steeply reducing fecundity (Horne et al., 2017; Hopkins et al., 2021). Prolonged glowing periods could also increase predation risk, although few predators are able or willing to overcome glow-worm chemical defenses (see section "Predator-prey interactions" below; De Cock and Matthysen, 2001; Lewis et al., 2011). Small females may be especially vulnerable to artificial light, both because their glows tend to be dimmer (Borshagovski et al., 2020), and therefore less visible under artificial illumination, and because they have fewer energy reserves with which to produce them. This may explain why smaller females cease glowing earlier under ALAN than do their larger counterparts (Elgert et al., 2021b; Hopkins et al., 2021).

Roving flashing fireflies

Fireflies that exchange precisely timed, discrete flashes as part of courtship (**Figure 3C**) seem unlikely to mistake continuously glowing artificial light sources for conspecifics. However, like other bioluminescent taxa, these species are nevertheless vulnerable to masking effects of artificial light.

Several recent studies have examined the impact of ALAN on courtship signaling by North American flashing fireflies, primarily in the genera *Photinus* and *Photuris* (**Table 1**). Under moderately dim artificial light, a mixed species assemblage reduced their courtship flash activity (number of flash patterns per minute) to 50% of the baseline rate (1.2 lux; Costin and Boulton, 2016). Males of the common crepuscular species *Photinus pyralis* flashed at 75% of their baseline rate when placed directly beneath a bright artificial light source (175 lux; Firebaugh and Haynes, 2019), while nocturnal *Photuris versicolor* fireflies flashed at 30% of baseline within 10 m of a similar source (300 lux; Firebaugh and Haynes, 2016). In the laboratory, males of the semi-nocturnal species *Photinus obscurellus* flashed at approximately 50% of baseline under dim and bright artificial light (24 or 240 lux; Owens and Lewis, 2021b; see also Owens and Lewis, 2022). The number of semi-nocturnal *Photinus sp1* males observed flashing in a transect 60 m from a bright floodlight was 13% of baseline (4 lux; Hagen et al., 2015). Similarly, at a popular firefly ecotourism site, males of the fully nocturnal, synchronous species *P. carolinus* flashed at only 4% of baseline within 5 m of an artificial light source (150 lux, broad-spectrum amber; Owens et al., 2022). Reductions in male courtship flash activity are frequently assumed to correspond to reductions in mate success, but a recent report suggests the strength of this relationship also varies by species (Owens and Lewis, 2022).

How ALAN impacts female courtship flash activity has been less well studied, perhaps because in flashing fireflies, females are typically more cryptic. In most species, however, female behavior determines mate success: the likelihood of any male successfully locating and mating with a female depends on how often she answers his advertisement flashes (Demary et al., 2005; Owens and Lewis, 2022). Recent studies on two *Photinus* species suggest that females are even more sensitive than males to downwelling ALAN (Firebaugh and Haynes, 2016; Owens and Lewis, 2021b). In *P. pyralis,* white light (175 lux) suppressed female response rates to around 50% of baseline (Firebaugh and Haynes, 2016, 2019). In *P. obscurellus*, female response rates response rates declined to only 10% of baseline under white light (24 or 240 lux), and 50% of baseline under dim red light (Owens and Lewis, 2021b). Females of these species perch near the ground and search the sky for male advertisements, and may therefore be more likely to look directly into an artificial light source than are patrolling males searching the ground for females. Whether subsequent light-adaptation of their compound eyes prevents females from perceiving male flashes entirely or reduces male attractiveness by making their courtship flashes appear dimmer remains unknown. Regardless, reductions in female response flash activity are once again assumed to correspond to reductions in mate success.

Direct investigations into the impact of ALAN on mate success in flashing fireflies have had surprisingly mixed results. In the field, males of the crepuscular species *Photinus greeni* almost never approached imitation females that were directly illuminated when there were unilluminated competitors nearby (5 lux; Owens and Lewis, 2022). However, an early study by Thancharoen (2007) found that pairs of crepuscular *Sclerotia aquatilis* fireflies enclosed together in the laboratory (theoretically obviating the effects of sexual competition and/or impaired mate location) mated successfully under very dim light (0.05–0.3 lux), although courtship and mounting behaviors were prolonged. *P. obscurellus* pairs enclosed together in the laboratory mated successfully under dim light (3 lux) but not bright light (30 lux; Owens and Lewis, 2022). Dim light (5 lux) was relatively more disruptive to the mate success of *P. obscurellus* females permitted to move freely in the field, yet even bright light (>20 lux) had no detectable impact on the mate success of females of the crepuscular species *P. pyralis* and *Photinus marginellus* (Owens and Lewis, 2022).

Some roving flashing fireflies appear therefore capable of behaviorally adapting to ALAN. *P. obscurellus* males in the laboratory plastically increased the intensity, duration, and density (number of flashes per flash pattern) of their advertisement flashes in response to increases in ambient light levels, although these behavioral adaptations were unable to rescue baseline female response rates (Owens and Lewis, 2021b; see also Owens and Lewis, 2022). Other species, especially those active near sunset, may be even more capable of competing with visual noise (but note Borshagovski et al., 2020) or may be able to rely on redundant, as yet undescribed pheromone cues when engaging in courtship and mating activity in a less informative visual environment.

Stationary synchronous fireflies

Among the most visually impressive of all animal courtships are the communal displays (**Figure 3D**) created by various lekking fireflies that range from East India through Thailand, Malaysia and Indonesia across to the Philippines and Papua New Guinea (Ballantyne and Lambkin, 2013; Jusoh et al., 2018; Jaikla et al., 2020a). Such fireflies include several *Pteroptyx* species, in which thousands of males gather in visually prominent display trees along tidal rivers and flash synchronously to create a collective courtship signal that attracts flying females from surrounding areas (Wong and Yeap, 2012; Jusoh et al., 2018; Jaikla et al., 2020a). These congregating fireflies are star attractions in a booming ecotourism sector throughout this region (Lewis et al., 2021), yet surprisingly little is known about how ALAN impacts their behavior or population persistence.

In addition to the disappearance of their mangrove forest habitat, Wong and Yeap (2012) mention bright artificial lights associated with shrimp farms and tourism infrastructure as possible threats to *Pteroptyx* fireflies in Malaysia. However, Prasertkul (2018) recorded large congregations of *Pteroptyx valida* and *P. malaccae* that remained year-round in close proximity to artificial illumination from fluorescent streetlights (3–14 lux) and house lights (0.5–5 lux) in an urban park near Bangkok, Thailand. It thus appears, at least in this urban setting, that light pollution does not prevent *Pteroptyx* males from congregating. It remains unknown whether artificial light might impact mate attraction in congregating fireflies, for example by disrupting male flash synchrony or decreasing female recruitment into congregations. Even after females arrive within *Pteroptyx* display trees, the sexes continue to communicate using flash signals (Case, 1980). In apparent competitive interactions, males flash while aiming their lanterns directly toward nearby males. Before attempting copulation, males also flash while curling their lanterns directly in front of the eyes of the focal female. Additional work is needed to determine whether ALAN affects these close-range sexual interactions.

When poorly managed, tourism exposes local firefly populations to artificial light in numerous ways: distant semi-permanent sources associated with infrastructure (e.g., signage, buildings, and roads) and close-range, transient flash photography. During field observations of *Pteroptyx* spp., Lloyd (1973) reported that males were readily attracted to flashes from a small incandescent penlight, including some males that approached from up to 75 feet away. Thancharoen and Masoh (2019) studied small aggregations of *P. malaccae* brought into the laboratory to examine how tourist photography might impact courtship and oviposition. Flash illumination from smartphones and digital cameras, as well as red autofocus lights, temporarily reduced male flash activity, although most males eventually resumed flashing and successfully mounted females. Males also exhibited decreased synchrony following all types of camera illumination, suggesting that unregulated tourist photography could impede their ability to synchronize in the field. Based on these findings, recently published guidelines for sustainable firefly tourism recommend limiting flashlight and phone use and prohibiting flash photography (Lewis et al., 2021).

Predator-prey interactions

Many, but not all, fireflies have protective chemical defenses (Eisner et al., 1997; Tyler et al., 2008; Fu et al., 2009; Berger et al., 2021) that prevent attack by most predators, especially vertebrates (Day, 2011). For firefly adults, bioluminescence is a main component of a multimodal aposematic signal that is also thought to include reflectance-based red, yellow, and black warning coloration (Stevens and Ruxton, 2012), wing beat frequencies (Leavell et al., 2018), and ultrasonic clicks (Krivoruchko et al., 2021). Firefly larvae employ bioluminescence exclusively as an aposematic signal, in combination with warning coloration (De Cock and Matthysen, 2001), and likely have done so for close to 150 million years (Martin et al., 2017; Powell et al., 2022). By interfering with the detection of these reflectance- and emission-based visual signals of unpalatability, ALAN has the potential to heighten predation on firefly adults and larvae (Briolat et al., 2021).

Owens et al. (2018) exposed male *Aquatica ficta* fireflies to artificial illumination of varying spectra and intensity while recording the aposematic alarm flashes they produced in response to the constraints of the experimental apparatus. Alarmed *A. ficta* males responded to ALAN much as did advertising *P. obscurellus* males in a later study (Owens and Lewis, 2021b): their flashes became more conspicuous (brighter and longer) under short- to mid-wavelength artificial light, but were produced at only 45% of the baseline rate. Similarly, an early field study found that *L. noctiluca* and *P. hemipterus* glow-worm larvae glowed less often under bright night skies, especially when passing clouds diffused moonlight and artificial light from nearby developments into their usually dark habitats (De Cock, 2004).

For roving flashing fireflies in the genus *Photuris,* bioluminescence functions in their roles as both predator and prey (Souto et al., 2019). Firebaugh and Haynes (2019) attempted to document interactions between female *P. versicolor* predators and male *P. pyralis* prey within artificially illuminated field enclosures (175 lux). ALAN reduced the flash activity of both species, but no predation was observed in either lit or unlit field enclosures; hence the influence of ALAN on these particular predator-prey interactions remains unclear (Lewis and Owens, 2019).

Development

The effects of ALAN on earlier firefly life stages are relatively unexplored (**Table 1**). Owens and Lewis (2021a) conducted laboratory experiments to investigate how ALAN affects the development of immature *Photuris* sp. and *P. obscurellus* fireflies. Long-term exposure to artificial light at night (50 lux) did not impact overall survivorship or the duration of egg, larval, and pupal stages in either species, both of which spend the majority of their larval lifespan underground. It did however, accelerate weight gain of *Photuris* larvae, perhaps by prolonging perceived daylength – assessed before or during nightly foraging bouts on the soil surface – and thus delaying diapause (Gaston et al., 2017). Rearing aquatic *A. ficta* larvae under very dim ALAN for 2 weeks resulted in high mortality both immediately following and several months after exposure (Chen et al., 2021). Subsequent gene expression profiling of these larvae suggested that ALAN may perturb hormone regulation and suppress reproductive development.

Three recent studies report on larval movements in response to ALAN. Wanjiru Mbugua et al. (2020) found that *Lamprigera* sp. larvae foraging in an urban park could often be found near paved trails, but avoided areas illuminated by streetlights ($\geq$ 3 lux); the few larvae found directly beneath lit streetlights tended to be immobile. Similarly, *Photuris* larvae exposed in the laboratory to point sources of ALAN (800 lux) at the start of their nightly surface foraging period moved toward darker areas, but a significant number burrowed beneath the surface instead of dispersing across it (Owens and Lewis, 2021a). Murphy and Moiseff (2020) also found that uniform ALAN ($\sim$915 lux) reduced surface movements of *Photuris* larvae. Such behavioral responses may arise because sufficiently bright ALAN appears to prolong daylength, and nocturnal larvae are inactive during the "day." By immobilizing larvae, ALAN could increase their chances of being trampled in heavily trafficked areas (an already frequent occurrence: Lehtonen et al., 2021) and impede their ability to disperse into darker habitats where they might enjoy greater reproductive success as adults.

In contrast to most species, the later larval instars of *L. noctiluca* glow-worms (Tyler, 2002; Tyler, 2013) and related species (De Cock and Matthysen, 2001) regularly exhibit diurnal activity. While preparing to pupate, these "walkabout" larvae can often be found crawling over bare soil, rocks, or pavement in broad daylight (e.g., Lehtonen et al., 2021), presumably searching for pupation sites and, among females, associated display sites (Tyler, 2013). If so, individuals may not detect ALAN until they reach their relatively philopatric adult stage, which would explain why adult females can so often be found signaling unsuccessfully from brightly lit display sites (Ineichen and Rüttimann, 2012; Van den Broeck et al., 2021a). In fact, late-instar females that actively search for open habitat (Lehtonen et al., 2021) may use environmental light as a cue for habitat quality, in which case ALAN sources operating before dusk or after dawn could act as ecological traps (*sensu* Robertson et al., 2017).

Further research should reveal which, if any, particular aspects of artificial light environments (spatial, temporal, spectral, etc.) most affect fireflies of all life stages.

Future directions

Visual ecology

Because the firefly visual system is highly optimized, what a species perceives can reveal a great deal about how they might respond to artificial light. Unfortunately, little is known about the vision of most species. Electroretinography and photoreceptor gene sequencing efforts have both thus far primarily revealed the spectral sensitivities of North American (Lall et al., 2009; Sander and Hall, 2015) and Asian (Eguchi et al., 1984) roving flashing fireflies (but see Booth et al., 2004). Data on changes in overall sensitivity due to light-adaptation of the compound eye are also urgently needed to understand the mechanisms underlying the impact of artificial light on courtship and mate success (but see Lall, 1993; Oba and Kainuma, 2009; Smith, 2011). For example, it remains unknown whether glow-worm males and roving flashing firefly females fail to respond to artificially illuminated conspecifics because they are blind to their courtship signals, because their courtship signals appear less bright against the background, or because ALAN transitions fireflies from a mode of nocturnal courtship behavior to one of diurnal resting behavior through temporal disorientation.

Movement ecology

Fireflies that are negatively impacted by artificial light may be able to persist by sheltering in shaded refuges or dispersing to darker areas of the landscape. For example, species that typically occupy open habitats such as lawns, meadows, or grasslands may aggregate along forest edges or in forest clearings where their signals are more likely to be detected. Whether light-polluted populations are capable of fully transitioning to new habitat types remains unknown, as are the associated fitness costs: even if adults are relatively flexible in their habitat requirements their juvenile stages may not be. Field studies of firefly movement under artificial light are rare and almost exclusively involve single generations of largely subterranean larvae (Wanjiru Mbugua et al., 2020; Owens and Lewis, 2021a; see also Kakehashi et al., 2014) or species that are relatively resilient to ALAN (Firebaugh and Haynes, 2016; Owens and Lewis, 2022; but see Gardiner and Didham, 2021). The primary dispersing life stage (larvae, adult males or females) is also unknown for nearly every species (but see Kaufmann, 1965), despite how crucial this information is for conservation planning (Schultz et al., 2019).

In the absence of long-term survey data (see below), genetic differentiation can reflect the movement of firefly populations not just on evolutionary timescales (Suzuki et al., 2004; Lower et al., 2018; Kim et al., 2021) but also the approximately 20–200 years timescales most relevant to artificial light (e.g., Dayton and Szczys, 2021). Population genetic studies could reveal whether different species have moved toward or away from intensely light-polluted habitats over time, and whether particular sources have resulted in population sinks or barriers to gene flow, e.g., if lines of regularly spaced streetlights along roads intensify habitat fragmentation. Comparative field surveys and behavioral studies could help confirm and elaborate upon these results. For example, female *L. noctiluca* glow-worms are significantly less abundant near artificial lights (Gardiner and Didham, 2021) but marked individuals rarely leave artificially illuminated display sites either in the field or the laboratory (Elgert et al., 2020; Van den Broeck et al., 2021a), meaning that the current distribution may be symptomatic of a population sink.

Nocturnal insects such as moths, mayflies, and gnats can be maladaptively attracted to artificial lights (Haynes and Robertson, 2021). While male glow-worms have repeatedly been shown to approach artificial lights that are sufficiently dim and long-wavelength so as to resemble female conspecifics (Schwalb, 1961; Booth et al., 2004; Bek, 2015; Pacheco et al., 2016), fireflies are not commonly thought to be attracted to the broad-spectrum artificial lights used for public safety, unless they somehow simulate conspecific courtship signals (i.e., flash patterns). Surprisingly, however, one recent study captured significant numbers of *P. pyralis* and *Photuris* fireflies at non-flashing broad-spectrum light traps (Firebaugh and Haynes, 2019). Because species that are attracted to or simply not repelled by ALAN could be at significantly greater risk of courtship disruption and subsequent population decline, broader understanding of the phototactic tendencies of fireflies should be a research priority.

Evolutionary adaptation

Few studies have investigated whether fireflies may be evolving physiologically or behaviorally in response to artificial light, a novel selection pressure (Hopkins et al., 2018). Several possibilities merit further investigation.

The amplification of intensity-based sexual selection in light-polluted habitats may result in firefly populations that emit more intense bioluminescent courtship signals, possibly at negligible metabolic cost (Woods et al., 2007). At higher latitudes, *L. noctiluca* females possess larger light organs that emit brighter glows, presumably to attract males during the relatively short and bright summer nights (Borshagovski et al., 2020; compare to Owens et al., 2018). Simulated conspecific courtship advertisements of greater intensity also garner more frequent responses from *P. pyralis* females (Vencl and Carlson, 1998) and attract more *L. noctiluca* males, both in darkness (Hopkins et al., 2015) and under artificial light (Elgert et al., 2021a). Evolutionary adaptations that increase the conspicuousness of bioluminescent signals in order to preserve lines of visual communication may be augmented *via* the addition of more filter pigments in the compound eye that further narrow long-wavelength sensitivity to the peak wavelength of conspecific bioluminescence.

Alternatively, firefly populations may shift away from a primarily visual communication system toward greater reliance on chemical signals. Pheromones are broadly used as sexual signals by diurnal dark fireflies (Stanger-Hall et al., 2018), and also contribute to the multimodal courtship signals of some nocturnal glow-worms (Ohba, 2004); their use by other firefly species has yet to be fully investigated. Chemical signals should be relatively unaffected by ALAN and therefore may more reliably attract conspecifics within severely light-polluted habitats. Comparisons of multiple urban and rural populations of the same firefly species (e.g., Santangelo et al., 2022) can disentangle the conflicting possibilities described above to reveal whether artificial light promotes or inhibits bioluminescent signaling behavior. If consistent differences are observed, common garden experiments will be able to reveal whether these differences are the result of phenotypic plasticity or rapid evolution. Artificial selection on species that can be reared in the laboratory could then help elucidate the timescales on which such differences are likely to arise and quantify their benefits for reproductive fitness.

A third, related possibility is that some firefly species are already relatively resilient to artificial light due to favorable life history traits (e.g., flightedness, lack of habitat specificity, diurnal or crepuscular temporal niche, use of multimodal courtship signals, asynchronicity, etc.), and are gradually replacing less resilient species within severely light-polluted habitats (see Khattar et al., 2022). For example, *P. pyralis*, the most widespread roving flashing firefly in North America, has a crepuscular courtship period and appears to be relatively unaffected by artificial light (Firebaugh and Haynes, 2016; Owens and Lewis, 2022); *P. pyralis* and other crepuscular species may even be able to capitalize on resultant expansions in their temporal or photic niche, while fully nocturnal species are left at a disadvantage. Although it can be difficult to track the abundances of individual firefly species within a community due to the skill required for species identification (e.g., Lloyd, 2017), long-term expert surveys may be able to document changes in species composition over time and by doing so help predict the future for firefly populations on an increasingly light-polluted planet.

Conservation recommendations

Clearly much remains to be done. Although long-term survey data are sparse, both anecdotal reports and expert opinion suggest certain firefly populations have recently undergone population declines (Jusoh and Hashim, 2012;

Khoo et al., 2016; Atkins et al., 2017; Faust, 2017; Lloyd, 2017; Gardiner and Didham, 2020; Lewis et al., 2020; Fallon et al., 2021; see also Chatragadda, 2020). A survey of international firefly experts ranked ALAN among the top three threats to fireflies in nearly every geographic region (Lewis et al., 2020). Even in the absence of evidence demonstrating that ALAN is a direct causal factor in firefly declines (but see Cornelisse et al., 2019), the myriad negative impacts of ALAN reviewed here suggest the urgent need for conservation action, especially as the extent and intensity of artificial light continues to grow (Vaz et al., 2021). Many straightforward measures have already been proposed to address the general catastrophe of insect declines (Harvey et al., 2020), and fireflies can act as flagship species to galvanize these efforts. We already know enough to recommend several high priority, no-regret conservation actions for keeping their magic alive, including:

- Prevent habitat degradation by removing or limiting ALAN within protected areas and other locations that support at-risk firefly species.
- Launch education and outreach programs to promote locally appropriate firefly-friendly lighting guidelines (see below).
- Establish standardized monitoring programs to track long-term population trends for fireflies occurring along an ALAN gradient.

Targeted community science monitoring programs (e.g., the UK Glow-worm Survey, Observatoire des vers luisants et lucioles, Night Lights of Kuala Selangor Nature Park, Mass Audubon Firefly Watch, etc.) can be of immense value in identifying firefly populations on the decline. These programs should be continued, expanded, and where possible unified into a single standardized platform that supports species identification (by trained observers or machine-learning algorithms, e.g., Goh et al., 2022). The responses of fireflies to ALAN are highly species-specific, and measures of general firefly activity without accompanying species information can be compromised by changes in species composition. Similarly, firefly surveys should aim to count individuals instead of flashes or glow signals, due to the known effects of ALAN on courtship signaling behavior.

The studies we have reviewed here also provide the basis for more specific lighting recommendations. The simplest and most effective conservation measure is to switch off any lights situated in and around known firefly and glow-worm habitats during mating seasons. On low-traffic roadways, motion sensors that switch off streetlights when vehicles are absent can offer an efficient conservation solution. Where removing artificial lights is not possible for safety reasons, proper shielding should be installed on streetlights to limit light spillage into firefly and glow-worm habitat. Given that extremely low levels of artificial light reduce mating success of *L. noctiluca* glow-worms (Van den Broeck et al., 2021b), simply dimming streetlights may not suffice to mitigate ALAN impact as it does for some other taxa. Gardiner and Didham (2021) recommend a minimum distance of 100 m between artificial light and glow-worm habitat. Vaz et al. (2021) point out the importance of establishing new protected areas within the darkest places where at-risk fireflies are found, as well as reducing ALAN within existing protected areas. Unfortunately, evidence suggests that no spectrum of artificial light is universally "firefly friendly" (Owens et al., 2018, 2022; Owens and Lewis, 2021b). However, red light is preferable to other colors as it is much less visible to insects generally (van der Kooi et al., 2020); green, yellow, and amber light should be avoided because they are likely to be especially disruptive due to their spectral overlap with firefly bioluminescence.

Author contributions

AO: conceptualization, writing – original draft, review and editing, and visualization. MV: writing – original draft, review and editing, and visualization. RD: writing – original draft, review and editing, and supervision. SL: conceptualization, writing – original draft, review and editing, and supervision. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by a 2021–2022 PEO Scholar Award to AO and a FWO Flanders Ph.D. fellowship (Grant ID: 1143322N) to MV.

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

References

Atkins, V., Bell, D., Bowker, A., Charig, M., Crew, J., Dale, M., et al. (2017). The status of the glow-worm *Lampyris noctiluca* L. in England. *Lampyrid* 4, 20–35.

Baldaccini, N. E., Fiaschi, V., and Papi, F. (1969). Rhythmic synchronous flashing in a Bosnian firefly. *Ital. J. Zool.* 3, 239–245.

Ballantyne, L. A., and Lambkin, C. L. (2013). Systematics and phylogenetics of Indo-Pacific Luciolinae fireflies (Coleoptera: Lampyridae) and the description of new genera. *Zootaxa* 3653, 1–162. doi: 10.11646/zootaxa.3653.1.1

Baudry, G., Hopkins, J., Watts, P. C., and Kaitala, A. (2021). Female sexual signaling in a capital breeder, the European glow-worm *Lampyris noctiluca*. *J. Insect Behav.* 34, 16–25. doi: 10.1007/s10905-020-09763-9

Bek, R. J. (2015). *Investigating the Impact of Artificial Night Lighting on the Common European Glow-Worm*. Leeds: University of Leeds.

Berger, A., Petschenka, G., Degenkolb, T., Geisthardt, M., and Vilcinskas, A. (2021). Insect collections as an untapped source of bioactive compounds-Fireflies and cardiotonic steroids as a proof of concept. *Insects* 12:689. doi: 10.3390/insects12080689

Bird, S., and Parker, J. (2014). Low levels of light pollution may block the ability of male glow-worms to locate females. *J. Insect Conserv.* 18, 737–743. doi: 10.1007/s10841-014-9664-2

Booth, D., Stewart, A. J. A., and Osorio, D. (2004). Colour vision in the glow-worm *Lampyris noctiluca*: Evidence for a green-blue chromatic mechanism. *J. Exp. Biol.* 207, 2373–2378. doi: 10.1242/jeb.01044

Borshagovski, A.-M., Saari, P., Lehtonen, T. K., and Kaitala, A. (2020). When night never falls: Female sexual signalling in a nocturnal insect along a latitudinal gradient. *Behav. Ecol. Sociobiol.* 74:153. doi: 10.1007/s00265-020-02927-9

Branham, M. A., and Faust, L. F. (2019). Firefly flashing activity during the totality phase of a solar eclipse. *Entomol. News* 128:191. doi: 10.3157/021.128.0207

Branham, M. A., and Wenzel, J. W. (2003). The origin of photic behavior and the evolution of sexual communication in fireflies (Coleoptera: Lampyridae). *Cladistics* 19, 1–22. doi: 10.1111/j.1096-0031.2003.tb00404.x

Briolat, E. S., Gaston, K. J., Bennie, J., Rosenfeld, E. J., and Troscianko, J. (2021). Artificial nighttime lighting impacts visual ecology links between flowers, pollinators and predators. *Nat. Commun.* 12:4163. doi: 10.1038/s41467-021-24394-0

Buck, J. B. (1937). Studies on the firefly. II. The signal system and color vision in *Photinus pyralis*. *Physiol. Zool.* 10, 412–419. doi: 10.1086/physzool.10.4.30151427

Case, J. F. (1980). Courting behavior in a synchronously flashing, aggregative firefly, *Pteroptyx tener*. *Biol. Bull.* 159, 613–625. doi: 10.2307/1540827

Cashmore, A. R., Jarillo, J. A., Wu, Y. J., and Liu, D. (1999). Cryptochromes: Blue light receptors for plants and animals. *Science* 284, 760–765. doi: 10.1126/science.284.5415.760

Chatragadda, R. (2020). Decline of luminous firefly Abscondita chinensis population in Barrankula, Andhra Pradesh, India. *Int. J. Trop. Insect Sci.* 40, 461–465. doi: 10.1007/s42690-019-00078-7

Chen, Y.-R., Wei, W.-L., Tzeng, D. T. W., Owens, A. C. S., Tang, H.-C., Wu, C.-S., et al. (2021). Effects of artificial light at night (ALAN) on gene expression of Aquatica ficta firefly larvae. *Environ. Pollut.* 281:116944. doi: 10.1016/j.envpol.2021.116944

Cheng, S., Faidi, M. A., Tan, S.-A., Vijayanathan, J., Malek, M. A., Bahashim, B., et al. (2021). Fireflies in Southeast Asia: Knowledge gaps, entomotourism and conservation. *Biodivers. Conserv.* 30, 925–944. doi: 10.1007/s10531-021-02129-3

Cornelisse, T., Jepsen, S., Fallon, C., and Tyler, J. (2019). *Petition for emergency listing of the Bethany Beach Firefly (Photuris bethaniensis) under the Endangered Species Act and to Concurrently Designate Critical Habitat.* Portland, OR: Xerces Society.

Costin, K. J., and Boulton, A. M. (2016). A field experiment on the effect of introduced light pollution on fireflies in the Piedmont Region of Maryland. *Coleopt. Bull.* 70, 84–86. doi: 10.1649/072.070.0110

Cratsley, C., Prasertkul, T., and Thancharoen, A. (2012). Male aggregation behaviour in the fireflies Photinus ignitus and *Pteroptyx malaccae*. *Lampyrid* 2, 87–98.

Cronin, T. W., Järvilehto, M., Weckström, M., and Lall, A. B. (2000). Tuning of photoreceptor spectral sensitivity in fireflies. *J. Comp. Physiol. A* 186, 1–12. doi: 10.1007/s003590050001

da Silveira, L. F. L., and Mermudes, J. R. M. (2014). Systematic review of the firefly genus Amydetes Illiger, 1807 (Coleoptera: Lampyridae), with description of 13 new species. *Zootaxa* 3765, 201–248. doi: 10.11646/zootaxa.3765.3.1

Day, J. C. (2011). Parasites, predators and defence of fireflies and glow-worms. *Lampyrid* 1, 70–102.

Dayton, J., and Szczys, P. (2021). Metapopulation connectivity retains genetic diversity following a historical bottleneck in a federally endangered seabird. *Ornithol. Appl.* 123:duab037. doi: 10.1093/ornithapp/duab037

De Cock, R. (2004). *The Adaptive Value of Luminescent Behaviour in Glow-Worms (Coleoptera: Lampyridae).* Ann Arbor, MI: ProQuest Dissertations Publishing.

De Cock, R. (2009). "Biology and behaviour of European lampyrids," in *Bioluminescence in Focus—A Collection of Illuminating Essays*, ed. V. B. Meyer-Rochow (Kerala: Research Signpost), 161–200.

De Cock, R., Faust, L., and Lewis, S. (2014). Courtship and mating in *Phausis reticulata*: Male flight behaviors, female glow displays, and male attraction to light traps. *Fla. Entomol.* 97, 1290–1307. doi: 10.1653/024.097.0404

De Cock, R., and Matthysen, E. (2001). Do glow-worm larvae use warning coloration? *Ethology* 107, 1019–1033. doi: 10.1046/j.1439-0310.2001.00746.x

De Cock, R., and Matthysen, E. (2005). Sexual communication by pheromones in a firefly. *Phosphaenus hemipterus* (Coleoptera: Lampyridae). *Anim. Behav.* 70, 807–818. doi: 10.1016/j.anbehav.2005.01.011

Demary, K., Michaelidis, C. I., and Lewis, S. M. (2005). Firefly courtship: Behavioral and morphological predictors of male mating success in *Photinus greeni*. *Ethology* 112, 485–492. doi: 10.1111/j.1439-0310.2005.01176.x

Dreisig, H. (1978). The circadian rhythm of bioluminescence in the glowworm, *Lampyris noctiluca* L. *Behav. Ecol. Sociobiol.* 3, 1–18. doi: 10.1007/BF00300044

Eguchi, E., Nemoto, A., Meyer-Rochow, V. B., and Ohba, N. (1984). A comparative study of spectral sensitivity curves in three diurnal and eight nocturnal species of Japanese fireflies. *J. Insect Physiol.* 30, 607–612. doi: 10.1016/0022-1910(84)90044-1

Eisner, T., Goetz, M. A., Hill, D. E., Smedley, S. R., and Meinwald, J. (1997). Firefly "femmes fatales" acquire defensive steroids (lucibufagins) from their firefly prey. *Proc. Natl. Acad. Sci. U.S.A.* 94, 9723–9728. doi: 10.1073/pnas.94.18.9723

Elgert, C., Hopkins, J., Kaitala, A., and Candolin, U. (2020). Reproduction under light pollution: Maladaptive response to spatial variation in artificial light in a glow-worm. *Proc. R. Soc. B* 287:20200806. doi: 10.1098/rspb.2020.0806

Elgert, C., Lehtonen, T. K., Kaitala, A., and Candolin, U. (2021a). Sexual selection for bright females prevails under light pollution. *Curr. Zool.* 67, 329–331. doi: 10.1093/cz/zoaa071

Elgert, C., Lehtonen, T. K., Kaitala, A., and Candolin, U. (2021b). The duration of artificial light defines sexual signalling in the common glow-worm. *Behav. Ecol. Sociobiol.* 75:154. doi: 10.1007/s00265-021-03093-2

Elvidge, C. D., Keith, D. M., Tuttle, B. T., and Baugh, K. E. (2010). Spectral identification of lighting type and character. *Sensors* 10, 3961–3988. doi: 10.3390/s100403961

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C. M., Elvidge, C. D., Baugh, K., et al. (2016). The new world atlas of artificial night sky brightness. *Sci Adv* 2:e1600377. doi: 10.1126/sciadv.1600377

Fallon, C. E., Walker, A. C., Lewis, S., Cicero, J., Faust, L., Heckscher, C. M., et al. (2021). Evaluating firefly extinction risk: Initial red list assessments for North America. *PLoS One* 16:e0259379. doi: 10.1371/journal.pone.0259379

Faust, L. (2012). Fireflies in the snow: Observations on two early-season arboreal fireflies Ellychnia corrusca and Pyractomena borealis. *Lampyrid* 2, 48–71.

Faust, L. F. (2017). *Fireflies, Glow-worms, and Lightning Bugs*. Athens: University of Georgia Press.

Firebaugh, A., and Haynes, K. J. (2016). Experimental tests of light-pollution impacts on nocturnal insect courtship and dispersal. *Oecologia* 182, 1203–1211. doi: 10.1007/s00442-016-3723-1

Firebaugh, A., and Haynes, K. J. (2019). Light pollution may create demographic traps for nocturnal insects. *Basic Appl. Ecol.* 34, 118–125. doi: 10.1016/j.baae.2018.07.005

Fu, X., Benno Meyer-Rochow, V., Tyler, J., Suzuki, H., and De Cock, R. (2009). Structure and function of the eversible organs of several genera of larval firefly. *Chemoecology* 19, 155–168. doi: 10.1007/s00049-009-0016-8

Fu, X. H., and Ballantyne, L. (2008). Taxonomy and behaviour of lucioline fireflies (Coleoptera: Lampyridae: Luciolinae) with redefinition and new species of Pygoluciola Wittmer from mainland China and review of Luciola LaPorte. *Zootaxa* 1733:1. doi: 10.11646/zootaxa.1733.1.1

Gardiner, T., and Didham, R. K. (2020). Glowing, glowing, gone? Monitoring long-term trends in glow-worm numbers in south-east England. *Insect Conserv. Divers.* 13, 162–174. doi: 10.1111/icad.12407

Gardiner, T., and Didham, R. K. (2021). Glow-worm abundance declines with increasing proximity to artificial lighting. *Entomol. Gaz.* 72, 1–11. doi: 10.31184/G00138894.721.1798

Gaston, K. J., Davies, T. W., Nedelec, S. L., and Holt, L. A. (2017). Impacts of artificial light at night on biological timings. *Annu. Rev. Ecol. Evol. Syst.* 48, 49–68. doi: 10.1146/annurev-ecolsys-110316-022745

Goh, K.-S., Lee, C.-M., and Wang, T.-Y. (2022). Species-specific flash patterns track the nocturnal behavior of sympatric Taiwanese fireflies. *Biology* 11:58. doi: 10.3390/biology11010058

Gunn, P., and Gunn, B. (2012). Lunar effects on the bioluminescent activity of the glow-worm *Lampyris noctiluca* and its larvae. *Lampyrid* 3, 1–16.

Hagen, O., Santos, R. M., Schlindwein, M. N., and Viviani, V. R. (2015). Artificial night lighting reduces firefly occurrence in Sorocaba, Brazil. *Adv. Entomol.* 03, 24–32. doi: 10.4236/ae.2015.31004

Hall, D. W., Sander, S. E., Pallansch, J. C., and Stanger-Hall, K. F. (2016). The evolution of adult light emission color in North American fireflies. *Evolution* 70, 2033–2048. doi: 10.1111/evo.13002

Hariyama, T. (2000). The brain as a photoreceptor: Intracerebral ocelli in the firefly. *Naturwissenschaften* 87, 327–330. doi: 10.1007/s001140050732

Harvey, J. A., Heinen, R., Armbrecht, I., Basset, Y., Baxter-Gilbert, J. H., Bezemer, T. M., et al. (2020). International scientists formulate a roadmap for insect conservation and recovery. *Nat. Ecol. Evol.* 4, 174–176. doi: 10.1038/s41559-019-1079-8

Haynes, K. J., and Robertson, B. A. (2021). A transdisciplinary research agenda for understanding insect responses to ecological light pollution informed by evolutionary trap theory. *Curr. Opin. Insect. Sci.* 45, 91–96. doi: 10.1016/j.cois.2021.02.004

Hopkins, G. R., Gaston, K. J., Visser, M. E., Elgar, M. A., and Jones, T. M. (2018). Artificial light at night as a driver of evolution across urban-rural landscapes. *Front. Ecol. Environ.* 114:8951. doi: 10.1002/fee.1828

Hopkins, J., Baudry, G., Candolin, U., and Kaitala, A. (2015). I'm sexy and I glow it: Female ornamentation in a nocturnal capital breeder. *Biol. Lett.* 11:20150599. doi: 10.1098/rsbl.2015.0599

Hopkins, J., Lehtonen, T. K., Baudry, G., and Kaitala, A. (2021). Costly mating delays drive female ornamentation in a capital breeder. *Ecol. Evol.* 11, 8863–8868. doi: 10.1002/ece3.7719

Horne, J., Horne, A., and Tyler, J. (2017). The influence of female body weight on egg production in the glow-worm *Lampyris noctiluca* (Coleoptera: Lampyridae). *Lampyrid* 4, 36–39.

Ineichen, S., and Rüttimann, B. (2012). Impact of artificial light on the distribution of the common European glow-worm, *Lampyris noctiluca*. *Lampyrid* 2, 31–36.

Jaikla, S., Lewis, S. M., Thancharoen, A., and Pinkaew, N. (2020a). Distribution, abundance, and habitat characteristics of the congregating firefly, Pteroptyx Olivier in Thailand. *J. Asia-Pacific Biodivers.* 13, 358–366. doi: 10.1016/j.japb.2020.06.002

Jaikla, S., Thancharoen, A., and Pinkaew, N. (2020b). Biology and rearing technique for the mangrove firefly, Pteroptyx valida, with discussion of additional instar in female. *J. Asia-Pacific Biodivers.* 13, 367–371. doi: 10.1016/j.japb.2020.05.002

Johnsen, S., Widder, E. A., and Mobley, C. D. (2004). Propagation and perception of bioluminescence: Factors affecting counterillumination as cryptic. *Biol. Bull.* 207, 1–16. doi: 10.2307/1543624

Jusoh, W. F. A., Ballantyne, L., Lambkin, C. L., Hashim, N. R., and Wahlberg, N. (2018). The firefly genus Pteroptyx Olivier revisited (Coleoptera: Lampyridae: Luciolinae). *Zootaxa* 4456, 1–71. doi: 10.11646/zootaxa.4456.1.1

Jusoh, W. F. A. W., and Hashim, N. R. (2012). The effect of habitat modification on firefly populations at the Rembau-Linggi estuary, Peninsular Malaysia. *Lampyrid* 2, 149–155.

Kakehashi, K., Kuranishi, R. B., and Kamata, N. (2014). Estimation of dispersal ability responding to environmental conditions: Larval dispersal of the flightless firefly, *Luciola parvula*. *Ecol. Res.* 29, 779–787. doi: 10.1007/s11284-014-1156-z

Kaufmann, T. (1965). Ecological and biological studies on the West African firefly *Luciola discicollis* (Coleoptera: Lampyridae). *Ann. Entomol. Soc. Am.* 58, 414–426. doi: 10.1093/aesa/58.4.414

Khattar, G., Vaz, S., Pereira Braga, P. H., Macedo, M., and da Silveira, L. F. L. (2022). Life history traits modulate the influence of environmental stressors on biodiversity: The case of fireflies, climate and artificial light at night. *Divers. Distrib.* 0, 1–12. doi: 10.1111/ddi.13584

Khoo, V., Kirton, L. G., and Nada, B. (2016). Firefly conservation: Monitoring the synchronous fireflies of the Selangor River in Malaysia. *Antenna* 40, 46–51.

Kim, T.-S., Kwon, K., and Jang, G.-S. (2021). Variations in the distribution and genetic relationships among Luciola unmunsana populations in South Korea. *Land* 10:730. doi: 10.3390/land10070730

Kivelä, L. (2022). *The Effect of Artificial Light Spectrum on Mate Attraction in the Common Glow-Worm, Lampyris noctiluca*. Helsinki: University of Helsinki.

Kok, P. J. R., van Doorn, L., and Dezfoulian, R. (2019). Predation by non-bioluminescent firefly larvae on a tepui-summit endemic toad. *Curr. Biol.* 29, R1170–R1171. doi: 10.1016/j.cub.2019.10.001

Krivoruchko, K., Goldshtein, A., Boonman, A., Eitan, O., Ben-Simon, J., Thong, V. D., et al. (2021). Fireflies produce ultrasonic clicks during flight as a potential aposematic anti-bat signal. *iScience* 24:102194. doi: 10.1016/j.isci.2021.102194

Kyba, C. C. M., Mohar, A., and Posch, T. (2017). How bright is moonlight? *A&G* 58, 31–31. doi: 10.1093/astrogeo/atx025

Kyba, C. C. M., Tong, K. P., Bennie, J., Birriel, I., Birriel, J. J., Cool, A., et al. (2015). Worldwide variations in artificial skyglow. *Sci. Rep.* 5:8409. doi: 10.1038/srep08409

Lall, A. B. (1981). Electroretinogram and the spectral sensitivity of the compound eyes in the firefly *Photuris versicolor*: A correspondence between green sensitivity and species bioluminescence emission. *J. Insect Physiol.* 27, 461–468. doi: 10.1016/0022-1910(81)90097-4

Lall, A. B. (1993). Nightly increase in visual sensitivity correlated with bioluminescent flashing activity in the firefly *Photuris versicolor*. *J. Exp. Zool.* 265, 609–612. doi: 10.1002/jez.1402650518

Lall, A. B., Chapman, R. M., Ovid Trouth, C., and Holloway, J. A. (1980a). Spectral mechanisms of the compound eye in the firefly *Photinus pyralis*. *J. Comp. Physiol.* 135, 21–27. doi: 10.1007/BF00660178

Lall, A. B., Seliger, H. H., Biggley, W. H., and Lloyd, J. E. (1980b). Ecology of colors of firefly bioluminescence. *Science* 210, 560–562. doi: 10.1126/science.210.4469.560

Lall, A. B., Cronin, T. W., Bechara, E. J. H., Costa, C., and Viviana, V. R. (2009). "Visual ecology of bioluminescent beetles: Visual spectral mechanisms and the colors of optical signaling in Coleoptera, Elateroidea: Lampyridae, Elateridae and Phengodidae," in *Bioluminescence in Focus - A Collection of Illuminating Essays*, Ed. Meyer-Rochow, V. B. (Kerala: Research Signpost).

Leavell, B. C., Rubin, J. J., McClure, C. J. W., Miner, K. A., Branham, M. A., and Barber, J. R. (2018). Fireflies thwart bat attack with multisensory warnings. *Sci. Adv.* 4:eaat6601. doi: 10.1126/sciadv.aat6601

Lehtonen, T. K., Babic, N. L., Piepponen, T., Valkeeniemi, O., Borshagovski, A.-M., and Kaitala, A. (2021). High road mortality during female-biased larval dispersal in an iconic beetle. *Behav. Ecol. Sociobiol.* 75:26. doi: 10.1007/s00265-020-02962-6

Lewis, S. M. (2009). "Bioluminescence and sexual signaling in fireflies," in *Bioluminescence in Focus: A Collection of Illuminating Essays*, ed. V. B. Meyer-Rochow (Kerala: Research Signpost).

Lewis, S. M., and Cratsley, C. K. (2008). Flash signal evolution, mate choice, and predation in fireflies. *Annu. Rev. Entomol.* 53, 293–321. doi: 10.1146/annurev.ento.53.103106.093346

Lewis, S. M., Faust, L., and De Cock, R. (2011). The dark side of the light show: Predators of fireflies in the Great Smoky Mountains. *Psyche* 2012:634027. doi: 10.1155/2012/634027

Lewis, S. M., and Owens, A. C. S. (2019). A critique of Firebaugh & Haynes (2018) - Light pollution may create demographic traps for nocturnal insects. *Basic Appl. Ecol.* 35, 65–66. doi: 10.1016/j.baae.2019.01.002

Lewis, S. M., Thancharoen, A., Wong, C. H., López-Palafox, T., Santos, P. V., Wu, C., et al. (2021). Firefly tourism: Advancing a global phenomenon toward a brighter future. *Conservat. Sci. Prac.* 3:e391. doi: 10.1111/csp2.391

Lewis, S. M., Wong, C. H., Owens, A. C. S., Fallon, C., Jepsen, S., Thancharoen, A., et al. (2020). A global perspective on firefly extinction threats. *BioScience* 70, 157–167. doi: 10.1093/biosci/biz157

Lloyd, J. E. (1972). Chemical communication in fireflies. *Environ. Entomol.* 1, 265–266. doi: 10.1093/ee/1.2.265

Lloyd, J. E. (1973). Model for the mating protocol of synchronously flashing fireflies. *Nature* 245, 268–270. doi: 10.1038/245268a0

Lloyd, J. E. (1980). Male *Photuris* fireflies mimic sexual signals of their females' prey. *Science* 210, 669–671. doi: 10.1126/science.210.4470.669

Lloyd, J. E. (2008). "Fireflies," in *Encyclopedia of Entomology*, ed. J. L. Capinera (New York, NY: Springer), 1429–1452.

Lloyd, J. E. (2017). *A Naturalist's Long Walk Among Shadows*. Gainesville, FL: Self published.

Lloyd, J. E., and Wing, S. R. (1983). Nocturnal aerial predation of fireflies by light-seeking fireflies. *Science* 222, 634–635. doi: 10.1126/science.222.4624.634

Lower, S. E., Stanger-Hall, K. F., and Hall, D. W. (2018). Molecular variation across populations of a widespread North American firefly, *Photinus pyralis*, reveals that coding changes do not underlie flash color variation or associated visual sensitivity. *BMC Evol. Biol.* 18:129. doi: 10.1186/s12862-018-1251-9

Luginbuhl, C. B., Lockwood, G. W., Davis, D. R., Pick, K., and Selders, J. (2009). From the ground up I: Light pollution sources in flagstaff. *Arizona. Publ. Astro. Soc. Pac.* 121, 185–203. doi: 10.1086/597625

Martin, G. J., Branham, M. A., Whiting, M. F., and Bybee, S. M. (2017). Total evidence phylogeny and the evolution of adult bioluminescence in fireflies. *Mol. Phylogenet. Evol.* 107, 564–575. doi: 10.1016/j.ympev.2016.12.017

Martin, G. J., Stanger-Hall, K. F., Branham, M. A., Da Silveira, L. F. L., Lower, S. E., Hall, D. W., et al. (2019). Higher-Level phylogeny and reclassification of Lampyridae (Coleoptera: Elateroidea). *Insect. Syst. Divers.* 3:11. doi: 10.1093/isd/ixz024

Murphy, F., and Moiseff, A. (2019). Anatomy of the stemmata in the *Photuris* firefly larva. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 205, 151–161. doi: 10.1007/s00359-018-01312-2

Murphy, F., and Moiseff, A. (2020). Ambient illumination influence on *Photuris* firefly larval surface movements is not mediated by the stemmata. *J. Insect Behav.* 33, 30–37. doi: 10.1007/s10905-020-09743-z

Oba, Y., and Kainuma, T. (2009). Diel changes in the expression of long wavelength-sensitive and ultraviolet-sensitive opsin genes in the Japanese firefly, *Luciola cruciata*. *Gene* 436, 66–70. doi: 10.1016/j.gene.2009.02.001

Oba, Y., and Schultz, D. T. (2014). Eco-evo bioluminescence on land and in the sea. *Adv. Biochem. Eng. Biotechnol.* 144, 3–36. doi: 10.1007/978-3-662-43385-0_1

Ohba, N. (2004). Flash communication systems of Japanese fireflies. *Integr. Comp. Biol.* 44, 225–233. doi: 10.1093/icb/44.3.225

Owens, A. C. S., Cochard, P., Durrant, J., Farnworth, B., Perkin, E. K., and Seymoure, B. (2020). Light pollution is a driver of insect declines. *Biol. Conserv.* 241:108259. doi: 10.1016/j.biocon.2019.108259

Owens, A. C. S., Dressler, C., and Lewis, S. M. (2022). Costs and benefits of "insect friendly" artificial lights are taxon specific. *Oecologia* 199, 487–497. doi: 10.1007/s00442-022-05189-6

Owens, A. C. S., and Lewis, S. M. (2018). The impact of artificial light at night on nocturnal insects: A review and synthesis. *Ecol. Evol.* 8, 11337–11358. doi: 10.1002/ece3.4557

Owens, A. C. S., and Lewis, S. M. (2021a). Effects of artificial light on growth, development, and dispersal of two North American fireflies. *J. Insect Physiol.* 130:104200. doi: 10.1016/j.jinsphys.2021.104200

Owens, A. C. S., and Lewis, S. M. (2021b). Narrow-spectrum artificial light silences female fireflies. *Insect Conserv. Divers.* 14, 199–210. doi: 10.1111/icad.12487

Owens, A. C. S., and Lewis, S. M. (2022). Artificial light impacts the mate success of female fireflies. *R. Soc. Open Sci.* 9:220468. doi: 10.1098/rsos.220468

Owens, A. C. S., Meyer-Rochow, V. B., and Yang, E.-C. (2018). Short- and mid-wavelength artificial light influences the flash signals of Aquatica ficta fireflies. *PLoS One* 13:e0191576. doi: 10.1371/journal.pone.0191576

Pacheco, Y. M., Martin, G. J., and Bybee, S. M. (2016). On the phototactic response of Rwandan Diaphanes Motschulsky to a trap with a 630 nm red light. *Coleopt. Bull.* 70, 559–561. doi: 10.1649/0010-065X-70.3.559

Powell, G. S., Saxton, N. A., Pacheco, Y. M., Stanger-Hall, K. F., Martin, G. J., Kusy, D., et al. (2022). Beetle bioluminescence outshines extant aerial predators. *Proc. R. Soc. B.* 289:20220821. doi: 10.1098/rspb.2022.0821

Prasertkul, T. (2018). Characteristics of Pteroptyx firefly congregations in a human dominated habitat. *J. Insect Behav.* 31, 436–457. doi: 10.1007/s10905-018-9687-8

Robertson, B. A., Campbell, D.-R., Durovich, C., Hetterich, I., Les, J., and Horváth, G. (2017). The interface of ecological novelty and behavioral context in the formation of ecological traps. *Behav. Ecol.* 28, 1166–1175. doi: 10.1093/beheco/arx081

Sánchez de Miguel, A., Bennie, J., Rosenfeld, E., Dzurjak, S., and Gaston, K. J. (2021). First estimation of global trends in nocturnal power emissions reveals acceleration of light pollution. *Remote Sens.* 13:3311. doi: 10.3390/rs13163311

Sander, S. E., and Hall, D. W. (2015). Variation in opsin genes correlates with signalling ecology in North American fireflies. *Mol. Ecol.* 24, 4679–4696. doi: 10.1111/mec.13346

Santangelo, J. S., Ness, R. W., Cohan, B., Fitzpatrick, C. R., Innes, S. G., Koch, S., et al. (2022). Global urban environmental change drives adaptation in white clover. *Science* 375, 1275–1281. doi: 10.1126/science.abk0989

Sarfati, R., Gaudette, L., Cicero, J. M., and Peleg, O. (2022). Statistical analysis reveals the onset of synchrony in sparse swarms of *Photinus knulli* fireflies. *J. R. Soc. Interface* 19:20220007. doi: 10.1098/rsif.2022.0007

Sarfati, R., Hayes, J. C., and Peleg, O. (2021). Self-organization in natural swarms of *Photinus carolinus* synchronous fireflies. *Sci. Adv.* 7:eabg9259. doi: 10.1126/sciadv.abg9259

Schultz, C. B., Haddad, N. M., Henry, E. H., and Crone, E. E. (2019). Movement and demography of at-risk butterflies: Building blocks for conservation. *Annu. Rev. Entomol.* 64, 167–184. doi: 10.1146/annurev-ento-011118-112204

Schwalb, H. H. (1961). Contributions to the biology of the domestic lampyridae *Lampyris noctiluca* and Phausis splendidula and experimental analysis of their predatory and sexual behavior. *Zoologiscbee Jahrbuch* 88, 99–550.

Sharkey, C. R., Fujimoto, M. S., Lord, N. P., Shin, S., McKenna, D. D., Suvorov, A., et al. (2017). Overcoming the loss of blue sensitivity through opsin duplication in the largest animal group, beetles. *Sci. Rep.* 7:8. doi: 10.1038/s41598-017-00061-7

Smith, S. R. (2011). *Changes in visual sensitivity as a function of bioluminescent flashing activity period in firefly species*. Washington, DC: Howard University.

Souto, P. M., Campello, L., Khattar, G., Miras Mermudes, J. R., Monteiro, R. F., and Lima da Silveira, L. F. (2019). How to design a predatory firefly? Lessons from the Photurinae. *Zool. Anz.* 278, 1–13. doi: 10.1016/j.jcz.2018.10.006

Stanger-Hall, K. F., and Lloyd, J. E. (2015). Flash signal evolution in Photinus fireflies: Character displacement and signal exploitation in a visual communication system. *Evolution* 69, 666–682. doi: 10.1111/evo.12606

Stanger-Hall, K. F., Sander Lower, S. E., Lindberg, L., Hopkins, A., Pallansch, J., and Hall, D. W. (2018). The evolution of sexual signal modes and associated sensor morphology in fireflies. *Proc. R. Soc. B* 285:20172384. doi: 10.1098/rspb.2017.2384

Stevens, M., and Ruxton, G. D. (2012). Linking the evolution and form of warning coloration in nature. *Proc. Biol. Sci.* 279, 417–426. doi: 10.1098/rspb.2011.1932

Stewart, A. J. A., Perl, C. D., and Niven, J. E. (2020). Artificial lighting impairs mate attraction in a nocturnal capital breeder. *J. Exp. Biol.* 223:jeb229146. doi: 10.1242/jeb.229146

Suzuki, H., Sato, Y., Ohba, N., Bae, J.-S., Jin, B.-R., Sohn, H.-D., et al. (2004). Phylogeographic analysis of the firefly, Luciola lateralis, in Japan and Korea based on mitochondrial cytochrome oxidase II gene sequences (Coleoptera: Lampyridae). *Biochem. Genet.* 42, 287–300. doi: 10.1023/B:BIGI.0000039805.75118.8f

Thancharoen, A. (2007). *The Biology and Mating Behaviour of An Aquatic Firefly Species, Luciola aquatilis sp. nov. Thancharoen (Coleoptera: Lampyridae)*. unpublished Ph.D thesis, Mahidol University, Bangkok.

Thancharoen, A., and Masoh, S. (2019). "Effect of camera illumination on flashing behavior of *Pteroptyx malaccae*," in *Bioluminescence*, Ed. K. Sung-Bae (Berlin: Springer). doi: 10.5772/intechopen.85796

Tyler, J. (2002). *The Glow-Worm*. Bengaluru: Lakeside Printing.

Tyler, J. (2013). What do glow-worms do on their day off? The diurnal behaviour of adult *Lampyris noctiluca* (L.). *Lampyrid* 3, 23–34.

Tyler, J., Mckinnon, W., Lord, G. A., and Hilton, P. J. (2008). A defensive steroidal pyrone in the glow-worm *Lampyris noctiluca* L. (Coleoptera: Lampyridae). *Physiol. Entomol.* 33, 167–170. doi: 10.1111/j.1365-3032.2007.00610.x

Van den Broeck, M., De Cock, R., Van Dongen, S., and Matthysen, E. (2021a). Blinded by the light: Artificial light lowers mate attraction success in female glow-worms. *Insects* 12:734. doi: 10.3390/insects12080734

Van den Broeck, M., De Cock, R., Van Dongen, S., and Matthysen, E. (2021b). White LED light intensity, but not colour temperature, interferes with mate-finding by glow-worm males. *J. Insect Conserv.* 25, 339–347. doi: 10.1007/s10841-021-00304-z van der Kooi, C. J., Stavenga, D. G., Arikawa, K., Belušič, G., and Kelber, A. (2020). Evolution of insect color vision: From spectral sensitivity to visual ecology. *Annu. Rev. Entomol.* 66, 23.1–23.38. doi: 10.1146/annurev-ento-061720-071644

Vaz, S., Manes, S., Gama-Maia, D., Silveira, L., Mattos, G., Paiva, P. C., et al. (2021). Light pollution is the fastest growing potential threat to firefly conservation in the Atlantic Forest hotspot. *Insect Conserv. Divers.* 14, 211–224. doi: 10.1111/icad.12481

Vencl, F. V., and Carlson, A. D. (1998). Proximate mechanisms of sexual selection in the firefly *Photinus pyralis*. *J. Insect Behav.* 11, 191–207. doi: 10.1023/A:1021091806472

Wanjiru Mbugua, S., Hay Wong, C., and Ratnayeke, S. (2020). Effects of artificial light on the larvae of the firefly Lamprigera sp. in an urban city park, Peninsular Malaysia. *J. Asia. Pac. Entomol.* 23, 82–85. doi: 10.1016/j.aspen.2019.10.005

Warrant, E. J. (2017). The remarkable visual capacities of nocturnal insects: Vision at the limits with small eyes and tiny brains. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 372:20160063. doi: 10.1098/rstb.2016.0063

Wing, S. R. (1989). Energetic costs of mating in a flightless female firefly, Photinus collustrans. *J. Insect Behav.* 2, 841–847. doi: 10.1007/BF01049405

Wong, C. H., and Yeap, C. A. (2012). Conservation of congregating firefly zones (CFZs) in peninsular Malaysia. *Lampyrid* 2, 174–187.

Woods, W. A. Jr., Hendrickson, H., Mason, J., and Lewis, S. M. (2007). Energy and predation costs of firefly courtship signals. *Am. Nat.* 170, 702–708. doi: 10.1086/521964

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 17 April 2023
DOI 10.3389/fevo.2023.1126253

EDITED BY
Ann Valerie Hedrick,
University of California, Davis,
United States

REVIEWED BY
Astrid T. Groot,
University of Amsterdam, Netherlands
Alex Baranowski,
University of Rhode Island, United States

*CORRESPONDENCE
Jiang Feng
✉ fengj@nenu.edu.cn
Tinglei Jiang
✉ jiangtl730@nenu.edu.cn

SPECIALTY SECTION
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 17 December 2022
ACCEPTED 30 March 2023
PUBLISHED 17 April 2023

CITATION
Zhang W, Liu Y, Wang Z, Lin T, Feng J and Jiang T (2023) Effects of predation risks of bats on the growth, development, reproduction, and hormone levels of *Spodoptera litura*. *Front. Ecol. Evol.* 11:1126253. doi: 10.3389/fevo.2023.1126253

Effects of predation risks of bats on the growth, development, reproduction, and hormone levels of *Spodoptera litura*

Wenjun Zhang[1,2], Yingying Liu[1,2], Zhiqiang Wang[1,2], Tingting Lin[1,2], Jiang Feng[1,2,3]* and Tinglei Jiang[1,2,4]*

[1]Jilin Provincial Key Laboratory of Animal Resource Conservation and Utilization, Northeast Normal University, Changchun, China, [2]Key Laboratory of Vegetation Ecology of Education Ministry, Institute of Grassland Science, Northeast Normal University, Changchun, China, [3]College of Life Science, Jilin Agricultural University, Changchun, China, [4]Jilin Songnen Grassland Ecosystem National Observation and Research Station, Northeast Normal University, Changchun, China

Predation is a powerful selection pressure that shapes predator–prey interactions. Due to long-term interactions, moths have developed hearing to detect the echolocation calls of bats. This allows bats to impose the non-consumptive effects of predation on moths. However, information on the changes in the growth, development, reproduction, and hormones of moths that are vulnerable to bat predation is limited. In this study, we used *Spodoptera litura* (Lepidoptera: Noctuidae) to determine the effects of the predation risks of bats on their growth, development, reproduction, and hormone titers. Our results showed that the larvae of *S. litura* increased food intake, accelerated the development of the larval stages, increased mortality and metamorphosis failure, and had a smaller body size under the risk of predation by bats. Additionally, the reproductive activity and fecundity decreased in the adults of *S. litura*, but the juvenile hormone titers and 20-Ecdydysone hormone titers increased. These results suggested an adaptive response in *S. litura* under bat predation risks at the cost of lower survival and reproduction. Finally, we found that *S. litura* moths showed different responses to different predation risk cues, which suggested that they might assess the magnitude of different predation risks to make the most suitable decision for survival and reproduction. Our results highlight the importance of the predation risk imposed by bats to negatively regulate the population dynamics of moths. Our findings indicated that the biological control of bugs using bats is a promising strategy.

KEYWORDS

predation risks, bats, moths, non-consumptive effects, biological control

Introduction

The risk of predation strongly influences predator–prey interactions (Carpenter et al., 2001; Frank, 2008) and leads to changes in population dynamics, community structure, and ecosystem function (Krebs et al., 1995; Zanette et al., 2011). Predator–prey interactions are generally quantified by comparing the changes in the abundance of prey populations due to the direct consumption effects (CEs) of predators (Sih et al., 1985; Sheriff et al., 2020). However, researchers have realized that predation risks can also exert strong non-consumptive effects (NCEs) on prey by changing prey traits (e.g., behavior, physiology, and morphology) and development through multiple signals (e.g., auditory, olfactory, and visual signals) (MacLeod et al., 2018; DeWitt et al., 2019) which in turn can negatively affect the adaptations and fitness of prey (Sheriff et al., 2020). Several studies have shown that NCEs associated with predator–prey interactions might be equally or even more important than CEs on the prey population and community dynamics (Peckarsky et al., 2008; Peacor et al., 2020). Several studies on NCEs have been conducted with insects (Bauman et al., 2019), fishes (Hughes et al., 2014; Mitchell and Harborne, 2020; Benti et al., 2021), amphibians (Zamzow et al., 2010), birds (Malone et al., 2017), and mammals (Abom and Schwarzkopf, 2016; Fauteux et al., 2018). For example, *Melanoplus femurrubrum* (Orthoptera: Acrididae) increases the mass-specific metabolic rate by 32%, with a concomitant increase in carbohydrate intake by 40% when they perceive the risk of predation by spiders (Hawlena and Schmitz, 2010). The presence of fish predators that cannot attack also might increase the failure rate of metamorphosis and larval mortality in *Leucorrhinia intacta* (Odonata: Libelulidae) (McCauley et al., 2011). Different ultrasound frequencies can negatively affect juvenile hormone titers in *Monochamus alternatus* (Coleoptera: Cerambycidae), which might further disrupt sexual maturation (Zha et al., 2021). However, these studies mainly focused on the changes in the traits of the prey (i.e., vigilance, body size, habitat selection), but studies on the effects of the changes in traits on fitness components or the abundance of the prey remains unclear (Peacor et al., 2022).

There are more than 1,400 species of bats, most of which occupy a unique nocturnal ecological niche (Kunz et al., 2011). Insectivorous bats with precise echolocation systems mainly hunt nocturnal insects (Wilson and Reeder, 2005). The interaction between bats and moths is a fascinating evolutionary “arms race” (Rubin et al., 2018). During coevolution, echolocating bats influenced the evolution of numerous characteristics of nocturnal insects, and many insects evolved ultrasound-sensitive ears, evasive behaviors, or other survival strategies in response to the selection pressure imposed by bats. Thus, even in the absence of predation, bats can still pose a strong predation risk to moths. However, the NCEs of the predation risk of bats on moths remain unclear.

The echolocation calls of bats vary among species and can be classified into three types: constant frequency (CF), frequency modulation (FM), and quasi-constant frequency (QCF) (Schnitzler et al., 2003). CF bats use CF acoustic signals for echolocation, and the duration of the acoustic signal is long, i.e., up to 30–60 ms per signal. FM bats use FM acoustic signals for echolocation, and the duration of the acoustic signal is short, about 0.5–5 ms per signal (Jones and Teeling, 2006). Exposure to the risk cues (e.g., ultrasound) of predators can have strong indirect effects on the physiology of the exposed moths and also the performance of the offspring, which indicates promising results for biological control strategies based on the ecology of fear (Cinel et al., 2020). For example, exposure of *Spodoptera litura* (Lepidoptera: Noctuidae) and *S. exigua* to pulsed ultrasound white noise elicited a flight-stopping response in moths with no or little auditory adaptations, which indicated that the moths tended to escape from the ultrasound (Nakano et al., 2022). In another study, ultrasonic treatment had strong negative effects on the biological parameters, such as longevity, body mass, and fecundity, of the immature life stages of *Sesamia cretica* (Lepidoptera: Noctuidae) (Agah-Manesh et al., 2021). These studies suggested that bat ultrasound might be used to effectively control agricultural pests. However, many studies have used synthetic ultrasound signals to recreate a stimulus similar to the calls of bats (Lalita and McNeil, 1998; Huang and Subramanyam, 2004). These synthetic ultrasounds often lack the biological attributes of real bat ultrasounds, and thus, they may not be suitable for assessing the magnitude of bat NCEs on prey. Additionally, prey can detect predators using visual, tactile, chemical, physical, and other cues (Hermann and Thaler, 2014). As most studies have only focused on the acoustic cue of bats, it remains unclear whether the multimodal cues related to the risk of predation can cause a greater degree of negative effects on the prey.

Spodoptera litura (Lepidoptera: Noctuidae) is a polyphagous and important agricultural pest found worldwide (Xiao et al., 2021). It has evolved the ultrasound-sensitive tympanal organ in response to predation by echolocating bats. Thus, the predation risk of bats, especially using ultrasound stimuli, might be used for controlling this pest. Previously, we discovered that the CF-FM bat *Rhinolophus sinicus* (Chiroptera: Rhinolophidae) and the FM bat *Miniopterus fuliginosus* (Chiroptera: Vespertilionidae) could prey on a large number of *S. litura* (unpublished data), and the two bats have different foraging habitats, physiological ecology, and ultrasonic parameters. Though it is not clear if the bats prefer to eat this species or whether the prey cannot effectively evade predation, it still provides a good opportunity to study the NCEs of the predation risk of bats on the changes in the traits of these moths and the consequences of these changes on their fitness.

For prey, there is usually a trade-off between predator avoidance and growth (survival and reproduction) (Thaler et al., 2012). An increase in the survival of prey is associated with a reduction in foraging activity and an increase in vigilance when exposed to high predation risk (Lalita and McNeil, 1998; MacLeod et al., 2018). Conversely, prey allocates more energy to reproduction and adaptive changes in behavior, physiology, and life history traits (Sheriff et al., 2020). In this study, we used *R. sinicus* and *M. fuliginosus* and their echolocation calls related to foraging as predation risk cues to investigate the effects of different predation risks imposed by bats on the growth, development, reproduction, and hormone levels of *S. litura*. We hypothesized that the predation risks of bats would negatively affect the growth, development, reproduction, and physiology of *S. litura*. We tested the following predictions: (1) bat predation risk might be associated with changes in the growth and development of *S. litura,* including food intake, body mass, survival rate, death rate, pupation rate, and eclosion rate; (2) bat predation risk might be associated with a decrease in reproductive behavior and fecundity in *S. litura*; (3) the levels of the JH and 20-E hormone of *S. litura* might change after exposure to the risk of predation by bats.

Materials and methods

Capture and housing of bats

We collected 10 individuals of *R. sinicus* from Jiumen Cave, Lengshuijiang City, Hunan Province, in May 2021. We also collected 10 individuals of *M. fuliginosus* from Feilong Cave, Jingnan Town, Xingyi City, Guizhou Province, in June 2021. We placed mist nets at the entrance of the caves in the early morning to capture the bats when they returned from foraging (Gong et al., 2022). Each individual was placed in a sterile cloth bag and brought back to the laboratory (5 m × 10 m × 3 m) for rearing at Northeast Normal University. In the laboratory, the temperature and relative humidity were set at 21–22°C and 40%, respectively, and a 12-h/12-h light/dark cycle (light conditions of 07:00–19:00 h) was maintained to mimic the natural environment inside caves (Zhang et al., 2022). All bats were provided *ad libitum* freshwater and larvae of *Zophobas morio* (Coleoptera: Tenebrionidae) enriched with vitamins and minerals. Bats were kept healthy during the acclimation or experimental periods. All bats were released in good health at the location where they were captured, after completion of the experiments.

Recording of echolocation calls during foraging

Before the experiments, we recorded the foraging echolocation calls from each bat to use as acoustic stimuli in our experiment. In total, 20 wild-caught *S. litura* were periodically released in an acoustics laboratory (3 m × 5 m × 3 m), and we also hung 10 *S. litura* larvae with a thin wire (0.7 mm in diameter) 1 m from the top of the room to ensure that preys were available for bats. Before the experiment, the bats were fed 5 *Z. morio* to reach a semi-starved state to motivate the bats to feed on the *S. litura*. During each experiment, we brought one bat into the laboratory to record echolocation calls, from search-phase calls to feeding buzzes. The interactions were captured using two infrared cameras (FDR-AX60; Sony Corp., Tokyo, Japan) placed opposite to each other and two single-channel ultrasound recording devices (Ultra-Sound Gate 116, Avisoft Bioacoustics, Berlin, Germany). The sampling frequency was 375 kHz with a 16-bit resolution (Sun et al., 2018). The experiments were performed when the foraging activity was the highest (Appel et al., 2021) (between 20:00 and 06:00 h), and the calls of each bat were recorded for 1 h. After the calls were recorded, the bats were returned to their large flight cage in the husbandry room and given food as a reward.

Synthesis and editing of playback files

Each playback file was created by randomly mixing the echolocation calls from one individual. The echolocation call sequences (including searching, approaching, and feeding buzz phases) with a good signal-to-noise ratio were selected for editing. These calls were randomly stitched together using the Avisoft-SASLab Pro 5.2 (Avisoft Bioacoustics, Berlin, Germany) software to construct playback files of 1 min. Each playback file consisted of 14 echolocation call sequences with random silent intervals of 5–8 s between sequences to mimic the natural intervals of echolocation calls. The playback files were normalized, with the peak amplitude of the weakest call set at about −30 dB. Each group of playback files was randomly arranged based on the 14 echolocation call sequences to generate 10 playback files. All playback files were high-pass filtered at 2 kHz to minimize the effect of background noise. We constructed a white noise (0 ~ 100 kHz) stimulus to determine whether the response of the moths to bat echolocation calls was similar to that of white noise. The individuals of *S. litura* in each treatment group were stimulated with a group of playback files every night during the experimental period.

Collection and maintenance of *S. litura*

In total, 1,000 eggs of *S. litura* were purchased from Baiyun Industrial Co., Ltd. in Henan Province and housed in polypropylene plastic rearing boxes (20 cm × 14 cm × 8 cm) in an artificial climate chamber (PRX-450C, NingboSaifu, Ningbo, China) at 27 ± 1°C, 65 ± 5% relative humidity, and a 14-h/10-h light/dark photoperiod. All the newly-hatched larvae in the experiment were reared together on an artificial diet (Guanghong et al., 1998). They were isolated individually into finger tubes to prevent the effect of density on their growth and development. After pupation in the test tubes, the pupae were transferred to polypropylene plastic boxes (20 cm × 14 cm × 8 cm) individually and moisturized by spraying water every day until they emerged as adults. The adults were placed in a cage (40 cm × 40 cm × 40 cm) made of 120-mesh gauze and with a 10% honey solution applied on a ball of cotton in a glass dish for mating and laying eggs. The eggs on the wall of the cage of *S. litura* were collected on disinfected plastic sheets and disinfected with sodium hypochlorite solution (340.0–460.0 μg/L effective chlorine content) for 30 min to prevent pathogen infection (Xiong et al., 2015). Then, the eggs were rinsed with water for 3 min, air dried, placed in a rearing box as mentioned above, and incubated in an artificial climate chamber. The individuals of *S. litura* were reared for a generation under predator-free conditions for subsequent experiments.

The playback experiment

All experiments were performed in artificial climate chambers under the same physical conditions. To assess the differences between two acoustic bat cues and the NCEs of white noise on the *S. litura*, and to determine whether bats have only acoustic cues that can cause effects on *S. litura*, six treatment groups were established, including the control group (Control), white noise playback group (Whitenoise), CF echolocation call playback group (CF-call), *R. sinicus* exposure group (CF-exp), FM echolocation call playback group (FM-call), and *M. fuliginosus* exposure group (FM-exp). The Whitenoise, CF-call, and FM-call groups were the echolocation call playback groups, and the CF-exp and FM-exp groups were bat the exposure groups. We provided chronic stimulation throughout the life history stages of *S. litura* to determine the sustained effects of chronic stimulation. For the echolocation call playback groups, the microphone, and the loudspeaker were connected to an ultrasound recording system and an ultrasound playback interface (UltraSoundGate player 116), respectively. The speaker was set on a tripod 50 cm above the individuals of *S. litura*. These speakers provide an acoustic cue to *S. litura*. For the bat exposure groups, each group consisted of five adult bats in a cage (50 cm × 50 cm × 50 cm) 50 cm away from the *S. litura* moths to create an environment where the moths were exposed to bat predators. These bats might provide acoustic, visual, and olfactory cues to *S. litura.* All stimulation treatments were provided from 10:00 p.m. to 8:00 a.m. and stopped for the rest of the day, whereas the larvae in the control group were placed in the same environment in the absence of stimuli.

Development and food intake of *S. litura* larvae under the risk of predation

Newly hatched larvae of *S. litura* were randomly assigned to one of the six above-mentioned treatment groups. We chose 300 newly hatched larvae from each group and divided them into six replicates of 50 larvae each. The larvae were reared individually in sterilized glass test tubes (20 mm × 150 mm) and fed an artificial diet. Each replicate had 50 test tubes in one test tube rack, counting a total of six test tube racks per group. The test tubes were sealed with sterilized gauze to ensure air permeability, and the tubes were placed in plastic containers (22 cm × 9 cm × 15 cm). The development progress of *S. litura* was recorded, specifically, the developmental stages, number of pupae, and number of emerging adults. After the third instar of the *S. litura* larvae, randomly 100 larvae in each treatment group were weighed daily using an electronic balance (BSA124S 120 g/0.1 mg, Sartorius Ltd., Germany) till they started to develop into pre-pupae. At this time point, larvae stopped feeding and made pupal chambers. The weight of the pupae in all treatment groups was recorded 3 days after pupation. The condition of the moths and wing abnormalities were recorded every day until all moths died. For each treatment type, we selected additional 100 third instar larvae and reared them individually in single tubes containing enough fresh artificial diet to satisfy their food intake when exposed to different stimuli. The remaining artificial feed was weighed each day and replaced with a sufficient amount of fresh artificial diet. The experiment was completed after all larvae entered the prepupal stage; the amount of food consumed was calculated for the larvae in each treatment group.

The reproductive behavior of *S. litura* under the risk of predation

The behavior of insects is complex and variable. We selected three main behaviors to determine the behavioral responses of moths to different types of treatments, which included movement, mating, and courtship (Svensson et al., 2003; Zhou et al., 2010). We selected moths of similar body condition that emerged on the same day from each of the treatment groups and placed them in plastic cups (10 cm × 10 cm × 10 cm) with 10% fresh honey solution applied on cotton balls to provide supplementary nutrients. Ten pairs of moths were assigned to each treatment group (Sandhyarani and Rani, 2013). The behaviors of the moths were recorded using an infrared camera (HDR-CX 760E; Sony Corp., Japan) every day within the duration in which the stimuli were provided (Wu et al., 2018). We recorded three nights total because the peak of *S. litura* movement, mating, and courtship occurred within 3 days of eclosion (Li et al., 2012). We used QvodPlayer (Version 5.0.80, ShenzhenQvod Technology Co., Ltd., Guangdong, China) to analyze the recorded video files of the moths in the six treatment groups and quantify their behavior. Based on the recorded data, the percentage of time spent performing each behavior was calculated. Following the methods described above, we selected another 10 pairs of moths to determine the effects of the different types of treatments on the oviposition of female moths (Wu et al., 2018). The eggs were collected and counted daily. From them, 100 eggs laid on the same day by females of the same age were randomly selected to calculate the hatching rate, and 10 replicates were used for each treatment (Wu et al., 2018).

The titers of JH and 20-E of *S. litura* under predation risk

To determine the effects of different types of treatments on the hormone levels of *S. litura*, we selected two hormones that are critical for insect growth and development and analyzed their changes. From the six treatment groups, the late fourth, late fifth, and late sixth instar larvae, as well as, 1-, 3-, and 5-day-old female and male moths, were selected to determine the titers of JH and 20-E. Furthermore, because the JH titer was generally low in pupae and the 20-E titer did not differ significantly (Plantevin et al., 1984), we chose 1-, 3-, and 5-day-old pupae without distinguishing between male and female to only determine the 20-E titers. Three biological replicates were set up for each treatment (Zha et al., 2021). The double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was performed to evaluate the levels of JH and 20-E. Insect JH and 20E ELISA kits (detection range of 6.25–200 pg./mL) (Shanghai Enzyme-Linked Biotechnology Co., Ltd., China) were used to determine JH and 20E levels, following the manufacturer's instructions. Each analysis phase was performed separately three times and averaged.

Data analysis

The Kolmogorov–Smirnov test was conducted to test the normality of the data, and parametric tests were performed to analyze normally distributed data. Non-parametric tests were performed to analyze the data that were not normally distributed. The data on development duration, mortality, adult longevity, pupation rate, eclosion rate, larval food intake, maximum body mass of last instar larvae, 3-day-old pupal weight, total egg production, hatching rate, titers of JH, and titers of 20-E were normally distributed in each treatment group, and the differences among the different treatment groups were analyzed by performing the one-way analysis of variance (ANOVA) with Tukey's multiple comparisons. The percentage of time spent moving (adults), courting, and mating was not normally distributed. For these three parameters, the Kruskal-Wallis and Wilcoxon rank sum tests were performed to compare the differences among/between different treatment groups. All statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, United States).

Results

Effects of bat predation risks on the growth and development of *S. litura*

The food intake of *S. litura* larvae exposed to different predation risks and white noise was significantly higher than that of the larvae in the Control group ($F_{5,\,449} = 28.976$, $p < 0.001$, Figure 1A), and the larvae in the CF-call, CF-exp, and FM-exp groups had the highest food intake, followed by those in the FM-call and Whitenoise groups. Additionally, larvae exposed to predation risk showed faster development of the larval stages compared to those in the Control group (Figure 1B), whereas their maximum fresh weight was not significantly different from those in the Control group (Figure 1C). However, the weight of pupae was significantly lower for those that were exposed to different predation risks compared to the weight of pupae in the Control group ($F_{5,\,436} = 11.017$, $p < 0.001$, Figure 1D). The weight of pupae was significantly lower in the FM-exp and Whitenoise groups ($p < 0.001$, Figure 1D), and although it was also lower in the CF-exp, CF-call, and FM-call groups, the differences were not significant.

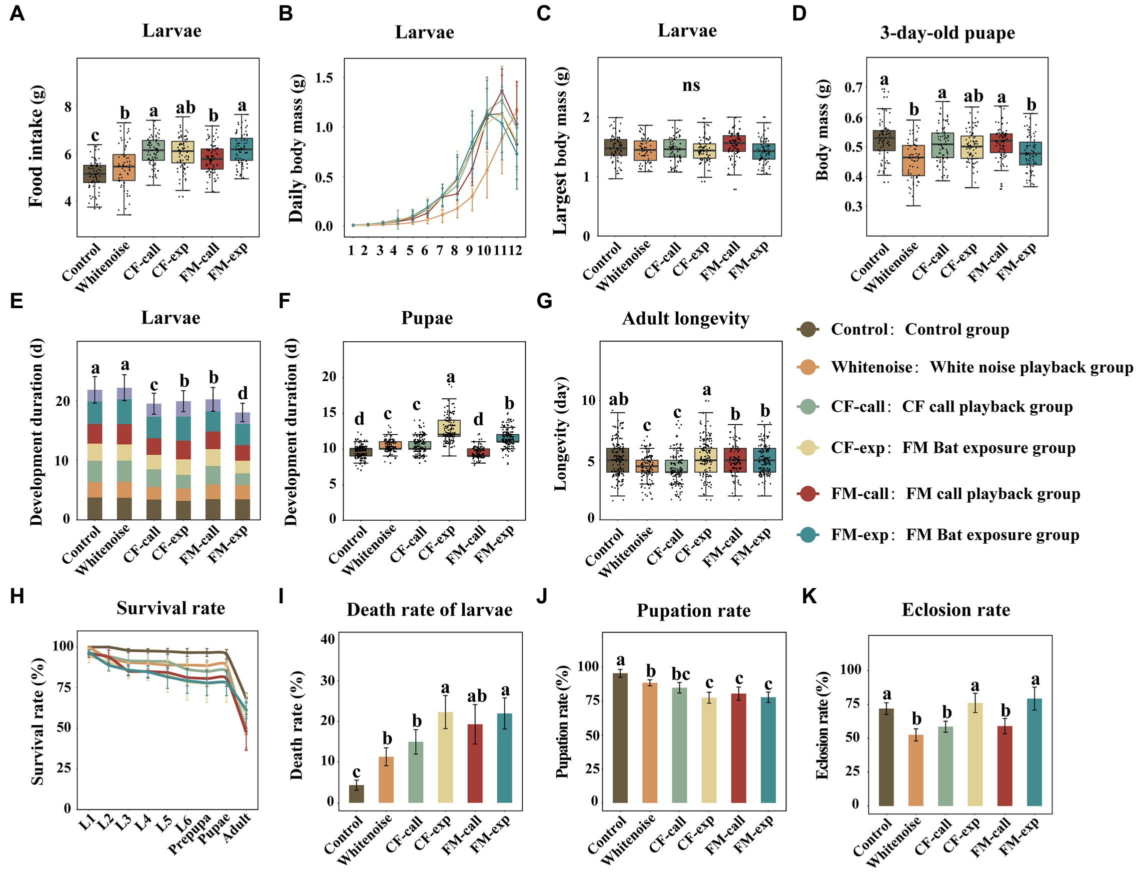


FIGURE 1
Effects of different treatments on the growth and development of *S. litura*. **(A)** Food intake of larvae in different treatment groups, **(B)** diurnal variation in the body mass of the larvae, **(C)** maximum fresh body mass of the larvae, **(D)** body mass of 3-day-old pupae, **(E)** the duration of larval development, **(F)** the duration of pupal development, **(G)** longevity of moths, **(H)** the survival rate at different life stages, **(I)** the death rate of larvae, **(J)** the pupation rate of larvae, and **(K)** the eclosion rate of pupae. The data are expressed as the mean±SD. Different colors represent data from different treatment groups. The different letters on the bar and box plots indicate significant differences between treatment groups (Tukey's test, $p<0.05$); ns indicates no significant difference between treatments.

The bat and echolocation playback call treatments significantly accelerated larval growth (Figure 1E) and decreased larval developmental duration ($F_{5,1,514} = 18.630$, $p < 0.001$, Figure 1E), but the growth of larvae was not significantly different between the Whitenoise and Control groups (Figure 1E). The duration of the pupal period also showed differences between treatments ($F_{5,733} = 18.630$, $p < 0.001$; Figure 1F). The pupae in the CF-exp group had the longest pupal period (12.62 ± 1.63 day), followed by those in the FM-exp (11.63 ± 1.19 day), CF-call (10.56 ± 1.19 day), and Whitenoise (10.27 ± 0.97 day) groups. The pupal period of the FM-call group was 9.57 ± 0.71 day, which was not significantly different from that of the pupae in the Control group. The longevity of *S. litura* adults was significantly lower for those in the CF-call and Whitenoise groups compared to the longevity of those in the Control group ($F_{5,733} = 16.285$, $p < 0.001$, Figure 1G). However, although the longevity of adults was lower for those in the FM-exp, FM-call, and CF-exp groups relative to those in the Control group, the differences were not significant (Figure 1G).

For the *S. litura* individuals exposed to different predation risks and white noise, their survival rate was significantly lower (Figure 1H), larval mortality was significantly higher ($F_{5,30} = 11.017$, $p < 0.001$, Figure 1I), and pupation rate was significantly lower ($F_{5,30} = 17.575$, $p < 0.001$, Figure 1J) than those of the individuals in the Control group. Overall, the moths exposed to the two bat playback treatments had the highest larval mortality ($p < 0.001$, Figure 1I), followed by those in the FM-call, CF-call, and Whitenoise groups ($p = 0.032$, Figure 1I). Similarly, the moths in the CF-exp and FM-exp groups had the lowest pupation rate ($p < 0.001$, Figure 1J), followed by those in the FM-call and CF-call groups ($p = 0.001$, Figure 1J), but no significant difference was found between the moths in the Whitenoise and Control groups. The eclosion rate was significantly lower ($F_{5,30} = 17.084$, $p < 0.001$, Figure 1K) only for pupae exposed to CF echolocation calls ($p = 0.014$, Figure 1K), FM echolocation calls ($p = 0.019$, Figure 1K), and white noise ($p < 0.001$, Figure 1K) relative to that of the pupae in the Control group, however, exposure to the two bat playback treatments had no significant effect on the eclosion rate of the pupae.

Effects of bat predation risks on the reproductive behavior and fecundity of *S. litura*

The percentage of time spent moving was significantly lower for *S. litura* individuals exposed to different bat predation risks and white noise ($H_{5,30} = 20.268$, $p = 0.001$, Figure 2A) than for the individuals in the Control group. The percentage of time spent moving for individuals in the FM-exp group was significantly lower than that for the individuals in the Control group ($p = 0.001$, Figure 2A). The proportion of mating behavior of *S. litura* individuals in all treatment groups was similar ($H_{5,30} = 6.792$, $p = 0.237$, Figure 2B). However, among all groups of individuals exposed to bats and bat echolocation playback calls, mating behavior was only observed once in the CF-call, FM-call, and FM-exp groups. The percentage of time spent courting for individuals in all treatment groups was similar to that for individuals in the Control group, but it was significantly lower for individuals in the FM-call group than that for individuals in the CF-call group ($p = 0.001$, Figure 2C).

The peak fecundity period of *S. litura* was the third day after laying, and then, the fecundity of the moths decreased, except for those in the CF-call group, where the daily fecundity was higher than that of the moths in the Control group on the fourth day after laying (Figure 2D). Overall, the total fecundity of *S. litura* decreased when exposed to different predation risks and white noise ($F_{5,54} = 3.855$, $p = 0.005$, Figure 2E). The moths in the FM-exp group produced the lowest number of eggs (1,935 ± 1,465), which was significantly lower than the number of eggs laid by moths of the other groups ($p < 0.001$, Figure 2E), i.e., the Whitenoise group (2,847 ± 1,149), the CF-call group (2,912 ± 1,507), and the FM-call group (3,397 ± 1,798). The number of eggs laid by moths in the CF-exp group (4,008 ± 864) was lower than that laid by moths in the Control group (4,138 ± 892), but the differences were not significant. The hatching rate of the moths exposed to different predation risks and white noise was significantly lower than that of the moths in the Control group ($F_{5,54} = 29.333$, $p < 0.001$, Figure 2F), with the lowest hatching rate (64.20%) recorded for moths in the FM-exp group ($p < 0.001$, Figure 2E), followed by those in the CF-call (67.80%), FM-call (75.50%), CF-exp (75.6%), Whitenoise (82.60%), and Control groups (89.90%).

Effects of the predation risk of bats on the titers of JH and 20-E in *S. litura*

Except for the late fifth instar in the Whitenoise group, the titers of JH in *S. litura* larvae under different predation risks and white noise were significantly higher at the late fourth instar ($F_{5,12} = 63.152$, $p < 0.001$, Figure 3A), late fifth instar ($F_{5,12} = 92.645$, $p < 0.001$, Figure 3A), and late sixth instar ($F_{5,12} = 92.645$, $p < 0.001$, Figure 3A) compared to that in the larvae of the Control group. The larvae in the FM-call group had the highest JH titers at the late fourth instar, but no significant differences were observed between the larvae in different predation risk groups at the late fifth instar. The highest titers of JH were found in the larvae of the FM-exp group ($p < 0.001$, Figure 3A) at the late sixth instar. The JH titers were significantly higher in the larvae of the Whitenoise group than that in the larvae of the Control group at both the late fourth and late sixth instars ($p < 0.001$, Figure 3A) but not in the late fifth instar.

Except for the 3- and 5-day-old female moths in the CF-exp group and the 5-day-old female moths in the Whitenoise group, the 1-day-old ($F_{5,12} = 344.102$, $p < 0.001$, Figure 3B), 3-day-old ($F_{5,12} = 406.822$, $p < 0.001$, Figure 3B), and 5-day-old ($F_{5,12} = 119.925$,

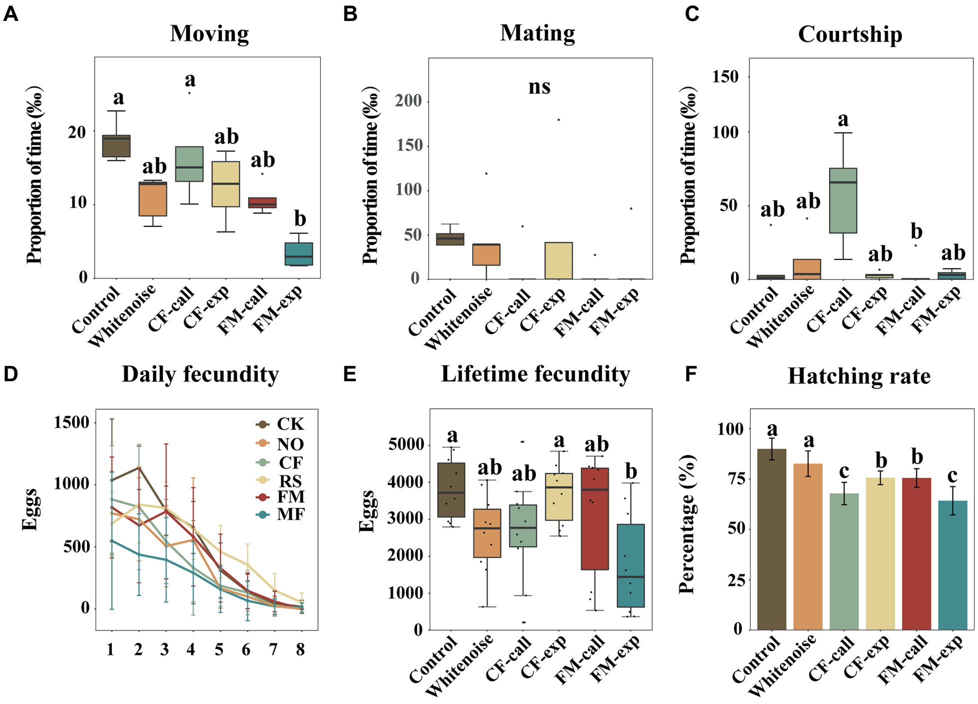


FIGURE 2
Reproductive behavior and fecundity of *S. litura* moths under different types of predation risk. **(A)** The proportion of time spent moving, **(B)** the proportion of time spent mating, **(C)** the proportion of time spent courting, **(D)** daily fecundity, **(E)** lifetime fecundity, and **(F)** the hatching rate. The data are expressed as the mean±SD. Different letters on the bars or box plots indicate significant differences between treatments (Tukey's test, $p<0.05$); ns indicates no significant difference between treatments.

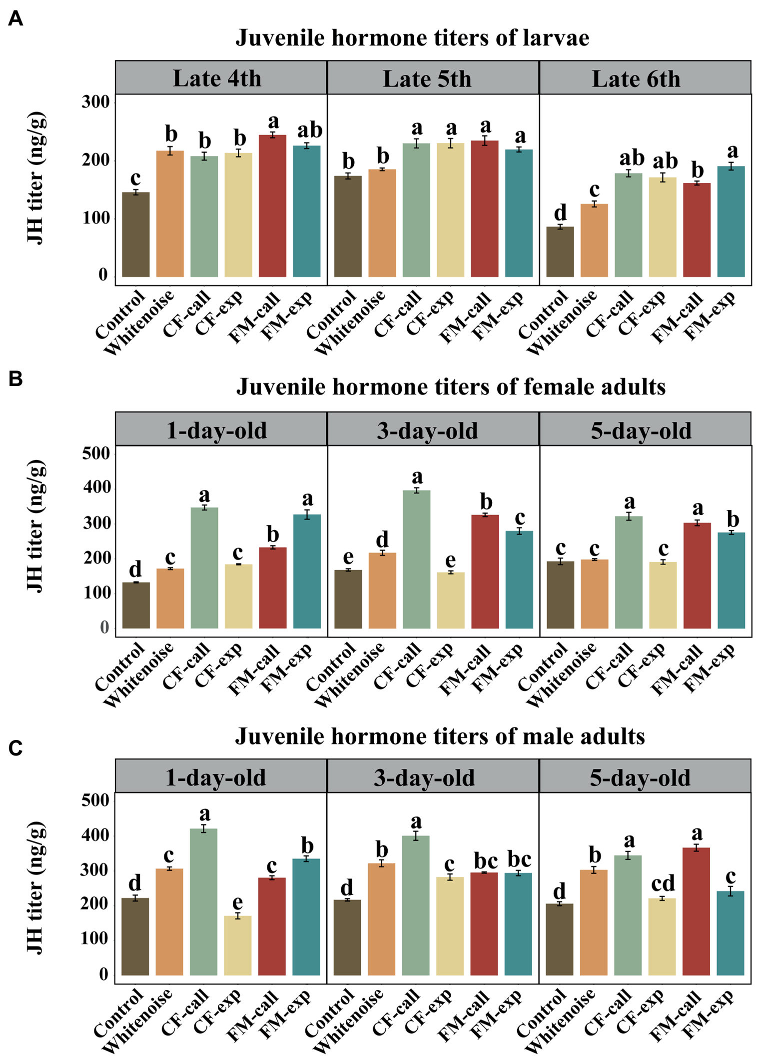


FIGURE 3
The JH titers of *S. litura* under different types of predation risk. The JH titers of **(A)** larvae of different ages, **(B)** female adult moths, and **(C)** male adult moths. The data are expressed as the mean±SD. Different letters on the bars indicate significant differences between treatments (Tukey's test, $p<0.05$).

$p < 0.001$, Figure 3B) female moths under the predation risk of bats and white noise treatment had significantly higher JH titers than those in the Control group. The highest JH titers were recorded in the 1-day-old female moths of the CF-call ($p < 0.001$, Figure 3B) and FM-exp groups ($p < 0.001$, Figure 3B). Additionally, the highest JH titers were also observed in the 3-day-old female adults of the CF-call group ($p < 0.001$, Figure 3B) and in the 5-day-old female moths of the CF-call and FM-call groups ($p < 0.001$, Figure 3B).

Except for the 1- and 5-day-old male moths in the CF-exp group, the 1-day-old ($F_{5,\ 12} = 344.102$, $p < 0.001$, Figure 3C), 3-day-old ($F_{5,12} = 406.822$, $p < 0.001$, Figure 3C), and 5-day-old ($F_{5,12} = 119.925$, $p < 0.001$, Figure 3C) adult male moths exposed to bat predation risk or white noise treatment had significantly higher JH titers than those in the Control group. The highest titers of JH were found in the moths of the CF-call group for both 1-day-old ($p < 0.001$, Figure 3C) and 3-day-old ($p < 0.001$, Figure 3C) male moths, but the highest titers of JH was observed in the 5-day-old moths of the FM-call and CF-call groups ($p < 0.001$ Figure 3C).

Effects of bat predation risks on the 20-E titers of *S. litura*

Except for the late fifth instar in the CF-exp group, the 20-E titers of larvae exposed to the predation risk of bats and white noise treatment were significantly higher than those in the larvae of the Control group at the late fourth instar ($F_{5,\ 12} = 105.267$, $p < 0.001$, Figure 4A), late fifth instar ($F_{5,12} = 79.6340$, $p < 0.001$, Figure 4A) and late sixth instar ($F_{5,12} = 28.492$, $p < 0.001$, Figure 4A). The highest 20-E titers were observed in the larvae of the CF-call group ($p < 0.001$, Figure 4A) at the late fourth instar, and in the larvae of the CF-call and FM-exp groups at the late fifth instar and late sixth instar ($p < 0.001$, Figure 4A).

The 20-E titers of 1-day-old ($F_{5,\ 12} = 128.045$, $p < 0.001$, Figure 4B), 3-day-old ($F_{5,12} = 122.559$, $p < 0.001$, Figure 4B), and 5-day-old ($F_{5,\ 12} = 218.364$, $p < 0.001$, Figure 4B) pupae in the CF-call, FM-call, and Whitenoise groups were significantly higher than that in the pupae of the Control group. The 20-E titers among

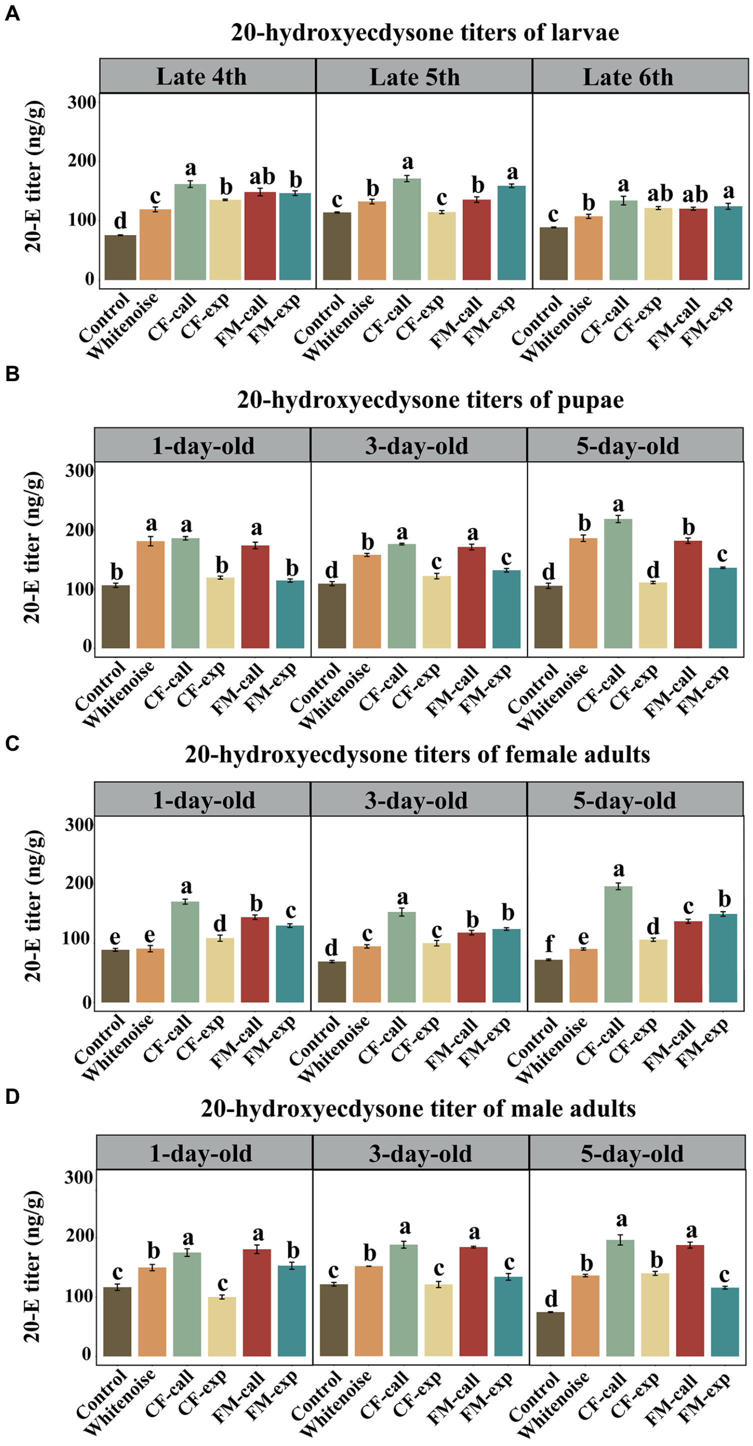


FIGURE 4
The 20-E titers in the different life stages of *S. litura* under different types of predation risk. The 20-E titers of **(A)** larvae, **(B)** pupae, **(C)** female adults, and **(D)** male adults. The data are expressed as the mean±SD. Different letters on the bars indicate significant differences between treatments (Tukey's test, $p<0.05$).

the three acoustic groups for the 1-day-old pupae were not significantly different (Figure 4B). The highest 20-E titers for 3-day-old pupae were recorded in the CF-call ($p < 0.001$, Figure 4B) and FM-call groups ($p < 0.001$, Figure 4B) and in the 5-day-old pupae of the CF-call group ($p < 0.001$, Figure 4B).

Except for the 1-day-old female adults in the Whitenoise group, the 1-day-old ($F_{5,\ 12} = 118.900$, $p < 0.001$, Figure 4C), 3-day-old ($F_{5,12} = 108.990$, $p < 0.001$, Figure 4C), and 5-day-old ($F_{5,12} = 336.080$, $p < 0.001$, Figure 4C) adult females exposed to predation by bats and white noise treatment had higher 20-E titers than the females in the Control group. The highest titers of 20-E were found in the 1-day-old

($p < 0.001$, Figure 4C), 3-day-old ($p < 0.001$, Figure 4C), and 5-day-old ($p < 0.001$, Figure 4C) female adults of the CF-call group.

Except for the 1- and 3-day-old male adults of *S. litura* in the CF-exp group and the 3-day-old male adults in the FM-exp group, the 1-day-old ($F_{5,\ 12} = 61.749$, $p < 0.001$, Figure 4D), 3-day-old ($F_{5,12} = 104.083$, $p < 0.001$, Figure 4D), and 5-day-old ($F_{5,\ 12} = 202.927$, $p < 0.001$, Figure 4D) adult male moths exposed to predation risk of bats and white noise treatment had significantly higher 20-E titers than those in the Control group. The highest titers of 20-E were found in the 1-day-old ($p < 0.001$; Figure 4C), 3-day-old ($p < 0.001$; Figure 4C), and 5-day-old ($p < 0.001$; Figure 4C) adult males of the CF-call and FM-call groups.

Discussion

Our results showed that the larvae of *S. litura* responded to predation risks by increasing their food intake and accelerating their development, but their body mass did not increase. The duration of larval development in the presence of predation risk was shorter than that without predation risk, and the duration of pupal development was longer. In addition, mortality and the rate of metamorphosis failure, which included pupation and eclosion failure, were higher at all stages for the moths exposed to predation risk. These results supported our first prediction that bat predation risk might be associated with changes in the growth and development of *S. litura,* including food intake, body mass, survival rate, death rate, pupation rate, and eclosion rate. The predation risk decreased the reproductive behavior, fecundity, and adult longevity of *S. litura,* which supported our second prediction that bat predation risk might be associated with a decrease in reproductive behavior and fecundity in *S. litura*. Finally, larvae and pupae of *S. litura* exposed to predation risk cues showed significantly higher titers of JH and 20-E, which supported our third prediction that the levels of the JH and 20-E hormone of *S. litura* might change after exposure to the risk of predation by bats.

Predation risk can affect prey growth and development (Higginson and Ruxton, 2010; Moore et al., 2018). For example, in *Manduca sexta*, individuals exposed to predators decreased their food intake by 30–40% but developed faster and gained the same weight as the individuals with no predation risk (Thaler et al., 2012). Individuals of *Helicoverpa armigera* accelerate their development, enter the pupal and adult stages earlier, and have a bigger body size as adults when exposed to the risk of predation by *Harmonia axyridis* (Xiong et al., 2015). Individuals of *Aedes notoscriptus* have slower development and a smaller size as adults when exposed to the risk of predation by fish (van Uitregt et al., 2012). In this study, the predation risk of bats resulted in faster development, smaller size of adults, higher mortality and metamorphosis failure rates, and lower adult longevity of *S. litura* moths compared to the corresponding changes in the moths of the control group. Predation risk-induced rapid development in prey demands more energy for the maintenance of physiological functions, which in turn requires them to obtain more resources (Barton, 2010). In general, the large size of the body is, in many cases, positively correlated with fecundity and survival probability (Brodin and Johansson, 2004). However, our results were contrary to this pattern. Predation risk can increase stress in prey, decrease antioxidant defenses, and result in oxidative damage (Zha and Lei, 2012; Janssens and Stoks, 2013, 2014; Roux et al., 2021; Venkanna et al., 2021). Oxidative damage is a major determinant of the life histories of animals and can influence organisms over a long period in many ways, e.g., decrease fertility, accelerate aging (Monaghan et al., 2009), and increase mortality and metamorphosis failure rates (Baranowski and Preisser, 2018). Predation risk also induces faster metabolism and increases consumption rates in prey (Schmitz et al., 2016), which might explain the increased food intake but smaller body size of *S. litura* moths exposed to predation risk. Additionally, some studies have found a trade-off between prey stress and immune function under predation risk (Duong and McCauley, 2016; Schwenke et al., 2016). When exposed to predation risk, the energy allocated to immunity decreases in prey, which decreases the immune function and increases mortality. In this study, the larvae of *S. litura* were vulnerable to the environment, and the adults were more susceptible to predation by bats. This caused individuals to increase their food intake, accelerate larval development, and extend the pupal stage, as an adaptive response to the risk of predation by bats. The increase in the mortality and metamorphosis failure rates were associated with the adaptive response of *S. litura*.

Predation risk affects prey reproduction by decreasing foraging behavior or by adversely affecting physiological effects, which in turn decreases their fecundity and the fitness of their offspring (Peckarsky et al., 1993; Mukherjee et al., 2014; Villalobos-Sambucaro et al., 2020). Usually, the resources accumulated by lepidopterans in the larval stage are used for reproduction (Fox and Czesak, 2000). An increase in body size increases fitness, which might be positively related to higher fecundity, probability of survival, and mating success (Brodin and Johansson, 2004). For example, bigger males of *S. litura* have greater chances of mating (Fox and Czesak, 2000; Okuzaki, 2021). A smaller body might decrease reproductive behavior and reproductive success. A decrease in the fecundity of *S. litura* individuals under predation risk might be related to a decrease in the reproductive success of *S. litura*. Furthermore, all of *S. litura's* activities took place at night (Li et al., 2012). In our study, no mating behavior was observed in the *S. litura* exposed to the risk of predation by bats, but egg production was observed, which could be the *S. litura* started to lay eggs at one point, whether they were mated or not, and thus these eggs were unfertilized, which may explain why the predation risk reduces the hatching rate of the egg of the *S. litura*. Additionally, a trade-off might occur between reproduction and immunity in insects (Schwenke et al., 2016; Guo et al., 2022). Mating promotes reproductive activity, and an increase in reproductive activity might decrease immune function (Gao et al., 2020). For example, delayed or no mating in adult *S. litura* is associated with longer life, probably due to lower energy investment in reproduction; this effect is especially prominent among females, as they allocate more energy to reproduction (Wu et al., 2018). Additionally, individuals of *Helicoverpa armigera* (Xiong et al., 2015), *Mamestra brassicae* (Huang et al., 2003) and *Plodia interpunctella* (Huang and Subramanyam, 2004) also had lower fecundity when exposed to ultrasound. The predation risk of bats decreased reproductive behavior, fecundity, and adult longevity in *S. litura,* which was similar to the findings of previous studies. We speculated that *S. litura* exposed to bat predation risk might be under chronic stress; oxidative stress might lead to a smaller body size and an increase in energy requirements allocated to survival. This, in turn, might cause lesser energy to be allocated to immunity and reproduction. However, there is a trade-off between reproduction and immunity in *S. litura*, where energy is preferentially allocated to immunity; this leads to a decrease in reproductive activity and fecundity.

Besides morphological and reproductive changes, physiological changes might occur in prey exposed to predation risk (Duong and McCauley, 2016). For example, the antioxidant metabolism and oxidoreductase activity increased in *Spodoptera frugiperda* after exposure to bat ultrasound (Cinel and Taylor, 2019). In our study, larvae, pupae, and adults of *S. litura* exposed to predation risk showed an increase in the levels of JH and 20-E to different degrees. Hormones are regulatory signaling factors and coordinate multiple developmental and physiological processes in insects (Cherbas et al., 1989; Flatt et al., 2005). JH and 20-E synergistically regulate insect growth and development, metamorphosis, reproduction, and different behaviors (Kim et al., 2019; Santos et al., 2019). The 20-E hormone can initiate and regulate molting and metamorphosis, while JH can regulate the direction of metamorphosis (Truman and Riddiford, 2002). High levels of JH not only promote oocyte maturation (Fleig, 1995) and the expression of the yolk protein genes but also suppress stress resistance and immune function (Parthasarathy et al., 2010; Süren-Castillo et al., 2012). High levels of 20-E, however, increase the expression of immune-related genes and lead to egg resorption of immature yolk (Soller et al., 1999; Schwenke et al., 2016). Therefore, maintaining the balance between JH and 20-E levels is essential for insect metamorphosis, reproduction, and immunity. In our study, exposure to the predation risk of bats increased larval 20-E titers, which accelerated development by advancing the molting time of *S. litura*. However, an increase in JH titers ensured that *S. litura* could maintain its larval form under predation risk. Exposure to white noise, CF-call, and FM-call treatments significantly increased the 20-E titers relative to that after exposure to the control and bat treatments; the eclosion rate increased significantly. We speculated that the increase in the 20-E titers in the pupal stage might be responsible for the decrease in the eclosion rate. An increase in the JH and 20-E titers in the adult stage was closely related to the reproductive and immune activities of adults. An increase in the JH titers under predation risk can promote the reproductive activities of adults, while an increase in 20-E titers can promote the immune activities of adults. However, although individuals showed physiological adaptation to predation risk, those in different life stages still experienced high mortality, higher failure of metamorphosis, and lower fecundity and longevity.

The strength of NCEs depends on the ability of the prey to perceive predators, and prey perceive predators through various sense organs (Hermann and Thaler, 2014). They might use multiple sensory systems individually or simultaneously (Rosier and Langkilde, 2011) and assess the magnitude of predation risk through multiple sensory systems (Gonzálvez and Rodríguez-Gironés, 2013). The FM-bat exposure group developed the fastest and had the lowest reproduction ability in this study, followed by the CF-bat exposure group, FM-call playback group, CF-call playback group, and White noise playback group. We discovered that exposure to bat predators had a greater negative impact than a single acoustic cue, with FM calls and FM bat predators having a greater negative impact than CF calls and CF bat predators. Previous research has shown that moth behavior and physiology vary with predation risk (Lalita and McNeil, 1998). *M. fuliginosus* prefers to forage in open farmland (Alberdi et al., 2020), *R. sinicus* prefers to forage in complex habitats (Aldridge and Rautenbach, 1987), and the former has a broader range of calls. The tympanic membranes of nocturnal moths can detect ultrasound frequencies from 10 to 100 kHz, but they are better at detecting frequencies between 20 and 50 kHz (Zha and Lei, 2012). The bandwidth of the echolocation calls of *M. fuliginosus* was larger than that of the CF bat *R. sinicus*. Thus, FM calls might pose a higher predation risk than CF calls. Noise is also a common abiotic environmental stress factor affecting organisms, which has a significant negative impact on the physiological ecology of organisms (Zha and Lei, 2012). In our study, the white noise frequency was 0–100 kHz which was wider than the bandwidth of two bats echolocation calls. However, the effects were lesser than those associated with bat predators and bat echolocation calls. Additionally, exposure to bats might be perceived as a greater predation risk by individuals of *S. litura* because they might detect chemical and visual cues of bats through smell or vision. We also found that acoustic cues had a greater effect on the pupae, which might be because the pupal shell prevented the detection of some of the chemical components, but acoustic signals could still be detected by *S. litura* through physical vibrations. Additionally, the effects of exposure to bats during the larval and adult stages were greater than the effects of exposure to a single acoustic cue, suggesting that *S. litura* might also perceive predation risk *via* vision and smell. These findings imply that *S. litura* can recognize various predation risks, assess the magnitude of predation risk, and devise appropriate defense strategies.

In conclusion, our results indicated that *S. litura* moths exposed to bat predation risk were in a state of chronic oxidative stress and suffered oxidative damage. Although the individuals showed varying degrees of adaptive responses to stress, they still had lower survival and reproduction. We found that the presence of bats might impose a greater risk of predation on *S. litura* than the presence of only their ultrasound calls. Our study showed that the NCEs associated with the risk of predation strongly influenced the size of the *S. litura* population in the laboratory, which might provide a novel approach to the biological control of *S. litura* in the field.

However, the echolocation calls used in this study were recorded while the bats were semi-starved, and the bats in the exposed group were also semi-starved, and it is unclear whether the hungry bats made the moths more terrified, which would require further investigation. The effects of bats on the population of *S. litura* moths in the field might be greater, which needs to be confirmed through field trials. Additionally, nocturnal predation by bats can significantly decrease agricultural pests on farmland (Rodríguez-San Pedro et al., 2020; Charbonnier et al., 2021), and the combination of direct and indirect effects of bats might be more effective in controlling pest populations than each effect in isolation. Field experiments will be conducted in the future to assess the effects of bat CEs and NCEs on moths, as well as the ability of bat biological control and the potential economic benefits.

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 authors.

Ethics statement

The animal study was reviewed and approved by Bat capture methods and experiments conformed to the Northeast Normal

University guidelines for animal behavior research. All husbandry and experimental procedures adhered to with the ASAB/ABS Guidelines for the Treatment of Animals in Behavioral Research and were approved by the Wildlife Conservation Office of Jilin Forestry Department, China.

Author contributions

TJ, WZ, and YL conceived the ideas and designed methodology. WZ and TL collected the samples and data. WZ analyzed the data. WZ and YL wrote the manuscript. TJ and JF revised the original draft. All authors contributed critically to the drafts and gave final approval for publication.

Funding

This research was supported by the National Natural Science Foundation of China (Grant Nos. 31922050, 31872680, and 32071492) and the Fund of the Jilin Province Science and Technology Development Project (Grant No. 20220101273JC).

Acknowledgments

We sincerely thank YinLi Hu and Jiqian Li for their great contributions to the recording of foraging echolocation calls. We are also grateful to Yang Gen and Yinli Hu for their contributions to larval and bat rearing.

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

References

Abom, R., and Schwarzkopf, L. (2016). Differential behavioural flexibility in response to predation risk in native and introduced tropical savannah rodents. *Anim. Behav.* 122, 117–124. doi: 10.1016/j.anbehav.2016.10.005

Agah-Manesh, H., Rajabpour, A., Yarahmadi, F., and Farsi, A. (2021). Potential of ultrasound to control *Sesamia cretica* (Lepidoptera: Noctuidae). *Environ. Entomol.* 50, 1393–1399. doi: 10.1093/ee/nvab103

Alberdi, A., Razgour, O., Aizpurua, O., Novella-Fernandez, R., Aihartza, J., Budinski, I., et al. (2020). DNA metabarcoding and spatial modelling link diet diversification with distribution homogeneity in European bats. *Nat. Commun.* 11:1154. doi: 10.1038/s41467-020-14961-2

Aldridge, H., and Rautenbach, I. (1987). Morphology, echolocation and resource partitioning in insectivorous bats. *J. Anim. Ecol.* 56, 763–778. doi: 10.2307/4947

Appel, G., López-Baucells, A., Rocha, R., Meyer, C. F., and Bobrowiec, P. E. D. (2021). Habitat disturbance trumps moonlight effects on the activity of tropical insectivorous bats. *Anim. Conserv.* 24, 1046–1058. doi: 10.1111/acv.12706

Baranowski, A. K., and Preisser, E. L. (2018). Predator cues increase silkmoth mortality. *Front. Ecol. Evol.* 6:220. doi: 10.3389/fevo.2018.00220

Barton, B. T. (2010). Climate warming and predation risk during herbivore ontogeny. *Ecology* 91, 2811–2818. doi: 10.1890/09-2278.1

Bauman, A. G., Seah, J. C. L., Januchowski-Hartley, F. A., Hoey, A. S., Fong, J., and Todd, P. A. (2019). Fear effects associated with predator presence and habitat structure interact to alter herbivory on coral reefs. *Biol. Lett.* 15:20190409. doi: 10.1098/rsbl.2019.0409

Benti, B., Miller, P. J. O., Biuw, M., and Curé, C. (2021). Indication that the behavioural responses of humpback whales to killer whale sounds are influenced by trophic relationships. *Mar. Ecol. Prog. Ser.* 660, 217–232. doi: 10.3354/meps13592

Brodin, T., and Johansson, F. (2004). Conflicting selection pressures on the growth/predation-risk trade-off in a damselfly. *Ecology* 85, 2927–2932. doi: 10.1890/03-3120

Carpenter, S. R., Cole, J. J., Hodgson, J. R., Kitchell, J. F., Pace, M. L., Bade, D., et al. (2001). Trophic cascades, nutrients, and lake productivity: Whole-lake experiments. *Ecol. Monogr.* 71, 163–186. doi: 10.1890/0012-9615(2001)071[0163:Tcnalp]2.0.Co;2

Charbonnier, Y., Papura, D., Touzot, O., Rhouy, N., Sentenac, G., and Rusch, A. (2021). Pest control services provided by bats in vineyard landscapes. *Agric. Ecosyst. Environ.* 306:107207. doi: 10.1016/j.agee.2020.107207

Cherbas, L., Koehler, M. M. D., and Cherbas, P. (1989). Effects of juvenile hormone on the ecdysone response of drosophila Kc cells. *Dev. Genet.* 10, 177–188. doi: 10.1002/dvg.1020100307

Cinel, S. D., Hahn, D. A., and Kawahara, A. Y. (2020). Predator-induced stress responses in insects: A review. *J. Insect Physiol.* 122:104039. doi: 10.1016/j.jinsphys.2020.104039

Cinel, S. D., and Taylor, S. J. (2019). Prolonged bat call exposure induces a broad transcriptional response in the male fall armyworm (*Spodoptera frugiperda*; Lepidoptera: Noctuidae) brain. *Front. Behav. Neurosci.* 13:36. doi: 10.3389/fnbeh.2019.00036

DeWitt, P. D., Visscher, D. R., Schuler, M. S., and Thiel, R. P. (2019). Predation risks suppress lifetime fitness in a wild mammal. *Oikos* 128, 790–797. doi: 10.1111/oik.05935

Duong, T. M., and McCauley, S. J. (2016). Predation risk increases immune response in a larval dragonfly (*Leucorrhinia intacta*). *Ecology* 97, 1605–1610. doi: 10.1890/15-1964.1

Fauteux, D., Gauthier, G., Berteaux, D., Palme, R., and Boonstra, R. (2018). High Arctic lemmings remain reproductively active under predator-induced elevated stress. *Oecologia* 187, 657–666. doi: 10.1007/s00442-018-4140-4

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

Fleig, R. (1995). Role of the follicle cells for yolk uptake in ovarian follicles of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae). *Int. J. Insect Morphol. Embryol.* 24, 427–433. doi: 10.1016/0020-7322(95)98841-Z

Fox, C. W., and Czesak, M. E. (2000). Evolutionary ecology of progeny size in arthropods. *Ann. Rev. Entom.* 45, 341–369. doi: 10.1146/annurev.ento.45.1.341

Frank, D. A. (2008). Evidence for top predator control of a grazing ecosystem. *Oikos* 117, 1718–1724. doi: 10.1111/j.1600-0706.2008.16846.x

Gao, B., Song, X. Q., Yu, H., Fu, D. Y., Xu, J., and Ye, H. (2020). Mating-induced differential expression in genes related to reproduction and immunity in *Spodoptera litura* (Lepidoptera: Noctuidae) female moths. *J. Insect Sci.* 20, 1–10. doi: 10.1093/jisesa/ieaa003

Gong, L., Geng, Y., Wang, Z., Lin, A., Wu, H., Feng, L., et al. (2022). Behavioral innovation and genomic novelty are associated with the exploitation of a challenging dietary opportunity by an avivorous bat. *iScience* 25:104973. doi: 10.1016/j.isci.2022.104973

Gonzálvez, F. G., and Rodríguez-Gironés, M. A. (2013). Seeing is believing: Information content and behavioural response to visual and chemical cues. *Proc. R. Soc. B Biol. Sci.* 280:20130886. doi: 10.1098/rspb.2013.0886

Guanghong, L., Qijin, C., and Yi, P. (1998). Studies of artificial diets for the beet armyworm, *Spodoptera exigua*. *Acta Sci. Nat. Univ. Sunyatseni* 37, 1–5.

Guo, P., Yu, H., Xu, J., Li, Y. H., and Ye, H. (2022). Flight muscle structure and flight capacity of females of the long-distance migratory armyworm, *Spodoptera frugiperda*: Effect of aging and reproduction, and trade-offs between flight and fecundity. *Entomol. Exp. Appl.* 1–11. doi: 10.1111/eea.13248

Hawlena, D., and Schmitz, O. J. (2010). Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *Proc. Natl. Acad. Sci. U. S. A.* 107, 15503–15507. doi: 10.1073/pnas.1009300107

Hermann, S. L., and Thaler, J. S. (2014). Prey perception of predation risk: Volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. *Oecologia* 176, 669–676. doi: 10.1007/s00442-014-3069-5

Higginson, A., and Ruxton, G. (2010). Adaptive changes in size and age at metamorphosis can qualitatively vary with predator type and available defenses. *Ecology* 91, 2756–2768. doi: 10.1890/08-2269.1

Huang, F., and Subramanyam, B. (2004). Behavioral and reproductive effects of ultrasound on the Indian meal moth, *Plodia interpunctella*. *Entomol. Exp. Appl.* 113, 157–164. doi: 10.1111/j.0013-8703.2004.00217.x

Huang, F., Subramanyam, B., and Taylor, R. (2003). Ultrasound affects spermatophore transfer, larval numbers, and larval weight of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). *J. Stored Prod. Res.* 39, 413–422. doi: 10.1016/s0022-474x(02)00035-8

Hughes, A. R., Mann, D. A., and Kimbro, D. L. (2014). Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. *Proc. Biol. Sci.* 281:20140715. doi: 10.1098/rspb.2014.0715

Janssens, L., and Stoks, R. (2013). Predation risk causes oxidative damage in prey. *Biol. Lett.* 9:20130350. doi: 10.1098/rsbl.2013.0350

Janssens, L., and Stoks, R. (2014). Chronic predation risk reduces escape speed by increasing oxidative damage: A deadly cost of an adaptive antipredator response. *PLoS One* 9:e101273. doi: 10.1371/journal.pone.0101273

Jones, G., and Teeling, E. C. (2006). The evolution of echolocation in bats. *Trends Ecol. Evol.* 21, 149–156. doi: 10.1016/j.tree.2006.01.001

Kim, K. N., Sin, U. C., Jo, Y. C., Huang, Z. J., Hassan, A., Huang, Q. Y., et al. (2019). Influence of green light at night on juvenile hormone in the oriental armyworm *Mythimna separata* (Lepidoptera: Noctuidae). *Physiol. Entomol.* 44, 245–251. doi: 10.1111/phen.12300

Krebs, C. J., Boutin, S., Boonstra, R., Sinclair, A. R. E., Smith, J. N. M., Dale, M. R. T., et al. (1995). Impact of food and predation on the snowshoe hare cycle. *Science* 269, 1112–1115. doi: 10.1126/science.269.5227.1112

Kunz, T. H., Braun de Torrez, E., Bauer, D., Lobova, T., and Fleming, T. H. (2011). Ecosystem services provided by bats. *Ann. N. Y. Acad. Sci.* 1223, 1–38. doi: 10.1111/j.1749-6632.2011.06004.x

Lalita, A., and McNeil, J. N. (1998). Predation risk and mating behavior: The responses of moths to bat-like ultrasound. *Behav. Ecol.* 9, 552–558. doi: 10.1093/beheco/9.6.552

Li, C., Yu, J.-F., Xu, J., Liu, J.-H., and Ye, H. (2012). Reproductive rhythms of the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *GSTF J. Biosci.* 2, 25–29. doi: 10.5176/2251-3140_2.1.18

MacLeod, K. J., Krebs, C. J., Boonstra, R., and Sheriff, M. J. (2018). Fear and lethality in snowshoe hares: The deadly effects of non-consumptive predation risk. *Oikos* 127, 375–380. doi: 10.1111/oik.04890

Malone, K. M., Powell, A. C., Hua, F., and Sieving, K. E. (2017). Bluebirds perceive prey switching by Cooper's hawks across an urban gradient and adjust reproductive effort. *Écoscience* 24, 21–31. doi: 10.1080/11956860.2017.1346449

McCauley, S. J., Rowe, L., and Fortin, M.-J. (2011). The deadly effects of "nonlethal" predators. *Ecology* 92, 2043–2048. doi: 10.1890/11-0455.1

Mitchell, M. D., and Harborne, A. R. (2020). Non-consumptive effects in fish predator–prey interactions on coral reefs. *Coral Reefs* 39, 867–884. doi: 10.1007/s00338-020-01920-y

Monaghan, P., Metcalfe, N. B., and Torres, R. (2009). Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecol. Lett.* 12, 75–92. doi: 10.1111/j.1461-0248.2008.01258.x

Moore, M. P., Lis, C., and Martin, R. A. (2018). Larval body condition regulates predator-induced life-history variation in a dragonfly. *Ecology* 99, 224–230. doi: 10.1002/ecy.2056

Mukherjee, S., Heithaus, M. R., Trexler, J. C., Ray-Mukherjee, J., and Vaudo, J. (2014). Perceived risk of predation affects reproductive life-history traits in *Gambusia holbrooki*, but not in *Heterandria formosa*. *PLoS One* 9:e88832. doi: 10.1371/journal.pone.0088832

Nakano, R., Ito, A., and Tokumaru, S. (2022). Sustainable pest control inspired by prey-predator ultrasound interactions. *Proc. Natl. Acad. Sci. U. S. A.* 119:e2211007119. doi: 10.1073/pnas.2211007119

Okuzaki, Y. (2021). Effects of body size divergence on male mating tactics in the ground beetle *Carabus japonicus*. *Evolution* 75, 2269–2285. doi: 10.1111/evo.14302

Parthasarathy, R., Sun, Z., Bai, H., and Palli, S. R. (2010). Juvenile hormone regulation of vitellogenin synthesis in the red flour beetle, *Tribolium castaneum*. *Insect. Biochem. Mol. Biol.* 40, 405–414. doi: 10.1016/j.ibmb.2010.03.006

Peacor, S. D., Barton, B. T., Kimbro, D. L., Sih, A., and Sheriff, M. J. (2020). A framework and standardized terminology to facilitate the study of predation-risk effects. *Ecology* 101:e03152. doi: 10.1002/ecy.3152

Peacor, S. D., Dorn, N. J., Smith, J. A., Peckham, N. E., Cherry, M. J., Sheriff, M. J., et al. (2022). A skewed literature: Few studies evaluate the contribution of predation-risk effects to natural field patterns. *Ecol. Lett.* 25, 2048–2061. doi: 10.1111/ele.14075

Peckarsky, B. L., Abrams, P. A., Bolnick, D. I., Dill, L. M., Grabowski, J. H., Luttbeg, B., et al. (2008). Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator-prey interactions. *Ecology* 89, 2416–2425. doi: 10.1890/07-1131.1

Peckarsky, B. L., Cowan, C. A., Penton, M. A., and Anderson, C. (1993). Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. *Ecology* 74, 1836–1846. doi: 10.2307/1939941

Plantevin, G., De Reggi, M., and Nardon, C. (1984). Changes in ecdysteroid and juvenile hormone titers in the hemolymph of *Galleria mellonella* larvae and pupae. *Gen. Comp. Endocrinol.* 56, 218–230. doi: 10.1016/0016-6480(84)90034-0

Rodríguez-San Pedro, A., Allendes, J. L., Beltrán, C. A., Chaperon, P. N., Saldarriaga-Córdoba, M. M., Silva, A. X., et al. (2020). Quantifying ecological and economic value of pest control services provided by bats in a vineyard landscape of Central Chile. *Agric. Ecosyst. Environ.* 302:107063. doi: 10.1016/j.agee.2020.107063

Rosier, R., and Langkilde, T. (2011). Behavior under risk: How animals avoid becoming dinner. *Nat. Educ. Knowl.* 2:8.

Roux, O., Renault, D., Mouline, K., Diabaté, A., and Simard, F. (2021). Living with predators at the larval stage has differential long-lasting effects on adult life history and physiological traits in two anopheline mosquito species. *J. Insect Physiol.* 131:104234. doi: 10.1016/j.jinsphys.2021.104234

Rubin, J. J., Hamilton, C. A., McClure, C. J., Chadwell, B. A., Kawahara, A. Y., and Barber, J. R. (2018). The evolution of anti-bat sensory illusions in moths. *Sci. Adv.* 4:eaar7428. doi: 10.1126/sciadv.aar7428

Sandhyarani, K., and Rani, P. U. (2013). Morphometric and developmental consequences in *Spodoptera litura* due to feeding on three varieties of sweet potato plants (*Ipomoea batata*). *Phytoparasitica* 41, 317–325. doi: 10.1007/s12600-013-0292-2

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

Schmitz, O. J., Rosenblatt, A. E., and Smylie, M. (2016). Temperature dependence of predation stress and the nutritional ecology of a generalist herbivore. *Ecology* 97, 3119–3130. doi: 10.1002/ecy.1524

Schnitzler, H. U., Moss, C. F., and Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends Ecol. Evol.* 18, 386–394. doi: 10.1016/S0169-5347(03)00185-X

Schwenke, R. A., Lazzaro, B. P., and Wolfner, M. F. (2016). Reproduction-immunity trade-offs in insects. *Annu. Rev. Entomol.* 61, 239–256. doi: 10.1146/annurev-ento-010715-023924

Sheriff, M. J., Peacor, S. D., Hawlena, D., and Thaker, M. (2020). Non-consumptive predator effects on prey population size: A dearth of evidence. *J. Anim. Ecol.* 89, 1302–1316. doi: 10.1111/1365-2656.13213

Sih, A., Crowley, P., McPeek, M., Petranka, J., and Strohmeier, K. (1985). Predation, competition, and prey communities: A review of field experiments. *Annu. Rev. Ecol. Syst.* 16, 269–311. doi: 10.1146/annurev.es.16.110185.001413

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

Sun, C., Jiang, T., Kanwal, J. S., Guo, X., Luo, B., Lin, A., et al. (2018). Great Himalayan leaf-nosed bats modify vocalizations to communicate threat escalation during agonistic interactions. *Behav. Process.* 157, 180–187. doi: 10.1016/j.beproc.2018.09.013

Süren-Castillo, S., Abrisqueta, M., and Maestro, J. L. (2012). FoxO inhibits juvenile hormone biosynthesis and vitellogenin production in the German cockroach. *Insect Biochem. Mol. Biol.* 42, 491–498. doi: 10.1016/j.ibmb.2012.03.006

Svensson, G. P., Skals, N., and Lofstedt, C. (2003). Disruption of the odour-mediated mating behaviour of *Plodia interpunctella* using high-frequency sound. *Entomol. Exp. Appl.* 106, 187–192. doi: 10.1046/j.1570-7458.2003.00022.x

Thaler, J. S., McArt, S. H., and Kaplan, I. (2012). Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proc. Natl. Acad. Sci. U. S. A.* 109, 12075–12080. doi: 10.1073/pnas.1208070109

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

van Uitregt, V. O., Hurst, T. P., and Wilson, R. S. (2012). Reduced size and starvation resistance in adult mosquitoes, *Aedes notoscriptus*, exposed to predation cues as larvae. *J. Anim. Ecol.* 81, 108–115. doi: 10.1111/j.1365-2656.2011.01880.x

Venkanna, Y., Suroshe, S. S., and Dahuja, A. (2021). Non-consumptive effects of the zigzag ladybird beetle, *Cheilomenes sexmaculata* (fab.) on its prey, the cotton aphid, *Aphis gossypii* glover. *Biocontrol Sci. Tech.* 31, 1204–1219. doi: 10.1080/09583157.2021.1937521

Villalobos-Sambucaro, M. J., Nouzova, M., Ramirez, C. E., Eugenia Alzugaray, M., Fernandez-Lima, F., Ronderos, J. R., et al. (2020). The juvenile hormone described in *Rhodnius prolixus* by Wigglesworth is juvenile hormone III skipped bisepoxide. *Sci. Rep.* 10:3091. doi: 10.1038/s41598-020-59495-1

Wilson, D. E., and Reeder, D. M. (2005). *Mammal species of the world: A taxonomic and geographic reference*. Maryland, USA: JHU Press.

Wu, C. X., Liu, J. F., Di, X. Y., and Yang, M. F. (2018). Delay in mating reduces Reproductivity but increases life span in tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 111, 1650–1657. doi: 10.1093/jee/toy108

Xiao, H.-Y., Li, G.-C., Wang, Z.-Q., Guo, Y.-R., and Liu, N.-Y. (2021). Combined transcriptomic, proteomic and genomic analysis identifies reproductive-related proteins and potential modulators of female behaviors in *Spodoptera litura*. *Genomics* 113, 1876–1894. doi: 10.1016/j.ygeno.2021.04.006

Xiong, X., Michaud, J. P., Li, Z., Wu, P., Chu, Y., Zhang, Q., et al. (2015). Chronic, predator-induced stress alters development and reproductive performance of the cotton bollworm, *Helicoverpa armigera*. *BioControl* 60, 827–837. doi: 10.1007/s10526-015-9689-9

Zamzow, J. P., Amsler, C. D., McClintock, J. B., and Baker, B. J. (2010). Habitat choice and predator avoidance by Antarctic amphipods: The roles of algal chemistry and morphology. *Mar. Ecol. Prog. Ser.* 400, 155–163. doi: 10.3354/meps08399

Zanette, L. Y., White, A. F., Allen, M. C., and Clinchy, M. (2011). Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* 334, 1398–1401. doi: 10.1126/science.1210908

Zha, Y.-P., and Lei, C.-L. (2012). Effects of ultrasound-stress on antioxidant enzyme activities of *Helicoverpa Armigera* (Hübner) (Lepidoptera: Noctuidae). *J. Agric. Urban Entomol.* 28, 34–41. doi: 10.3954/1523-5475-28.1.34

Zha, Y. P., Wu, X. L., Zhang, Z. Y., Chen, J. Y., and Chen, Q. C. (2021). Influence of ultrasound on juvenile hormone titers in *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). *Sci. Rep.* 11:1450. doi: 10.1038/s41598-021-81227-2

Zhang, C. M., Sun, C. N., Lucas, J. R., Feng, J., and Jiang, T. L. (2022). Acoustic signal dominance in the multimodal communication of a nocturnal mammal. *Curr. Zool.* 68, 592–599. doi: 10.1093/cz/zoab089

Zhou, Z. S., Chen, Z. P., and Xu, Z. F. (2010). Relations between air temperatures and mating behaviour of *Spodoptera litura* adults in nighttime in the tobacco field. *Afr. J. Agric. Res.* 5, 2994–2996.

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 21 April 2023
DOI 10.3389/fevo.2023.1150736

OPEN ACCESS

EDITED BY
Silvio Erler,
Julius Kühn-Institut, Germany

REVIEWED BY
Harmen P. Hendriksma,
Wageningen University & Research,
Netherlands
David W. Inouye,
University of Maryland, College Park,
United States

*CORRESPONDENCE
Jessie A. Thuma
✉ jessie.thuma@tufts.edu

RECEIVED 24 January 2023
ACCEPTED 03 April 2023
PUBLISHED 21 April 2023

CITATION
Thuma JA, Duff C, Pitera M, Januario N, Orians CM and Starks PT (2023) Nutrient enrichment and rainfall affect plant phenology and floral resource availability for pollinators. *Front. Ecol. Evol.* 11:1150736.
doi: 10.3389/fevo.2023.1150736

Nutrient enrichment and rainfall affect plant phenology and floral resource availability for pollinators

Jessie A. Thuma*, Christopher Duff, Madeline Pitera,
Nicholas Januario, Colin M. Orians and Philip T. Starks

Department of Biology, Tufts University, Medford, MA, United States

Wild pollinators are critical to maintaining ecosystem services and facilitating crop production, but habitat degradation and resource loss are leading to worldwide pollinator declines. Nutrient enrichment and changes in rainfall due to global warming are drivers of global environmental change, and likely to impact pollinator foraging behavior and reproductive success through changes to the growth and phenology of flowering plants. Here, we provide a short review of pollinator conservation in the context of nutritional ecology and plant-pollinator interactions. Then, we present novel research into the effects of nutrient and rainfall variation on plant phenology. In this study, we experimentally manipulated the amount of water and supplemental nutrients available to wild sunflower (*Helianthus annuus*) and goldenrod (*Solidago* spp.) throughout their growing season. We evaluated how changes in growth and bloom time could impact resource availability for bumble bee (*Bombus impatiens*) queens preparing to overwinter. We found that fertilizer and rainfall alter plant bloom time by 2–18 days, though flowering response was species-specific. Fertilizer did not significantly affect plant growth or number of flowers produced when plants were grown under drought conditions. When water was not limiting, fertilized sunflowers bloomed in floral pulses. These findings carry important implications for growers and land managers, providing insight into potential drivers of wild pollinator decline and possible conservation strategies.

KEYWORDS

pollination, drought, rainfall, nutrient enrichment, agriculture, bumble bees, phenology, global change biology

Introduction

Wild pollinators are essential to ecosystem function in natural systems and provide critical ecosystem services in agricultural systems (Vanbergen et al., 2013; Goulson et al., 2015). However, insect pollinators are in decline around the world, and their loss could have profound environmental, economic, and social consequences (Vanbergen et al., 2013; Goulson et al., 2015). Though managed honey bees (*Apis mellifera* Linnaeus) may provide adequate pollination to most crops, a diverse and abundant wild, native insect community can double fruit set even when honey bees account for half of all crop visitation (Garibaldi et al., 2013). Wild pollinators are particularly important to agricultural production as the majority of food crops require pollination to set fruit (Garibaldi et al., 2013). Though native bee communities could provide full pollination services to crops with heavy pollination requirements, the wild bee community on most farms is currently too small to provide sufficient pollination on their own due to lack of semi-natural habitat (Kremen et al., 2002).

Habitat loss and degradation due to climate change and land use intensification are in part responsible for insect pollinator decline (Vanbergen et al., 2013). Habitat alteration can directly impact pollinators or indirectly affect their performance through changes in floral abundance. For example, eutrophication may shift the abundance and timing of flowering in ways that may cause a phenological mismatch between flowering time and pollinator activity (David et al., 2019). Phenological mismatch as a potential driver of pollinator decline is generating increased attention (Ogilvie and Forrest, 2017; Stemkovski et al., 2020). Generalist pollinators rely on an abundant and diverse floral community for food resources from spring through fall in temperate regions (Woodard and Jha, 2017; Leach and Drummond, 2018). Since environmental change may alter plant nutritional chemistry, floral production, and flowering phenology, this may reduce both the quality and quantity of floral resources available to pollinators at critical periods in the foraging season (Ogilvie and Forrest, 2017). Resource loss and nutritional deficiencies leave wild insects more vulnerable to disease and can prohibit reproduction, reducing the pollinator community and pollination services to crops and other flowering plants (Roger et al., 2017). Climate change and agricultural intensification are two major drivers of habitat change that are rapidly altering growing conditions on regional and local scales (Brown et al., 2016; Ogilvie and Forrest, 2017; David et al., 2019; Descamps et al., 2021). Research in the field of nutritional ecology, the study of how an organism interacts with its environment to meet its nutritional needs, could provide a critical link in understanding how landscape-level changes directly impact pollinator health and behavior (Lihoreau et al., 2015; Woodard and Jha, 2017).

Inorganic chemical fertilizer, composed mainly of nitrogen, phosphorus, and potassium, is used widely in agriculture and land management to supplement nutrient-poor soil (Vitousek et al., 1997; Li et al., 2019). Nutrient enrichment from agrochemical runoff and industrial waste, a major driver of habitat change worldwide (David et al., 2019), may affect pollinators in diverse ways. While fertilizer application can result in plants that grow larger, faster, and yield more fruit (Muñoz et al., 2005; Burkle and Irwin, 2009; Li et al., 2019), fertilizer can also alter flower production and bloom time, and nectar and pollen quality (Hoover et al., 2012; David et al., 2019; Russo et al., 2020).

When fertilizer is applied in excess or at the wrong time in the growing season, nutrients can affect non-target plants and habitats through runoff (Shepherd et al., 2018; Russo et al., 2020). The result could be a change in the chemical landscape that reduces floral abundance by promoting nitrogen-limited fast-growth plants and shifting the phenology of those non-target plants (Hunter, 2016; David et al., 2019). Most agricultural fields produce flowers in synchronous pulses that do not offer a continuous supply of flowering plants throughout the growing season and may not offer a full range of essential nutrients (Goulson et al., 2008; Winfree et al., 2008). Wild pollinators must therefore rely on seminatural areas surrounding farms to fill these gaps in plant bloom (Goulson et al., 2008; Winfree et al., 2008).

When fertilizer runs into these adjacent seminatural fields, the timing of bloom may change to potentially widen gaps in resource availability. A change in flowering alters the availability of nectar and pollen to bees and nutrient enrichment can affect the quality of those resources. For example, fertilized *Succisa pratensis* (Devil's-Bit) plants, compared against unfertilized plants, produced pollen with a higher total amino acid concentration and altered amino acid profile that was associated with increased larval mortality in bumble bees (Ceulemans et al., 2017).

The effects of chemical fertilizer must be considered in the context of climate change, as environmental conditions alter how plants take up and use soil nutrients (Bassirirad, 2000; Walter, 2018). Though many plants can survive periods of low or high rainfall, extended dry or wet periods can be detrimental to plant growth and to pollinators relying on those plants (Lawson and Rands, 2019; Descamps et al., 2021). Climate change is predicted to bring increasingly long droughts and more frequent extreme precipitation events to many parts of the globe (Trenberth, 2008). Drought-stressed plants tend to bloom earlier, produce fewer flowers, restrict nutrient uptake from soil, and reduce nectar and pollen production (Shavrukov et al., 2017; Walter, 2018; Descamps et al., 2021). While adequate water will increase plant biomass and flower production (Zhang et al., 2020), excessive water that leaves soil saturated for extended periods can inhibit plant growth through reduced soil oxygen, root loss, nitrogen leaching, and limited nutrient uptake by plants (Bedard-Haughn, 2009). As such, fertilizer is likely to affect plants and their pollinators differently when applied under low vs. high rainfall conditions.

Studies in pollinator nutritional ecology examine the distribution and diversity of plants across the landscape and the quality of their floral rewards, which drives foraging behavior, delivery of pollination services, and population stability of both pollinators and plants (Woodard and Jha, 2017). Though insects need food throughout their lives, nutritional deficiencies during certain life cycle stages may have a disproportionate effect on reproductive success and population size. Using wild bees as an example: larvae cannot develop into functional adults without adequate food (Leach and Drummond, 2018); egg-laying females need the protein and fat in pollen to develop and maintain their ovaries (Leach and Drummond, 2018); and diapausing insects have short time frames to build fat body stores ahead of months-long diapause (Hahn and Denlinger, 2011; Woodard and Jha, 2017). Improper nutrition during any of these periods can result in population declines and a breakdown of plant-pollinator networks. In eusocial species like bumble bees (*Bombus* spp.), which rely on a single queen to survive diapause, initiate nests, and reproduce, the effect of inadequate nutrition on population size and pollination may be magnified.

Bumble bees are among the most important native pollinators for agricultural fields in the US, serving as the primary pollinators of crops like tomatoes, blueberries, and melons, and in some regions providing the majority of crop visitation (Winfree et al., 2008). Several species of bumble bees are in decline in North America and local population distributions are changing, due in large part to habitat loss and disease (Cameron et al., 2011; Carvell et al., 2011). In Europe, bumble bee species declines are closely linked to habitat loss and a narrowing of floral resource diversity and abundance from agricultural intensification (Goulson et al., 2008). As generalist pollinators with months-long flight seasons, bumble bees depend on phenological variation in plant communities to provide diverse floral resources from spring to early fall (Rundlöf et al., 2014; Mallinger et al., 2016; Malfi et al., 2019). The bumble bee colony cycle lasts for approximately 1 year (Alford, 1975; Goulson, 2003). Solitary queens establish nests underground or in thatched grass in the spring and lay eggs that develop into workers. Once workers emerge, queens remain in the nest and no longer forage. During the summer, queens produce workers that forage for food or care for brood before switching from worker production to male and new queen production in the fall. A new queen mates and then diapauses underground through the winter before initiating her own nest in the spring (Alford, 1975; Goulson, 2003).

There are several important nutritional windows for bumble bees, with most research focusing on nest initiation in the spring and gyne production in the fall. Early season resources can dictate a colony's rate of growth, maximum size, and whether the colony will reach the reproductive switch point (Crone and Williams, 2016; Malfi et al., 2019). Late-season resource availability affects new queen production, and the success of these new queens in preparing for diapause (Rundlöf et al., 2014; Woodard et al., 2019; Timberlake et al., 2020). Bumble bees can store food in their colonies for just a few days, requiring food resources to be available near continuously (Goulson, 2003). Larvae require approximately 8 more days of feeding to develop into queens rather than workers (Cnaani et al., 2002). The feeding period for queen larvae is typically 14–20 days followed by approximately 10 days pupation, while worker feeding period lasts approximately 7–11 days followed by a 10-day pupation (Cnaani et al., 2002). Body size is strongly associated with nutrition during larval development, and small queens are unlikely to survive diapause (Owen, 1988; Couvillon and Dornhaus, 2009). For queens that eclose at appropriate body size and weight, nutrition after eclosion is a critical period as mass gain in the week post-eclosion is a strong predictor of overwintering survival (Woodard et al., 2019; Treanore and Amsalem, 2020). New queens have only 6–7 days within their natal nests after emergence to build the necessary fat and energy stores for 6–9 months of overwintering (Woodard et al., 2019).

If a new queen does not consume enough food during larval development or post-eclosion to build energy stores, she may not survive overwintering or will emerge from diapause too weak to initiate a nest (Woodard et al., 2019; Timberlake et al., 2020; Treanore and Amsalem, 2020). Insufficient food during larval development would result in small queens unable to build fat body stores post-eclosion (Owen, 1988; Couvillon and Dornhaus, 2009). Poor nutrition also leaves her more vulnerable to disease or parasitoids, like the gut parasite *Crithidia bombi*, that can diminish diapause survival and spring nest initiation (Brown et al., 2003; Schlüns et al., 2010). If fertilizer or rainfall extremes alter the timing or quality of blooms in the late summer or early fall, then colonies may not have the food resources needed to produce queens or for queens to survive diapause (Aldridge et al., 2011). In this study, we examine how agricultural practices and climate change are altering the timing of plant growth and flowering in ways that could reduce resource availability to bumble bees during colony reproduction.

Using the common eastern bumble bee (*Bombus impatiens*) as a model pollinator, we measured growth in wild sunflower (*Helianthus annuus*) and goldenrod (*Solidago* spp.) under different fertilizer and precipitation treatments, and tracked bumble bee forager activity and timing of gyne emergence, to identify phenological mismatch between bloom time and colony reproduction. Wild sunflower and goldenrod are both commonly found on farmland in New England and can bloom into early October (Dr. R. Malfi, Dr. L. Russo, personal communication). These species were selected to represent flowering plants on farms or along field margins that provide important late-season food resources to pollinators and could be target or non-target recipients of chemical fertilizer (Kremen and M'Gonigle, 2015; Russo et al., 2020). This study took place over 2 years and captured the effects of fertilizer as it interacted with rain along a continuum from months-long drought in 2020 to extremely high rainfall in 2021.

Several studies have found that fertilizer and changes in rainfall impact plant phenology. Fertilizer can accelerate flowering or extend a plant's bloom period (Burkle and Irwin, 2009; Russo et al., 2020), while drought can induce early flowering as a mechanism of drought escape (Nord and Lynch, 2009; Shavrukov et al., 2017; Phillips et al., 2018). Plants grown with sufficient water can increase flower production or bloom early, but excessive water can hinder growth (Bedard-Haughn, 2009; Huang et al., 2018). Plant responses to resource variation are species specific, as one study found that nitrogen addition together with water addition accelerated flowering onset in some species of desert annuals, while delaying onset in others (Huang et al., 2018). However, no studies have examined the combined effects of fertilizer and rainfall on plant phenology through the lens of pollinator nutritional ecology.

Given previous research, we predict that fertilizer and drought will create resource gaps in the late growing season that leave bumble bees at risk for nutritional stress as colonies switch to reproduction (**Figure 1**). Fertilized plants will bloom earlier than unfertilized plants regardless of water availability, with increased bloom duration and flower production in normal to high rainfall. We predict that bloom duration, flower production and plant growth will decline when plants are grown under both low and very high rainfall conditions. However, fertilizer may replace nutrients leached from soil under high rain, mitigating some of the negative effects of excessive water on growth.

Fertilizer and drought are two stressors affecting pollinators across biological scales, from changes in individual plant physiology and single colony nutrition that trickle up to affect pollinator foraging selection, delivery of pollination services, and population dynamics (Brown et al., 2016; Walter, 2018; David et al., 2019). Studying these drivers of global change biology as they affect pollinator health and reproduction, may provide important insight into disruptions in critical plant-pollination networks.

Materials and methods

Site description

Experiments to study the effects of nutrient enrichment and precipitation variation on plant growth took place in 2020 and 2021 at the Boston Area Climate Experiment (BACE) in Waltham, MA (42° 23′ 3″ N, 71° 12′ 52″ W). BACE was established in 2007 as

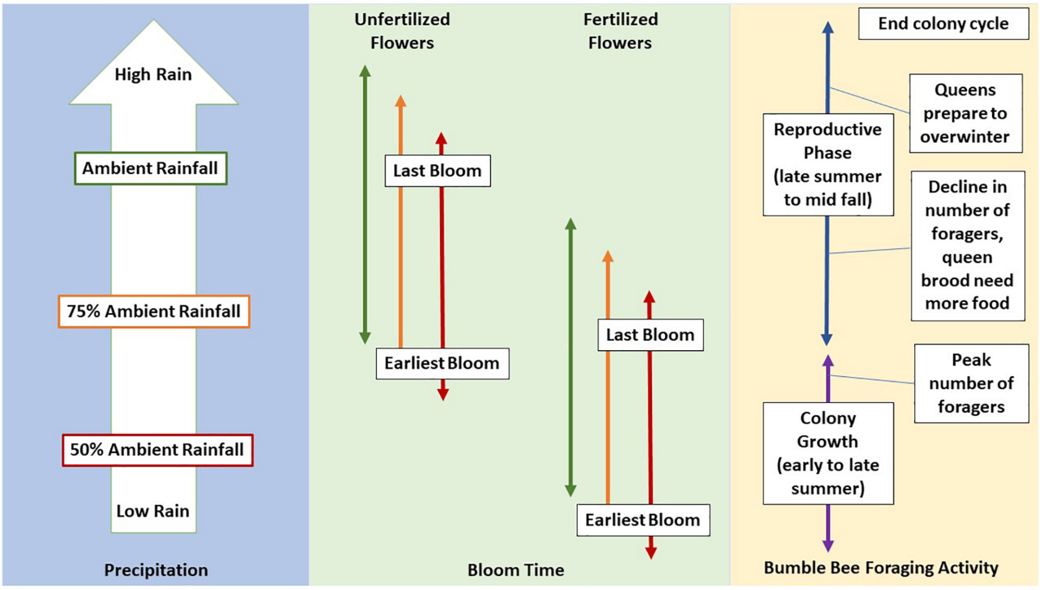


FIGURE 1
Predicted effect of fertilizer and drought on phenology. We predict that fertilizer and rain treatments will result in an increase in blooming floral resources earlier in the season and a reduction in resources available to reproductive colonies in the fall. Lines are colored by precipitation and represent time from summer to fall.

a long-term study site to learn how ecosystems would respond to changes in rainfall due to climate change (Hoeppner and Dukes, 2012). The field site is located on a four-acre organic farm with an active community garden. BACE is a previously managed old field system with three experimental blocks each consisting of three precipitation treatments: 100% ambient rainfall, 75% ambient rainfall, and 50% ambient rainfall. The latter two rain treatments represent drought conditions under average rainfall. Precipitation in each treatment was controlled by clear corrugated polycarbonate slats spaced at regular intervals above experimental plots to allow 75 or 50% rainfall to reach the plots below. The ambient treatment plots were covered with deer fencing to reduce photosynthetically active radiation by about 5% to approximate light interception by the polycarbonate slats in the drought treatments (Hoeppner and Dukes, 2012; Scott et al., 2019).

The study site receives approximately 8–11 cm of precipitation per month, based on the last 30 years of rainfall data in eastern Massachusetts (National Oceanic and Atmospheric Administration [NOAA], 2021). In the northeastern US, climate change is predicted to bring more frequent, prolonged droughts punctuated by heavy rainfall events (Runkle et al., 2017). In 2020, a prolonged drought affected BACE from July to October (Lombard et al., 2020), with 6.8 cm rain in June, 5 cm in July, and 5.8 cm in August (National Oceanic and Atmospheric Administration [NOAA], 2021). In 2021, eastern MA experienced the wettest July on record with 25.4 cm of rain. June 2021 received 6.5 cm of rain, and 17.8 cm of rain in August (National Oceanic and Atmospheric Administration [NOAA], 2021). The result is a precipitation continuum that ranges from extremely dry in the 2020 50% ambient (8.8 cm total rain June-August) rainfall treatment to extremely wet in the 2021 100% ambient rainfall treatment (49.7 cm total rain June-August), allowing us to examine the effects of both precipitation extremes predicted with climate change.

Study system

Pollinators

The common eastern bumble bee (*B. impatiens*) is a frequent forager in New England farm fields and a bumble bee species with one of the longest summer foraging periods (Novotny et al., 2021; Pugesek and Crone, 2021). *B. impatiens* fly until the end of October in Eastern Massachusetts, producing queens and males from August to October (Pugesek and Crone, 2021). As one of the few bees still active in September and October, growers may rely disproportionately on *B. impatiens* for fall crop and plant pollination. Though this species is not in decline in Massachusetts, a decline in local populations could create a problem for fall crop pollination.

Plants

Sunflower and goldenrod are native to North America, common to farm fields in New England, and flower in the late summer or early fall. Wild sunflowers are annuals and at times planted as crops for seeds, oil, or cut flowers (Kaya et al., 2012). Plants typically bloom from July to October in New England. Goldenrod are perennials and often grow along field margins in undisturbed ground, or are planted in pollinator habitat (Werner et al., 1980). Goldenrod occur in clonal stands, in which stems form at a rhizome node and grow outward from a central area (Werner et al., 1980). Goldenrod bloom from August to October. Both species are considered drought tolerant.

Goldenrod and sunflower provide important nutritional resources for late-season foragers and reproductives in bumble bee colonies. Sunflower pollen is typically low in protein but carries important medicinal properties that can reduce instances of the gut pathogen *Crithidia bombi*—a pathogen that can reduce queen overwintering survival and nest initiation in the spring

(Garibaldi et al., 2013; Adler et al., 2020). Goldenrod supplies an important source of pollen and nectar for bumble bees as new queens are produced in the fall at a time when overall floral abundance is declining (Oertel, 1967; Ziska et al., 2016).

Experimental approach

Plant propagation

For goldenrod (*Solidago* spp.), severed rhizomes were obtained from an existing clonal stand at BACE in both 2020 and 2021. To limit transplant shock the apical meristem was removed so that each stem was approximately 50 cm in height.

For wild sunflowers (*Helianthus annuus*), different seed sources were used for 2020 and 2021. In 2020, goldenrod were transplanted and sunflowers were planted from seed (Silver State seeds, Great Basin Supply) directly into pots in the field on June 12–13th, 2020. Seed germination was low in 2021, requiring us to source plants of three different sunflower varieties. These three varieties included: wild-type *H. annuus* seeds (American Meadows) planted in basic potting mix (Lambert Professional Growing Media, Germination and Seedlings) in a greenhouse on Tufts University campus; seedlings of the Soraya variety purchased from Russell's Garden Center in Wayland, MA, USA, and multi-headed wild sunflowers seedlings of unknown heritage sourced from a local grower in Burlington, MA, USA, and grown in basic potting mix. Hereafter, these varieties will be referred to as Wild-Type, Soraya, and S3.

Each sunflower treatment (2 m × 1 m plot) contained 1 Wild-Type, 2–3 S3, and 4 Soraya sunflowers. Sunflower were transplanted on June 24th and goldenrod on June 9th (goldenrod control transplanted June 19th). All plots were watered for the first 2 weeks after transplant as needed, and then watered only by rainwater. In watering control plots, plants were watered when soil moisture was low based on soil moisture measurements and visual inspection. Watering was needed only in June of 2021 as July–September saw record weekly rainfall.

Treatments

Sunflowers and goldenrod were grown under two fertilization treatments (fertilized and unfertilized) and three precipitation treatments (50, 75, and 100% ambient rainfall) (**Supplementary Table 1** and **Figure 1**). In each of the three precipitation treatments, sunflower and goldenrod were planted in separate 2 m × 2 m (meter) plots, separated by at least 2 m to avoid water pooling between plots, and each plot divided into two 2 m × 1 m sections. Twelve plastic 2-gallon pots were nested below the soil in each 2 m × 1 m section of the plot, with one section randomly assigned to the fertilized treatment (**Supplementary Table 1**). Plants were arranged in two rows within a treatment, and the orientation of those rows (North-South or East-West) alternated between treatment blocks to control for variation in sunlight. Fertilized plants received 15 mL of controlled-release fertilizer pellets (Osmocote, 14-14-14 NPK) mixed in the top 5 cm of soil on the day of planting. Plants were watered manually in the first 2 weeks after planting, and then watered only by rainfall for the remainder of the growing season.

In 2021, the same planting design was used in the 100, 75, and 50% rainfall plots as in 2020, with 12 plants per goldenrod plot and 8 plants per sunflower plot. An additional manually watered control treatment was added to BACE to ensure at least one treatment with adequate water in the case of drought. Each water control treatment consisted of a 2 m × 1 m plot, divided into two equally sized 1 m × 1 m halves that each contained either 6 pots of goldenrod, or 4 pots of sunflowers.

Plant measurements

In 2020, sunflower and goldenrod heights were measured twice per week throughout the month of August, and the number of flowering units within a treatment were counted throughout the bloom period. We recorded an average height of plants for each treatment replicate (2 m × 1 m plot) rather than record height for individual plants. During the month of August, pollen was manually removed from sunflower heads for a separate experiment, and data on flowering phenology were not recorded for sunflowers in 2020.

In 2021, height, flower number, and leaf size were recorded once per week for each sunflower from the time of transplanting through bloom until all flower heads were senesced. The number of leaves and nodes along the stalk were also recorded weekly until the first sunflower blooms appeared in July (**Supplementary Table 2**). Height and flower number were recorded once per week for each goldenrod plant from the time of transplanting to senescence. The width of 1–3 sunflower heads from each sunflower plant and the length of 1–3 goldenrod inflorescences from each goldenrod plant were recorded once during the growing period (**Supplementary Table 2**). Volumetric water content was measured weekly from each pot using a soil moisture probe (Campbell Scientific Hydrosense II) (**Supplementary Table 3**). When plants were in bloom, the number of flowering units in each sunflower or goldenrod treatment replicate was counted three times each week from the time of first bloom until final bloom, to provide more detailed phenology data.

Bumble bee surveys

To determine the timing of bumble bee reproductive activity, specifically the duration of queen development and timing of emergence in the field, we surveyed bumble bee visitation to flowers in the neighboring community garden and farm three times per week in 2021 (methods modified from Pollard, 1977). Males are produced before queens, and male emergence is thought to cue the rearing of queen larvae (Goulson, 2003; Belsky et al., 2020). Field observations of males would likely signal the start of queen production within the nest, and the time between male emergence and queen emergence would therefore represent queen larval development plus the 6–7 day preparation to leave the nest (Goulson, 2003; Woodard et al., 2019; Belsky et al., 2020; Treanore and Amsalem, 2020).

We surveyed bumble bee activity three times per week, weather permitting, from July 26th when the first sunflowers opened until October 22nd when the last goldenrod flowers senesced. Surveys took place between 9:00 and 16:00 when temperatures were above 60 degrees F, and never in rainy conditions. In total, we surveyed 4 community garden plots, and 3 locations on the farm. Survey plots were added in the community garden as the growing season

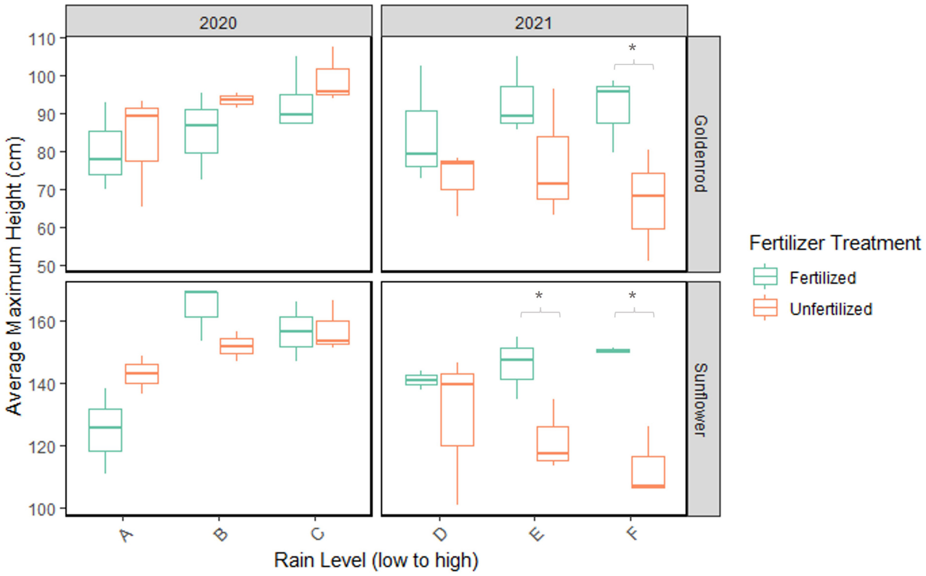


FIGURE 2
Maximum goldenrod and sunflower height (cm) in 2020 and 2021 from July to August. Rain level along the *x*-axis displays precipitation treatments (A–C represent 50–100% rainfall in 2020; D–F represent 50–100% rainfall in 2021). Boxplots represent the maximum height reached in each of 3 treatment replicates, measured by average plant height within the plot (n = 12 in goldenrod; n = 7 in sunflower). Averages for sunflower include three sunflower varieties in each plot. Fertilized treatments in green, unfertilized in orange. Rainfall totals for each treatment from June to August of each year from low to high: 8.8 cm (A), 13.2 cm (B), 17.6 cm (C), 24.9 cm (D), 37.3 cm (E), 49.7 cm (F). Each *represents statistical difference ($p < 0.05$) in average maximum height (cm) between fertilized vs. unfertilized plants.

progressed and some plants were no longer flowering while others started to bloom. On the farm, we surveyed a strip of pollinator garden in the middle of the farm field, one pollinator garden along the edge of the farm, and a selection of row flowers grown for cut flowers (**Supplementary Table 4**). For each survey, an observer walked slowly (approximately 20–25 steps/min) once along the perimeter of or within the survey area, depending on size and accessibility, and recorded each bumble bee that was on a flower. Bumble bees were identified to species (Three species largely found in this area from August to October: *Bombus impatiens*, *Bombus griseocollis*, and *Bombus vagans*) and caste (i.e., worker, male, queen), but only *B. impatiens* data were used for this study (**Supplementary Table 4**). We chose walking surveys rather than stationary timed surveys because we tested both methods before surveys began and found that walking surveys reduced the likelihood of counting the same individual more than once.

We also surveyed bumble bee visitation in experimental plots within BACE three times per week from the time of the first flower in a treatment replicate until the final flower senesced. Each survey lasted for 5 min, to capture a snapshot of bee visitation. Stationary, timed surveys were used for experimental plots because each plot had fewer flowering units overall than the farm or community garden, and plants were evenly spaced. We were thus unlikely to count the same bee multiple times.

Data analysis

We used generalized linear mixed models (GLMMs) to compare changes in plant growth and flower production as they varied with fertilizer and rain treatments. All statistical analyses were performed in R version 3.6.1. We used the functions glmer() or lmer() from the package "lme4"for all GLMMs (Bates et al., 2015). Separate analyses were performed for goldenrod and sunflower, and each sunflower variety was analyzed both in a single model and separately. Data from 2020 were analyzed separately from 2021 because rain totals varied dramatically (17.6 cm rain from June-August in 2020, 49.7 cm rain from June-August in 2021). Soil moisture content was not measured in 2020 so exact comparisons of rain treatments between years are not possible.

To measure maximum plant height (cm), leaf size (cm), and inflorescence length/width (cm), we used normally distributed GLMMs with fertilizer and rain as fixed effects, and either plot ID, greenhouse, and/or observer as random effects. Whether fertilizer and rain were included as an interactive effect, and which random effect was included, was decided using Akaike Information Criterion (AIC). Models to compare height in 2020 goldenrod included an offset for number of plants because height data were collected as an average value for the entire plot rather than for individual plants. In 2021, sunflower variety was included as a fixed effect when comparing all varieties in a single model. The number of leaves per plant was compared using GLMMS with a Poisson distribution, fertilizer and rain treatment as fixed effects, and greenhouse as a random effect.

To compare the number of flowers produced over the growing period in each treatment, we used either normally distributed or negative binomial GLMs, selecting the distribution that best fit the data using AIC. We summed all flowering unit counts over the full season for each plot and interpreted this value as the number of "flower days," a value that reflects both how many flowers were produced and how many days these flowers remained open. In this way, a single open flower can be counted multiple times if it remained open and available to pollinators for several days. This resulted in a single flower count for each treatment replicate. For some plant varieties, this count was large enough to fit a Gaussian distribution. Fertilizer and rain variety were included as fixed effects, with observer, greenhouse, or plot ID as random effects. Number of plants in each treatment replicate was included as an offset in all models of flower counts.

We used quantile regression to estimate patterns in the start and end of bloom in goldenrod and sunflowers across treatments, and to estimate timing of bumble bee foraging activity between workers, males and new queens (Cade and Noon, 2003). The onset of bloom or caste observations was estimated at the slope of the 0.2 quantile of flowering unit observations as a function of fertilizer and/or rain, or of the slope of the 0.2 quantile of bumble bee counts (Michielini et al., 2021). The end of bloom or bumble bee observations was estimated at the slope of the 0.8 quantile (Michielini et al., 2021). Predictor variables were compared using marginal hypothesis testing by hand, adding or removing variables from models and using anova.rq() to determine whether the main effects of rain and fertilizer or their interaction had a significant effect on bloom onset. If removing the variable did not result in a significantly worse model, the variable was removed from the analysis. Quantile regression was performed using the rq() function from the package “quantreg” (Koenker et al., 2019).

Phenology analysis of bumble bee activity used only data from farm and community garden surveys because these captured a wide variety of flowering plants and their bee visitors. Experimental plots had low visitation with single plant varieties in small survey areas relative to field surveys and may bias slope coefficients at 0.2 and 0.8 quantiles. The switch to reproduction in bumble bee colonies at this site was identified by the switch from majority workers to majority males in bumble bee counts during surveys. Since male production typically precedes queen production in bumble bee colonies (Goulson, 2003), we used the relative abundance of males to estimate reproductive switchpoint and queen development period.

Results

Plant growth

In 2020, when all plants grew under some level of water deficit, both goldenrod and sunflower height increased with rainfall while fertilizer did not increase plant growth (**Figure 2**). Rain had a significant effect on height in goldenrod (GLM, $\chi^2 = 7.0$, df = 2, p-value = 0.0303) and sunflower (GLM, $\chi^2 = 23.01$, df = 2, p-value < 0.0001), but there was no significant difference in height between fertilizer treatments in either species. Goldenrod height increased with rainfall in both fertilized and unfertilized treatments. Fertilized sunflowers grew larger in the 100% ($t = 3.319$) and 75% ($t = -4.601$) rain treatments than in the 50% rain treatments (pairwise comparison, Tukey’s adjustment, p-value < 0.0226).

In 2021, one of the wettest summers on record in Massachusetts, goldenrod and sunflower height generally increased with fertilizer, but plateaued or decreased as rain increased from 50 to 100% ambient rainfall (**Figure 2**). However, specific growth responses to fertilizer and rainfall varied between species and among sunflower varieties (**Figure 3**). Fertilizer had a significant effect on goldenrod height (GLM, $\chi^2 = 13.06$, df = 1, $p = 0.0003$) and fertilized goldenrod grew larger than unfertilized in 100% rain (pairwise comparison, Tukey’s adjustment, $t = 2.75$, $p = 0.0174$) (**Figure 2**). There was a significant effect of fertilizer (GLM, $\chi^2 = 51.21$, df = 1, p-value < 0.0001), plant variety (GLM, $\chi^2 = 157.02$, df = 2, p-value < 0.0001), and the interaction between fertilizer and plant variety (GLM, $\chi^2 = 18.07$, df = 2, p-value = 0.0024) on sunflower growth (**Figure 3**). The S3 sunflower variety showed no significant difference in growth between rain and fertilizer treatments. In the Soraya variety, there was a significant effect of fertilizer but not rainfall on growth (GLM, χ^2 10.99, df = 1, p-value = 0.0009), and fertilized plants in 100% ($t = 2.76$) and 75% ($t = 2.42$) rain grew larger than unfertilized (pairwise comparison, Tukey’s adjustment, p-value < 0.0328). And in the Wild-Type variety, fertilizer (GLM, $\chi^2 = 8.12$, df = 1, p-value = 0.0044) and rain (GLM, $\chi^2 = 7.82$, df = 2, p-value = 0.0200) main effects were significant predictors of plant growth but with no interaction.

For full summary of plant growth data, refer to **Supplementary Table 5**.

Total flower production

In 2020 we counted a total of 2,545 flowers on fertilized goldenrod and 2,709 flowers on unfertilized goldenrod. In 2021, we counted 2,453 flowers on fertilized goldenrod and 1,134 flowers on unfertilized goldenrod. Goldenrod produced more flowers with more water in both fertilized and unfertilized treatments, but only when water was limiting in 2020. When water was not limiting (2021), fertilized goldenrod produced more flowers than unfertilized and flower production increased with rainfall (**Figure 4**). In 2020, we found that rain has a significant effect on goldenrod flower production (negative binomial GLM, $\chi^2 = 8.04$, df = 2, p-value = 0.018) while fertilizer did not influence number of flowers. A total of 100% rain treatments produced significantly more flowers (348 ± 65 95% CI) than 50% rain treatment (245 ± 41) (pairwise comparison, Tukey’s adjustment, $t = 2.71$, p-value = 0.0182). In 2021, fertilizer (negative binomial GLM, $\chi^2 = 41.89$, df = 1, p-value < 0.0001) and the interaction between fertilizer and rain (negative binomial GLM, χ^2 7.28, df = 2, p-value = 0.0263) had significant effects on goldenrod flower production. Rain alone did not have a significant effect on flower production (negative binomial GLM, χ^2 0.422, df = 2, p-value = 0.8096). Fertilized goldenrod produced more flowers in 100% (241 ± 50 fertilized, 130 ± 25 unfertilized) ($t = 4.30$) and 75% (267 ± 46 fertilized, 126 ± 24 unfertilized) ($t = 5.31$) rain treatments (pairwise comparison, Tukey’s adjustment, p-value < 0.0001).

In 2021, we counted a total of 2,616 flowers in fertilized sunflowers and 2,765 flowers in unfertilized sunflowers. Sunflowers grown in 50% rain had significantly more “flower days” than those under 75 and 100% rainfall. However, specific responses to fertilizer and rainfall varied between sunflower varieties (**Figure 5**). Flower production of sunflowers in 2020 was not analyzed due to manipulation of flowering heads for a separate experiment that affected number of flowers per plant. In a model that includes all plant varieties, rainfall and plant variety had significant effects on the number of flowers produced (negative binomial GLM, $\chi^2 = 7.93, 203.55$; df = 2, 2; p-value < 0.0190). Each of the three

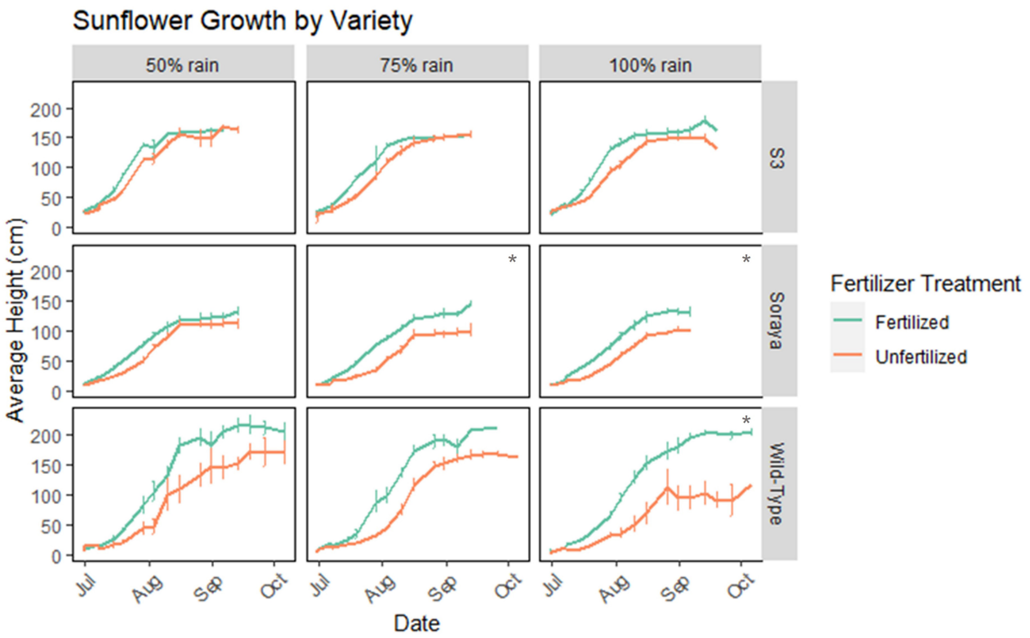


FIGURE 3

Average height (cm) of three sunflower varieties in July–October of 2021. Height is averaged over three replicate treatments of each rainfall and fertilizer treatment. Each replicate plot contained 4 Soraya plants, 2–3 S3 plants, and 1–2 Wild-Type plants. Each *represents statistical difference ($p < 0.05$) in average maximum height (cm) between fertilized vs. unfertilized plants. In all species, there was no significant difference in maximum growth between rain treatments. Error bars represent standard error.

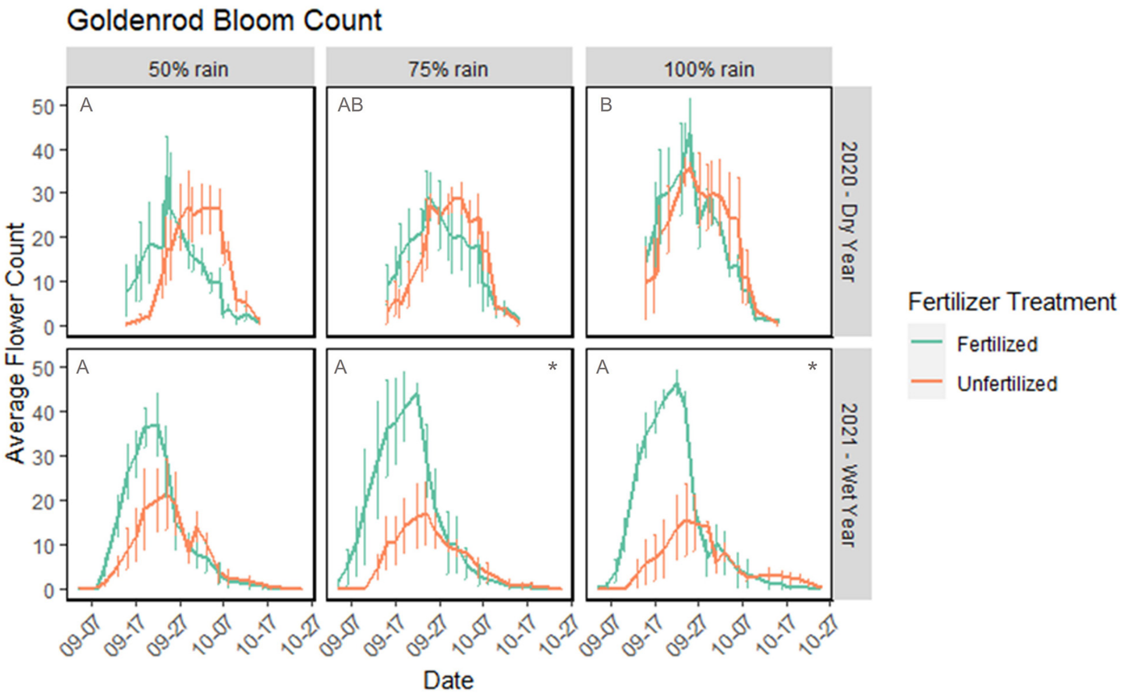


FIGURE 4

Average number of flowering units in goldenrod treatments over time in 2020 (dry year) and 2021 (wet year). Number of flowers averaged over three replicate treatments of each rainfall and fertilizer treatment. Each replicate contained approximately 12 plants. Fertilized treatments in green and unfertilized in orange. Each *represents statistical difference ($p < 0.05$) in total flowers produced between fertilized vs. unfertilized plants. Letters represent difference in flower production between rainfall treatments within a given year (row). Error bars represent standard error.

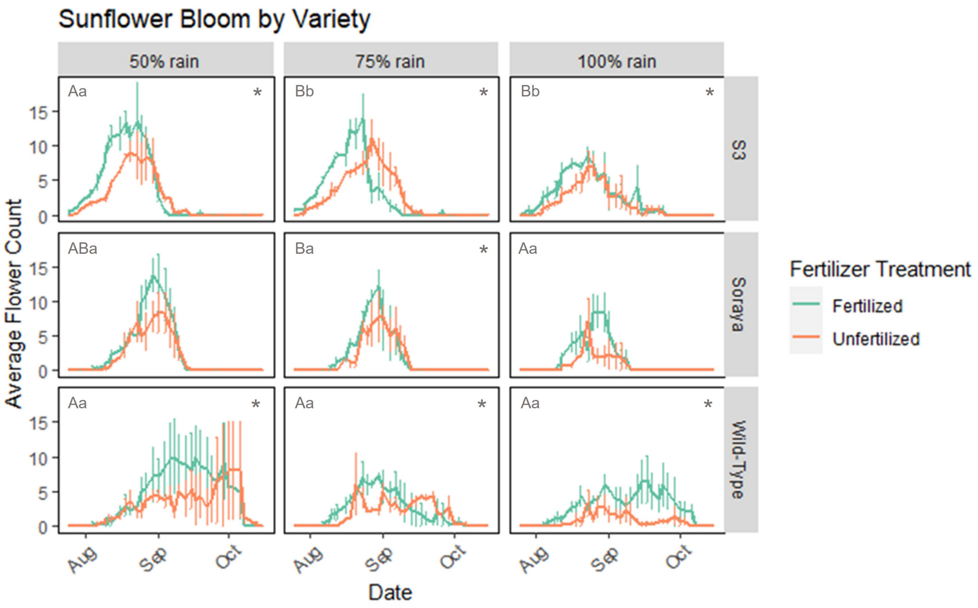


FIGURE 5
Average number of flowering units in three sunflower varieties over time in 2021 (wet year). Number of flowers averaged over three replicate treatments of each rainfall and fertilizer treatment. Each replicate plot contained 4 Soraya plants, 2–3 S3 plants, and 1–2 Wild-Type plants. Fertilized treatments in green and unfertilized in orange. Each *represents statistical difference ($p < 0.05$) in total flower days (not flowering rate) between fertilized vs. unfertilized plants. Upper case letters (AB) represent differences in total flower days with rain treatments among fertilized plants in a given sunflower variety (row). Lower case letters (ab) represent differences with rain treatment among unfertilized plants in a given variety. Error bars represent standard error.

varieties produced more flowers over the growing period in the 50% rain treatment than in the 75 and 100% rain treatments.

In analyzing each 2021 variety separately, fertilizer (GLM, $\chi^2 = 9.60$, df = 1, p-value = 0.0020) and rain (GLM, $\chi^2 = 16.72$, df = 2, p-value = 0.0002) had significant effects on number of flowers produced in S3 sunflowers. S3 sunflowers in 50% rain produced significantly more flowers than those growing in 75% ($t = 2.55$) and 100% ($t = -4.04$) rain and unfertilized S3 sunflowers produced significantly more flowers across all rain treatments (pairwise comparison, Tukey's adjustment, p-value < 0.0289).

In Soraya variety, fertilizer (negative binomial GLM, $\chi^2 = 5.02$, df = 1, p-value = 0.0250) and the interaction between fertilizer and rain (negative binomial GLM, $\chi^2 = 7.40$, df = 2, p-value = 0.0248) had significant effects on number of flowers produced. Unfertilized Soraya produced more flowers than fertilized in 75% rain (pairwise comparison, Tukey's adjustment, $t = -3.14$, $p = 0.0017$), and fertilized plants in 75% rain produced fewer flowers than fertilized in 100% ($t = 3.20$) and 50% ($t = 2.60$) rain (pairwise comparison, Tukey's adjustment, p-value < 0.0259). There was no significant difference in number of flowers produced between rain treatments in unfertilized plants, but overall unfertilized Soraya produced more flowers than fertilized.

In Wild-Type sunflowers, fertilizer but not rainfall had a significant effect on open flowers (negative binomial GLM, $\chi^2 = 7.33$, df = 1, p-value = 0.0068). Unfertilized Wild-Type plants produced fewer flowers than fertilized (pairwise comparison, Tukey's adjustment, $t = 2.20$, p-value = 0.0281).

For full summary of plant growth and flower data, refer to Supplementary Table 5.

Phenology—Goldenrod

In both 2020 and 2021, fertilized goldenrod bloomed and senesced earlier than unfertilized goldenrod across all rain treatments (Figure 6). In both years, fertilizer alone had a significant effect on bloom onset and end (quantile regression, marginal hypothesis test, p-value < 0.0306) with no significant interaction between rain and fertilizer. In 2020, goldenrod bloom onset and end occurred 4 days earlier in fertilized plants than unfertilized. Bloom duration was 2 days longer in unfertilized plants. In 2021, bloom began 4 days earlier in fertilized plants, and ended 2 days earlier. Thus, bloom duration was 2 days shorter in unfertilized plants. Fertilized goldenrod bloomed earlier under all rain treatments, and bloomed for a longer period than unfertilized goldenrod in 2021 when water was not limited.

Phenology—Sunflower

Sunflower bloom phenology data were collected only in 2021 due to a different experiment in 2020 that altered flowering. Therefore, sunflower bloom phenology was collected during the year of record rainfall but not in the drought year. In 2021, the timing bloom onset and end in sunflowers were highly variable among plant variety (Figure 6). In Wild-Type plants, there was no significant difference in date of bloom between different fertilizer and rain treatments, but both the interaction between rain and fertilizer (quantile regression, marginal hypothesis test, p-value = 0.0497) and the main effect of rain (p-value < 0.0001) had significant effects on the end of bloom. In fertilized Wild-Type

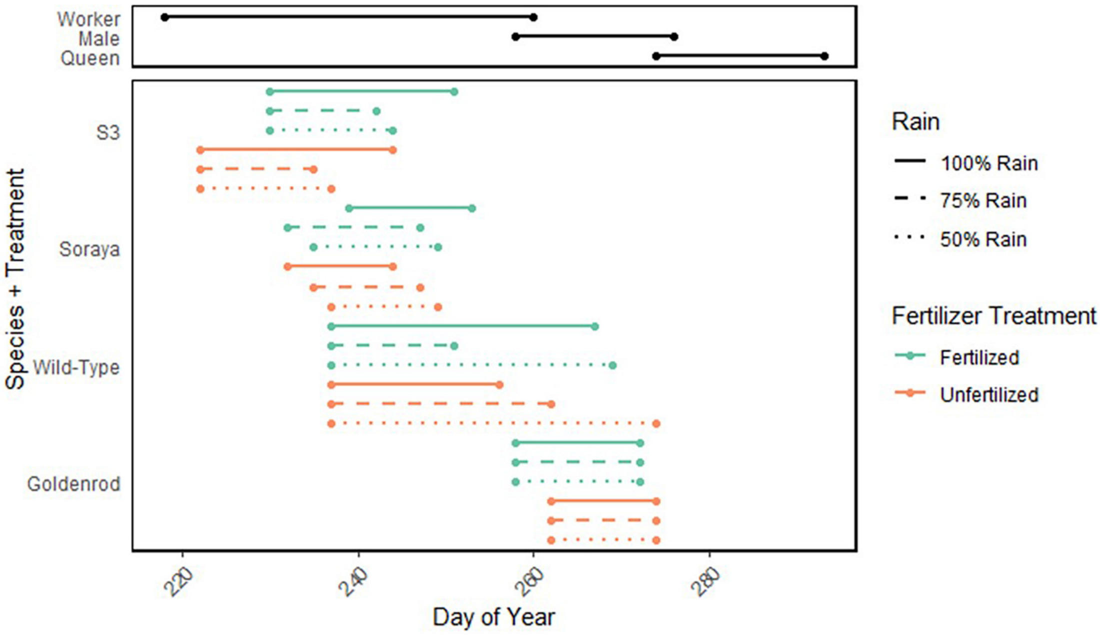


FIGURE 6
Activity period for *B. impatiens* and flowering period of four plant varieties influenced by fertilizer and rain treatments. Black lines represent abundance of *B. impatiens* workers, males and queens. As lab studies predict, queen development within the nest likely occurred during the male activity period. Green and orange lines represent bloom start and end points at 0.2 and 0.8 quantiles in 2021, color by fertilizer treatment and line style by rain treatment. *X*-axis represents number day of year, and *y*-axis represents plant species or variety. We observed 2,950 workers, 1,902 males, and 55 queens. Each goldenrod treatment represents flower counts from approximately 36 plants. Each sunflower treatment represents counts from 12 Soraya, 6–9 S3, and 3–5 Wild-Type.

sunflowers, plants in 75% rain ended bloom approximately 17 days before those in 50 and 100% rain. Among unfertilized Wild-Type sunflowers, plants grown under 50% rain reached the end of bloom approximately 18 days before those grown in 100% rain. Bloom duration in Wild-Type sunflowers ranged from 14 days in fertilized 75% rain to 37 days in unfertilized 50% rain. All Wild-Type plants, regardless of treatment, began to bloom at approximately the same time. However, end of bloom varied by up to 18 days between rain and fertilizer treatments.

In Soraya plants, the interaction of rain and fertilizer had a significant effect on the start and end of bloom (quantile regression, marginal hypothesis test, p-value < 0.0407), but the main effects of fertilizer and rain were not significant predictors of bloom time. Among fertilized Soraya, the dates of bloom and senescence were latest in 100% rain and earliest in 75% rain, by a difference of approximately 7 days. In unfertilized Soraya, the dates of bloom and senescence were earliest in 100% rain and latest in 50% rain, by a difference of approximately 5 days. Bloom duration in Soraya was approximately 2 days longer in fertilized plants. The interaction between fertilizer and rainfall resulted in a non-additive effect on bloom onset and end in Soraya sunflowers, without any pattern that tracks with rain or fertilizer treatment alone.

In the S3 variety, fertilizer had a significant effect on bloom onset (quantile regression, marginal hypothesis test, p-value = 0.0022), while both rain (p-value = 0.0014) and fertilizer (p-value = 0.0006) had significant effects on bloom end, though there was no significant interaction between the two predictors. Fertilized S3 plants bloomed 8 days later than unfertilized S3 plants. Unfertilized S3 senesced 8 days earlier than fertilized, and bloom end occurred approximately 8 days later in 100% rain than in 75 and 50% rain in both fertilized and unfertilized plants. Bloom duration in S3 plants was longest in 100% rain, with bloom lasting approximately 8 days longer in 100% rain in both fertilized and unfertilized treatments than 75 and 50% of ambient rain. On average, bloom onset and bloom end was later in fertilized S3 sunflowers than in unfertilized S3 sunflowers.

Bumble bee reproductive switch point

In 2021 we spent approximately 68 h surveying bumble bees and observed a total of 2,950 *B. impatiens* workers, 1902 males *B. impatiens*, and 55 *B. impatiens* queens. The observed activity periods for males and queens in the field suggest that there is a period of approximately 16 days, measured by the number of days between the 0.2 quantile slope of male activity and that of queen activity, in which most colonies have reached the point of queen larval development and feeding (Figure 6). If measured by the 0.1 quantile slope of male and queen emergence, queen development occurs over approximately 23 days, which provides an estimate of queen development in this field site that likely encompasses colonies that switched to reproduction early or late. We used the slopes from 0.2 to 0.8 quantiles to estimate the reproductive period with which the majority of colonies coincide.

Observations prior to July 30th were removed from the dataset, along with observations by a single observer on August 20th and 21st due to mistakes in species and/or caste identification. The first 20% of male observations occurred on day 258, just 2 days before

80% of worker observations were recorded, and at which point we estimate most colonies reached the reproductive switch point. The first 20% of queen observations occurred on day 274 and queens were observed through the end of the survey period. These queens were likely developing from larvae to eclosion and then feeding prior to foraging within the nest (Cnaani et al., 2002; Woodard et al., 2019) during the 16-day period in which most males were observed from September 15th (day 258) to October 3rd (day 276) (**Supplementary Table 6**).

Discussion

We experimentally manipulated the amount of water and supplemental nutrients available to plants throughout their growing season to understand the effects of these treatments on flowering phenology. We then evaluated how these changes could impact resource availability for *B. impatiens* queens preparing to overwinter. We found that fertilizer and rain interact to affect the growth and phenology of sunflower and goldenrod, altering bloom time between 2 and 18 days, but that this effect varies between species and varieties. Our original hypothesis was that fertilized plants would grow more and bloom earlier than unfertilized plants because fertilizer would accelerate growth (Burkle and Irwin, 2009; Russo et al., 2020). We expected that under average conditions, plants grown in 50% rainfall would grow smaller and bloom earlier than those grown in 100% rainfall, since water limitation can reduce growth and result in drought escape (Nord and Lynch, 2009; Shavrukov et al., 2017).

However, neither 2020 nor 2021 were average rainfall years: the summer and fall of 2020 brought a prolonged drought, and summer 2021 was one of the wettest summers on record in the Boston area. We found that fertilizer can increase both sunflower and goldenrod growth when water is sufficient, but fertilizer does not affect maximum height or flowers produced when water is limiting. In sunflower, fertilized plants produce most flowers within a narrow window, resulting in a floral resource pulse. In goldenrod, fertilizer results in earlier bloom and senescence regardless of rainfall. Finally, field observations of bumble bees suggest the majority of colonies are simultaneously producing and feeding new queens over an approximately 16-day window. This indicates a short, critical nutritional window in which the most colonies would benefit from available floral resources.

Finding no effect of fertilizer on plant growth under drought conditions somewhat contradicts some studies that find fertilizer to increase plant growth under moderate drought, mitigating the negative effects of drought on plant growth (Garg et al., 2004; Barbosa et al., 2014; Kelso et al., 2020). We may have seen this difference because the total rainfall in 2020 surpassed moderate drought intensity: rainfall totals in July and August of 2020 were 50 and 68% of average rainfall, respectively, leaving the 50% ambient rainfall treatments at just 25 and 34% average rainfall (National Oceanic and Atmospheric Administration [NOAA], 2021). Plants tend to reduce uptake of nutrients during drought (Bista et al., 2018) and as soil dries it becomes primed to leach nutrients during the first significant rewetting (Shepherd et al., 2018). It is possible that in 2020, additional nutrients provided by fertilizer did not affect growth because plants were restricting nutrient uptake to conserve water, and that nutrients were lost from soil during the first significant rainfall. In 2021, growth may have declined in 100% rainfall among unfertilized plants because heavy rainfall leached nutrients from the soil (Bedard-Haughn, 2009) which were supplemented in fertilized treatments, resulting in no growth decline in fertilized plants as water increased. This finding carries important implications for growers and land managers, suggesting that fertilizer should not be applied as insurance against damage from dry conditions (Shepherd et al., 2018).

Flowering phenology was also altered in the experiments. Fertilizer resulted in floral pulses—narrow windows of resource availability (Hemberger and Gratton, 2018)—in sunflower in 2021 but not in goldenrod. Specifically, sunflower blooms were produced all at once rather than produced at an even rate over the bloom period. Comparing the sum of open flowers over time revealed that S3 and Soraya varieties produced significantly more flowers in unfertilized treatments regardless of rainfall. We concluded that fertilizer may have induced accelerated bloom in which plants produce more flowers over a short period, so that most flowers are open and available to pollinators over a shorter window of time than in unfertilized plants. Goldenrod, on the other hand, produced flowers at a similar rate between fertilizer treatments rather than in floral pulses. The number of goldenrod flowers and flower days did not differ significantly between fertilizer treatments in 2020, but was significantly higher in fertilized treatments in 2021. Flowering and flower days increased with rainfall in both study years.

Plants may respond to stress or external stimuli in two ways: flower to produce seed for the next generation, or delay flowering through slowed metabolism (Cho et al., 2017). Sunflowers are considered drought tolerant largely due to drought escape, which results in earlier and more rapid flowering (Hussain et al., 2018). It is possible that these sunflower varieties respond to other stimuli with rapid flowering as well, which could increase the number of flowers produced all at once and decrease the number of flowers produced later in the season. Goldenrod responds to drought and herbivory stress by reallocating resources to asexual reproduction via rhizome maintenance rather than increased or rapid flowering (Shibel and Heard, 2016; Rosenblatt, 2021). This stress response would explain why we observed a similar flowering rate across treatments but higher overall flowers and flower days with more rainfall and, in 2021, fertilizer addition.

Floral pulses that produce abundant food resources can support bumble bee colony growth, but may not increase colony reproduction (Riedinger et al., 2015; Hemberger et al., 2020). Hemberger et al. (2020) found that bumble bee microcolony reproduction was maximized when food rations were high and constantly available, but may be resilient to pulsed food sources if those pulses produce abundant food. Microcolony growth suffered under low abundance regardless of temporal availability. However, this study does not examine queen production, which is more costly than male production (Rundlöf et al., 2014), and colonies can store food for only a few days in the nest (Goulson, 2003) which could result in reduced resiliency to food pulses during queen reproduction. In field studies, bumble bee colony reproduction did not benefit from mass-flowering crops when colony flight periods extended beyond the floral pulse (Riedinger et al., 2015), but queen and male abundance increased when this floral pulse coincided with colony reproduction (Rundlöf et al., 2014).

Bloom time in each plant species and variety grown in this study varied with fertilizer and rain treatments by as much as 18 days. This species-specific response is a common finding among resource manipulation studies that make broad predictions difficult (Tilman and Wedin, 1991; Burkle and Irwin, 2009; Burkle and Runyon, 2016; Cho et al., 2017). Burkle and Irwin (2009) tested the effects of nutrient addition on floral characteristics in two subalpine plant species and found that life-history traits likely played a role in determining the growth response of each plant to soil nutrients. Even within a single species, individual response to resource availability can differ (Burkle et al., 2013; Alvarez-Maldini et al., 2020).

Though there was no single pattern in plant response across species, both fertilizer and rainfall significantly alter the timing and duration of bloom, creating potential phenological mismatch between late-season flowering plants and reproductive bumble bee colonies. Food availability and nectar quality in the fall is one of the strongest predictors of bumble bee populations in the spring (Timberlake et al., 2020). Because the effects of fertilizer and rainfall vary dramatically with plant species, it is essential that habitat surrounding farmland include a diverse community of fall-blooming plants. In this way, low levels of nutrient runoff will increase heterogeneity in bloom time rather than uniformly shift the bloom time of a few species in any direction.

Field observations of bumble bee activity suggest a period of just 16 days between male and queen emergence in which most colonies have reached the reproductive switch point. Though each colony varies in the exact timing of reproduction, these finding suggest that available resources during this short period would support reproduction for the largest number of colonies. Colony reproductive success hinges on a short window with two sensitive nutritional periods: queen larval development, and post-eclosion mass-gain (Owen, 1988; Couvillon and Dornhaus, 2009; Woodard et al., 2019). Food reserves within the nest last only 24–48 h (Goulson, 2003; Rotheray et al., 2017), so food resources outside the nest must be continuously available during reproduction. If food is insufficient during the development period, the colony fails to reproduce or queens do not prepare effectively for winter, leading to lower winter survival and fewer nests in the spring (Goulson, 2003; Fliszkiewicz and Wilkaniec, 2007; Woodard et al., 2019). The changes we observed in plant bloom—most notably the 8-day delay in fertilized S3 sunflower bloom and 4-day acceleration in fertilized goldenrod bloom—may be biologically significant to reproductive bumble bee colonies and warrant further investigation.

Changes in the timing of food availability locally can be overcome if workers fly farther to forage for their queens, or if queens delay diapause to forage after leaving the nest. Queens cannot fly for the first 3–5 days post-eclosion but can delay mating and overwintering by several days to forage for themselves farther from the nest if they have not gained needed energy stores (Watrous et al., 2021). Overwintering survival is optimized when queens enter diapause between 6 and 17 days post-eclosion (Treanore and Amsalem, 2020). However, the sublethal effects of delayed weight gain and the energy expenditure for queens to fly further from the nest to find food are unknown (Watrous et al., 2021). Further, a shift in colony foraging range to overcome local misalignment in bloom time and reproduction could result in changes to local pollination networks. Bumble bees show strong floral constancy (Ogilvie et al., 2017), and this shift in range could persist through the end of the season, resulting in new pollination patterns and potentially fewer visitors to some late-blooming plants.

Nutrient pollution from fertilizer is a major problem and its interactive effect with rain on plant growth may be exacerbated as rainfall frequency and intensity become more variable each year (Vitousek et al., 1997; Lawson and Rands, 2019). Chemical fertilizer has increased farm yield, but at the cost of eutrophication in soils and waterways, and at high economic expense to growers. Methods to make fertilizer use more efficient and reduce its use altogether are therefore important to the sustainability of food production (Watrous et al., 2021). More research is needed into how plant communities, rather than single plant species, respond to fertilizer and rainfall variation. This study does not capture the potential effects of belowground interactions between plants and competition for resources that would also drive changes in bloom in plant communities. Research is also needed to study changes not only in resource availability, but in resource quality measured by pollen and nectar nutritional value.

There is no pinpointing a single cause of pollinator decline, largely because it is the combined effects of stressors that have the largest impact on pollinator foraging, health and population size (Goulson et al., 2015). It is therefore essential that more studies examine how global drivers of change interact to affect potentially sensitive life cycle stages of pollinators through changes to plant growth and physiology. Overall, our study suggests that nutrient enrichment and changes in rainfall affect plant bloom time, and in some plant species this shift falls uniformly along nutrient treatments. Variation in the floral landscape determines pollinator foraging behavior, delivery of pollination services, and gyne production (Cardoza et al., 2012; Mallinger et al., 2016; Ceulemans et al., 2017; Adler et al., 2020; Timberlake et al., 2020). Anthropogenic nutrient deposition and increasingly varied rainfall alter this landscape, threatening the plant-pollinator interactions that support biodiversity and plant productivity (Brown et al., 2016; David et al., 2019). Wild pollinators are critical to successful and reliable crop pollination, especially under an increasingly unpredictable climate (Winfree et al., 2007). Our findings suggest that nutrient addition on managed landscapes without consideration for environmental factors like climate change could further reduce suitable habitat for already threatened pollinators. The results of our study have important implications for agricultural management in a time of increasingly variable climate that can prioritize both crop yield and native pollinator conservation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JT lead author on this manuscript and led the research. CD, MP, and NJ assisted in field work, data collection and entry, and editing. CO helped with project development and manuscript editing. PS advised the project, helped with project development, and manuscript editing. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Science Foundation Research Experience for Undergraduates Program (NSF DBI 1852438).

Acknowledgments

We gratefully acknowledge the assistance of Dr. Elizabeth Crone for help and guidance with statistical analysis, and Heidy Acevedo, Avalon Owens, Isaac Weinberg, and Nicholas Dorian for additional field and data collection support. We thank DI and HH reviewers for the comments on this manuscript. Dr. Lynn Adler of UMass Amherst, Dr. Laura Russo of the University of Tennessee, and Dr. Rosemary Malfi of UMass Amherst consulted on plant selection and study design. The Waltham Fields Community Farm, the University of Massachusetts Boston Area Climate Experiment, and the City of Waltham provided access to study sites.

Conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1150736/full#supplementary-material

References

Adler, L. S., Fowler, A. E., Malfi, R. L., Anderson, P. R., Coppinger, L. M., Deneen, P. M., et al. (2020). Assessing chemical mechanisms underlying the effects of sunflower pollen on a gut pathogen in bumble bees. *J. Chem. Ecol.* 46, 649–658. doi: 10.1007/s10886-020-01168-4

Aldridge, G., Inouye, D. W., Forrest, J. R. K., Barr, W. A., and Miller-Rushing, A. J. (2011). Emergence of a mid-season period of low floral resources in a montane meadow ecosystem associated with climate change. *J. Ecol.* 99, 905–913. doi: 10.1111/j.1365-2745.2011.01826.x

Alford, D. V. (1975). *Bumblebees*. Washington, DC: Davis-Poynter.

Alvarez-Maldini, C., Acevedo, M., Dumroese, R. K., González, M., and Cartes, E. (2020). Intraspecific variation in drought response of three populations of *Cryptocarya alba* and *Persea lingue*, two native species from Mediterranean central Chile. *Front. Plant Sci.* 11:1042. doi: 10.3389/fpls.2020.01042

Barbosa, E. R. M., Tomlinson, K. W., Carvalheiro, L. G., Kirkman, K., de Bie, S., Prins, H. H. T., et al. (2014). Short-term effect of nutrient availability and rainfall distribution on biomass production and leaf nutrient content of savanna tree species. *PLoS One* 9:e92619. doi: 10.1371/journal.pone.0092619

Bassirirad, H. (2000). Kinetics of nutrient uptake by roots: Responses to global change. *New Phytol.* 147, 155–169. doi: 10.1046/j.1469-8137.2000.00682.x

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bedard-Haughn, A. (2009). Managing excess water in Canadian prairie soils: A review. *Can. J. Soil Sci.* 89, 157–168. doi: 10.4141/CJSS07071

Belsky, J. E., Camp, A. A., and Lehmann, D. M. (2020). The importance of males to bumble bee (*Bombus* Species) nest development and colony viability. *Insects* 11:506. doi: 10.3390/insects11080506

Bista, D. R., Heckathorn, S. A., Jayawardena, D. M., Mishra, S., and Boldt, J. K. (2018). Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and -tolerant grasses. *Plants* 7:28. doi: 10.3390/plants7020028

Brown, M. J. F., Dicks, L. V., Paxton, R. J., Baldock, K. C. R., Barron, A. B., Chauzat, M.-P., et al. (2016). A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ* 4:e2249. doi: 10.7717/peerj.2249

Brown, M. J. F., Schmid-Hempel, R., and Schmid-Hempel, P. (2003). Strong context-dependent virulence in a host–parasite system: Reconciling genetic evidence with theory. *J. Anim. Ecol.* 72, 994–1002. doi: 10.1046/j.1365-2656.2003.00770.x

Burkle, L. A., and Irwin, R. E. (2009). The effects of nutrient addition on floral characters and pollination in two subalpine plants, *Ipomopsis aggregata* and *Linum lewisii*. *Plant Ecol.* 203, 83–98. doi: 10.1007/s11258-008-9512-0

Burkle, L. A., and Runyon, J. B. (2016). Drought and leaf herbivory influence floral volatiles and pollinator attraction. *Glob. Chang. Biol.* 22, 1644–1654.

Burkle, L. A., Souza, L., Genung, M. A., and Crutsinger, G. M. (2013). Plant genotype, nutrients, and G × E interactions structure floral visitor communities. *Ecosphere* 4:art113. doi: 10.1890/ES13-00039.1

Cade, B. S., and Noon, B. R. (2003). A gentle introduction to quantile regression for ecologists. *Front. Ecol. Environ.* 1, 412–420. doi: 10.1890/1540-92952003001[0412:AGITQR]2.0.CO;2

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., et al. (2011). Patterns of widespread decline in North American bumble bees. *Proc. Natl. Acad. Sci. U.S.A.* 108, 662–667. doi: 10.1073/pnas.1014743108

Cardoza, Y. J., Harris, G. K., and Grozinger, C. M. (2012). Effects of soil quality enhancement on pollinator-plant interactions. *Psyche* 2012:581458. doi: 10.1155/2012/581458

Carvell, C., Osborne, J. L., Bourke, A. F. G., Freeman, S. N., Pywell, R. F., and Heard, M. S. (2011). Bumble bee species' responses to a targeted conservation measure depend on landscape context and habitat quality. *Ecol. Applic.* 21, 1760–1771. doi: 10.1890/10-0677.1

Ceulemans, T., Hulsmans, E., Ende, W. V., and Honnay, O. (2017). Nutrient enrichment is associated with altered nectar and pollen chemical composition in *Succisa pratensis* Moench and increased larval mortality of its pollinator *Bombus terrestris* L. *PLoS One* 12:e0175160. doi: 10.1371/journal.pone.0175160

Cho, L.-H., Yoon, J., and An, G. (2017). The control of flowering time by environmental factors. *Plant J.* 90, 708–719. doi: 10.1111/tpj.13461

Cnaani, J., Schmid-Hempel, R., and Schmidt, J. O. (2002). Colony development, larval development and worker reproduction in *Bombus impatiens* Cresson. *Insectes Sociaux* 49, 164–170. doi: 10.1007/s00040-002-8297-8

Couvillon, M. J., and Dornhaus, A. (2009). Location, location, location: Larvae position inside the nest is correlated with adult body size in worker bumble-bees (*Bombus impatiens*). *Proc. R. Soc. B Biol. Sci.* 276, 2411–2418.

Crone, E. E., and Williams, N. M. (2016). Bumble bee colony dynamics: Quantifying the importance of land use and floral resources for colony growth and queen production. *Ecol. Lett.* 19, 460–468. doi: 10.1111/ele.12581

David, T. I., Storkey, J., and Stevens, C. J. (2019). Understanding how changing soil nitrogen affects plant–pollinator interactions. *Arthropod Plant Interact.* 13, 671–684. doi: 10.1007/s11829-019-09714-y

Descamps, C., Quinet, M., and Jacquemart, A.-L. (2021). The effects of drought on plant–pollinator interactions: What to expect? *Environ. Exp. Bot.* 182:104297. doi: 10.1016/j.envexpbot.2020.104297

Fliszkiewicz, M., and Wilkaniec, Z. (2007). Fatty acids and amino acids in the fat body of bumblebee *Bombus terrestris* (L.) in diapausing and non-diapausing queens. *J. Apicult. Sci.* 51, 55–63.

Garg, B., Burman, U., and Kathju, S. (2004). The influence of phosphorus nutrition on the physiological response of moth bean genotypes to drought. *J. Plant Nutr. Soil Sci.* 167, 503–508. doi: 10.1002/JPLN.200320368

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., et al. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339, 1608–1611. doi: 10.1126/science.1230200

Goulson, D. (2003). *Bumblebees: Their behaviour and ecology*. Oxford: Oxford University Press.

Goulson, D., Lye, G. C., and Darvill, B. (2008). Decline and conservation of bumble bees. *Annu. Rev. Entomol.* 53, 191–208.

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347:1255957. doi: 10.1126/science.1255957

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

Hemberger, J., and Gratton, C. (2018). Floral resource pulse decreases bumble bee foraging trip duration in central Wisconsin agroecosystem. *Ecol. Entomol.* 43, 447–457. doi: 10.1111/een.12516

Hemberger, J., Frappa, A., Witynski, G., and Gratton, C. (2020). Saved by the pulse? Separating the effects of total and temporal food abundance on the growth and reproduction of bumble bee microcolonies. *Basic Appl. Ecol.* 45, 1–11. doi: 10.1016/j.baae.2020.04.004

Hoeppner, S. S., and Dukes, J. S. (2012). Interactive responses of old-field plant growth and composition to warming and precipitation. *Glob. Chang Biol.* 18, 1754–1768. doi: 10.1111/j.1365-2486.2011.02626.x

Hoover, S. E. R., Ladley, J. J., Shchepetkina, A. A., Tisch, M., Gieseg, S. P., and Tylianakis, J. M. (2012). Warming, CO2, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecol. Lett.* 15, 227–234. doi: 10.1111/j.1461-0248.2011.01729.x

Huang, G., Li, C., and Li, Y. (2018). Phenological responses to nitrogen and water addition are linked to plant growth patterns in a desert herbaceous community. *Ecol. Evol.* 8, 5139–5152. doi: 10.1002/ece3.4001

Hunter, M. (2016). *The phytochemical landscape: Linking trophic interactions and nutrient dynamics*. Princeton, NJ: Princeton University Press.

Hussain, M., Farooq, S., Hasan, W., Ul-Allah, S., Tanveer, M., Farooq, M., et al. (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agric. Water Manage.* 201, 152–166. doi: 10.1016/j.agwat.2018.01.028

Kaya, Y., Jocic, S., and Miladinovic, D. (2012). "Sunflower," in *Technological innovations in major world oil crops, volume 1: Breeding*, ed. S. K. Gupta (Berlin: Springer), 85–129. doi: 10.1007/978-1-4614-0356-2_4

Kelso, M. A., Wigginton, R. D., and Grosholz, E. D. (2020). Nutrients mitigate the impacts of extreme drought on plant invasions. *Ecology* 101:e02980. doi: 10.1002/ecy.2980

Koenker, R., Portnoy, S., Ng, P. T., Zeileis, A., Grosjean, P., and Ripley, B. D. (2019). *Package 'quantreg*. Available online at: https://www.vps.fmvz.usp.br/CRAN/web/packages/quantreg/quantreg.pdf (accessed December 2022).

Kremen, C., and M'Gonigle, L. K. (2015). EDITOR'S CHOICE: Small-scale restoration in intensive agricultural landscapes supports more specialized and less mobile pollinator species. *J. Appl. Ecol.* 52, 602–610. doi: 10.1111/1365-2664.12418

Kremen, C., Williams, N. M., and Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16812–16816. doi: 10.1073/pnas.262413599

Lawson, D. A., and Rands, S. A. (2019). The effects of rainfall on plant–pollinator interactions. *Arthropod Plant Interact.* 13, 561–569. doi: 10.1007/s11829-019-09686-z

Leach, M. E., and Drummond, F. (2018). A review of native wild bee nutritional health. *Int. J. Ecol.* 2018:9607246. doi: 10.1155/2018/9607246

Li, Z., Zhang, R., Xia, S., Wang, L., Liu, C., Zhang, R., et al. (2019). Interactions between N, P and K fertilizers affect the environment and the yield and quality of satsumas. *Glob. Ecol. Conserv.* 19:e00663. doi: 10.1016/j.gecco.2019.e00663

Lihoreau, M., Buhl, J., Charleston, M. A., Sword, G. A., Raubenheimer, D., and Simpson, S. J. (2015). Nutritional ecology beyond the individual: A conceptual framework for integrating nutrition and social interactions. *Ecol. Lett.* 18, 273–286. doi: 10.1111/ele.12406

Lombard, P. J., Barclay, J. R., and McCarthy, D.-A. E. (2020). *2020 drought in New England*. Reston, VA: U.S. Geological Survey, doi: 10.3133/ofr20201148

Malfi, R. L., Crone, E., and Williams, N. (2019). Demographic benefits of early season resources for bumble bee (*B. vosnesenskii*) colonies. *Oecologia* 191, 377–388. doi: 10.1007/s00442-019-04472-3

Mallinger, R. E., Gibbs, J., and Gratton, C. (2016). Diverse landscapes have a higher abundance and species richness of spring wild bees by providing complementary floral resources over bees' foraging periods. *Landsc. Ecol.* 31, 1523–1535. doi: 10.1007/s10980-015-0332-z

Michielini, J. P., Dopman, E. B., and Crone, E. E. (2021). Changes in flight period predict trends in abundance of Massachusetts butterflies. *Ecol. Lett.* 24, 249–257. doi: 10.1111/ele.13637

Muñoz, A., Celedon-Neghme, C., Cavieres, L. A., and Arroyo, M. T. K. (2005). Bottom-up effects of nutrient availability on flower production, pollinator visitation, and seed output in a high-Andean shrub. *Oecologia* 143, 126–135. doi: 10.1007/s00442-004-1780-3

National Oceanic and Atmospheric Administration [NOAA] (2021). *NOAA's national weather service. Climate. NOWData*. Available online at: https://www.weather.gov/wrh/Climate?wfo=box (accessed September 27, 2021).

Nord, E. A., and Lynch, J. P. (2009). Plant phenology: A critical controller of soil resource acquisition. *J. Exp. Bot.* 60, 1927–1937. doi: 10.1093/jxb/erp018

Novotny, J. L., Reeher, P., Varvaro, M., Lybbert, A., Smith, J., Mitchell, R. J., et al. (2021). Bumble bee species distributions and habitat associations in the Midwestern USA, a region of declining diversity. *Biodivers. Conserv.* 30, 865–887. doi: 10.1007/s10531-021-02121-x

Oertel, E. (1967). Nectar and pollen plants. *US Dept. Agric.* 335, 16–23.

Ogilvie, J. E., and Forrest, J. R. (2017). Interactions between bee foraging and floral resource phenology shape bee populations and communities. *Curr. Opin. Insect Sci.* 21, 75–82. doi: 10.1016/j.cois.2017.05.015

Ogilvie, J. E., Griffin, S. R., Gezon, Z. J., Inouye, B. D., Underwood, N., Inouye, D. W., et al. (2017). Interannual bumble bee abundance is driven by indirect climate effects on floral resource phenology. *Ecol. Lett.* 20, 1507–1515. doi: 10.1111/ele.12854

Owen, R. E. (1988). Body size variation and optimal body size of bumble bee queens (*Hymenoptera*: *Apidae*). *Can. Entomol.* 120, 19–27. doi: 10.4039/Ent12019-1

Phillips, B. B., Shaw, R. F., Holland, M. J., Fry, E. L., Bardgett, R. D., Bullock, J. M., et al. (2018). Drought reduces floral resources for pollinators. *Glob. Chang. Biol.* 24, 3226–3235. doi: 10.1111/gcb.14130

Pollard, E. (1977). A method for assessing changes in the abundance of butterflies. *Biol. Conserv.* 12, 115–134. doi: 10.1016/0006-3207(77)90065-9

Pugesek, G., and Crone, E. E. (2021). Contrasting effects of land cover on nesting habitat use and reproductive output for bumble bees. *Ecosphere* 12:e03642. doi: 10.1002/ecs2.3642

Riedinger, V., Mitesser, O., Hovestadt, T., Steffan-Dewenter, I., and Holzschuh, A. (2015). Annual dynamics of wild bee densities: Attractiveness and productivity effects of oilseed rape. *Ecology* 96, 1351–1360. doi: 10.1890/14-1124.1

Roger, N., Michez, D., Wattiez, R., Sheridan, C., and Vanderplanck, M. (2017). Diet effects on bumblebee health. *J. Insect Physiol.* 96, 128–133. doi: 10.1016/j.jinsphys.2016.11.002

Rosenblatt, A. E. (2021). Drought rewires an old field food web through shifts in plant nutrient content and herbivore feeding behaviors. *Clim. Change Ecol.* 2:100019. doi: 10.1016/j.ecochg.2021.100019

Rotheray, E. L., Osborne, J. L., and Goulson, D. (2017). Quantifying the food requirements and effects of food stress on bumble bee colony development. *J. Apicult. Res.* 56, 288–299. doi: 10.1080/00218839.2017.1307712

Rundlöf, M., Persson, A. S., Smith, H. G., and Bommarco, R. (2014). Late-season mass-flowering red clover increases bumble bee queen and male densities. *Biol. Conserv.* 172, 138–145. doi: 10.1016/j.biocon.2014.02.027

Runkle, J., Kunkel, K., Frankson, R., Easterling, D., DeGaetano, A. T., Stewart, B., et al. (2017). *Massachusetts state climate summary*. Washington, DC: NOAA.

Russo, L., Buckley, Y. M., Hamilton, H., Kavanagh, M., and Stout, J. C. (2020). Low concentrations of fertilizer and herbicide alter plant growth and interactions with flower-visiting insects. *Agric. Ecosyst. Environ.* 304:107141. doi: 10.1016/j.agee.2020.107141

Schlüns, H., Sadd, B. M., Schmid-Hempel, P., and Crozier, R. H. (2010). Infection with the trypanosome *Crithidia bombi* and expression of immune-related genes in the bumblebee *Bombus terrestris*. *Dev. Comp. Immunol.* 34, 705–709. doi: 10.1016/j.dci.2010.02.002

Scott, E. R., Li, X., Kfoury, N., Morimoto, J., Han, W.-Y., Ahmed, S., et al. (2019). Interactive effects of drought severity and simulated herbivory on tea (*Camellia sinensis*) volatile and non-volatile metabolites. *Environ. Exp. Bot.* 157, 283–292. doi: 10.1016/j.envexpbot.2018.10.025

Shavrukov, Y., Kurishbayev, A., Jatayev, S., Shvidchenko, V., Zotova, L., Koekemoer, F., et al. (2017). Early flowering as a drought escape mechanism in plants: How can it aid wheat production? *Front. Plant Sci.* 8:1950. doi: 10.3389/fpls.2017.01950

Shepherd, M., Lucci, G., Vogeler, I., and Balvert, S. (2018). The effect of drought and nitrogen fertiliser addition on nitrate leaching risk from a pasture soil; an assessment from a field experiment and modelling. *J. Sci. Food Agric.* 98, 3795–3805.

Shibel, Z., and Heard, S. B. (2016). Synergistic and additive effects of drought stress and simulated herbivory on two goldenrods, *Solidago altissima* and *S. gigantea*. *Botany* 94, 635–642. doi: 10.1139/cjb-2016-0060

Stemkovski, M., Pearse, W. D., Griffin, S. R., Pardee, G. L., Gibbs, J., Griswold, T., et al. (2020). Bee phenology is predicted by climatic variation and functional traits. *Ecol. Lett.* 23, 1589–1598. doi: 10.1111/ele.13583

Tilman, D., and Wedin, D. (1991). Plant traits and resource reduction for five grasses growing on a nitrogen gradient. *Ecology* 72, 685–700. doi: 10.2307/2937208

Timberlake, T. P., Vaughan, I. P., Baude, M., and Memmott, J. (2020). Bumblebee colony density on farmland is influenced by late-summer nectar supply and garden cover. *J. Appl. Ecol.* 1, 1006–1016. doi: 10.1111/1365-2664.13826

Treanore, E., and Amsalem, E. (2020). The effect of intrinsic physiological traits on diapause survival and their underlying mechanisms in an annual bee species *Bombus impatiens*. *Conserv. Physiol.* 8:coaa103. doi: 10.1093/conphys/coaa103

Trenberth, K. E. (2008). "The impact of climate change and variability on heavy precipitation, floods, and droughts," in *Encyclopedia of hydrological sciences*, eds M. G. Anderson and J. J. McDonnell (Hoboken, NJ: John Wiley & Sons, Ltd), hsa211. doi: 10.1002/0470848944.hsa211

Vanbergen, A. J., Baude, M., Biesmeijer, J. C., Britton, N. F., Brown, M. J. F., Bryden, J., et al. (2013). Threats to an ecosystem service: Pressures on pollinators. *Front. Ecol. Environ.* 11, 251–259. doi: 10.1890/120126

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., et al. (1997). Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Applic.* 7, 737–750. doi: 10.1890/1051-07611997007[0737:HAOTGN]2.0.CO;2

Walter, J. (2018). Effects of changes in soil moisture and precipitation patterns on plant-mediated biotic interactions in terrestrial ecosystems. *Plant Ecol.* 219, 1449–1462. doi: 10.1007/s11258-018-0893-4

Watrous, K. M., Costa, C. P., Diaz, Y. R., and Woodard, S. H. (2021). Flexibility in the Critical Period of Nutrient Sequestration in Bumble Bee Queens. *Integr. Organ. Biol.* 3:obab009. doi: 10.1093/iob/obab009

Werner, P. A., Gross, R. S., and Bradbury, I. K. (1980). THE BIOLOGY OF CANADIAN WEEDS.: 45. *Solidago canadensis* L. *Can. J. Plant Sci.* 60, 1393–1409. doi: 10.4141/cjps80-194

Winfree, R., Williams, N. M., Dushoff, J., and Kremen, C. (2007). Native bees provide insurance against ongoing honey bee losses. *Ecol. Lett.* 10, 1105–1113. doi: 10.1111/j.1461-0248.2007.01110.x

Winfree, R., Williams, N. M., Gaines, H., Ascher, J. S., and Kremen, C. (2008). Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *J. Appl. Ecol.* 45, 793–802. doi: 10.1111/j.1365-2664.2007.01418.x

Woodard, S. H., and Jha, S. (2017). Wild bee nutritional ecology: Predicting pollinator population dynamics, movement, and services from floral resources. *Curr. Opin. Insect Sci.* 21, 83–90. doi: 10.1016/j.cois.2017.05.011

Woodard, S. H., Duennes, M. A., Watrous, K. M., and Jha, S. (2019). Diet and nutritional status during early adult life have immediate and persistent effects on queen bumble bees. *Conserv. Physiol.* 7:coz048. doi: 10.1093/conphys/coz048

Zhang, J., Zuo, X., Zhao, X., Ma, J., and Medina-Roldán, E. (2020). Effects of rainfall manipulation and nitrogen addition on plant biomass allocation in a semiarid sandy grassland. *Sci. Rep.* 10:9026. doi: 10.1038/s41598-020-65922-0

Ziska, L. H., Pettis, J. S., Edwards, J., Hancock, J. E., Tomecek, M. B., Clark, A., et al. (2016). Rising atmospheric CO2 is reducing the protein concentration of a floral pollen source essential for North American bees. *Proc. R. Soc. B Biol. Sci.* 283:20160414. doi: 10.1098/rspb.2016.0414

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 22 May 2023
DOI 10.3389/fevo.2023.1119452

The organizational impact of chronic heat: diffuse brood comb and decreased carbohydrate stores in honey bee colonies

Isaac P. Weinberg*, Jaya P. Wetzel, Eleanor P. Kuchar, Abigail T. Kaplan, Rebecca S. Graham, Jonah E. Zuckerman and Philip T. Starks

Department of Biology, Tufts University, Medford, MA, United States

Check for updates

OPEN ACCESS

EDITED BY
Neelendra K. Joshi,
University of Arkansas, United States

REVIEWED BY
David Tarpy,
North Carolina State University, United States
Michael Lattorff,
University of Nairobi, Kenya

*CORRESPONDENCE
Isaac Weinberg
✉ Isaac.Weinberg@tufts.edu

RECEIVED 08 December 2022
ACCEPTED 02 May 2023
PUBLISHED 22 May 2023

CITATION
Weinberg IP, Wetzel JP, Kuchar EP, Kaplan AT, Graham RS, Zuckerman JE and Starks PT (2023) The organizational impact of chronic heat: diffuse brood comb and decreased carbohydrate stores in honey bee colonies. *Front. Ecol. Evol.* 11:1119452.
doi: 10.3389/fevo.2023.1119452

Insect pollinators are vital to the stability of a broad range of both natural and anthropogenic ecosystems and add billions of dollars to the economy each year. Honey bees are perhaps the best studied insect pollinator due to their economic and cultural importance. Of particular interest to researchers are the wide variety of mechanisms honey bees use for thermoregulation, such as fanning cool air currents around the hive and careful selection of insulated nest sites. These behaviors help honey bees remain active through both winter freezes and summer heatwaves, and may allow honey bees to deal with the ongoing climate crisis more readily than other insect species. Surprisingly, little is known about how honey bee colonies manage chronic heat stress. Here we provide a review of honey bee conservation behavior as it pertains to thermoregulation, and then present a novel behavior displayed in honey bees—the alteration of comb arrangement in response to 6 weeks of increased hive temperature. We found that while overall quantities of brood remained stable between treatments, brood were distributed more diffusely throughout heated hives. We also found that heated hives contained significantly less honey and nectar stores than control hives, likely indicating an increase in energy expenditure. Our results support previous findings that temperature gradients play a role in how honey bees arrange their comb contents, and improves our understanding of how honey bees modify their behavior to survive extreme environmental challenges.

KEYWORDS
conservation behavior, honey bees, insect architecture, thermoregulation, climate change

Introduction

Understanding the way insect behavior is affected by changing environmental conditions is vital to developing effective conservation strategies. Arguably the most culturally and economically important insect species is the honey bee, which provides vital pollination services to crops and wild flowering plants, and contributes tens of billions of dollars to the United States economy and hundreds of billions to the world economy each year (Gallai et al., 2009; Calderone, 2012; Hung et al., 2018; Reilly et al., 2020). Honey bee colonies are affected by many stressors including pesticides, disease, parasites, poor forage quality, and aggressive management strategies (Potts et al., 2010; Goulson et al., 2015; Wood and Goulson, 2017; Sánchez-Bayo and

Wyckhuys, 2019; Hristov et al., 2020; Panziera et al., 2022). No stressor, however, may be as threatening to honey bee colonies as increased global temperatures caused by the ongoing climate crisis. The behavioral strategies honey bees use to deal with acute bouts of heat stress are well documented (Jones and Oldroyd, 2006; Perez and Aron, 2020), but how honey bees respond to chronic heat stress, which is becoming more common, is less well understood. Improving our knowledge of how honey bee colonies are affected by chronic heat stress is vital, as the effectiveness of their response may impact their susceptibility to other stressors. In this manuscript, we provide a review of honey bee conservation behavior as it relates to their ability thermoregulate their hives. We then present a novel study on the effect of chronic heat stress on honey bee comb store arrangement.

The importance of thermoregulation

Temperature maintenance is important for all individuals in a hive, but it is particularly important for brood (Wang et al., 2016). While adult worker bees can withstand brief exposure to temperatures between 46 and 50°C (Heinrich, 1980; Coelho, 1991; Gonzalez et al., 2022), worker brood develop optimally in a relatively tight temperature range between 32 and 36°C (Becher et al., 2009), with the ideal temperature generally stated as 35°C (Szentgyörgyi et al., 2018). Prolonged exposure to temperatures below 32°C increase the likelihood of workers developing malformed wings, legs and abdomens (Himmer, 1932; Chacon-Almeida et al., 1999), and even brief exposure to temperatures of 20°C significantly reduces brood survival, and decreases their lifespan as adults (Wang et al., 2016). High temperatures may be even more detrimental for brood health, as pupal survival drops to zero at incubation temperatures of just 38°C (Groh et al., 2004).

Within the optimal temperature range, even small differences can have lasting effects on adult physiology and behavior. Bees reared at the higher end of their optimal range show accelerated pupal development, forage earlier in life, dance more frequently, have improved short term memory, and are better at in-hive tasks (Lin and Winston, 1998; Tautz et al., 2003; Petz et al., 2004; Jones et al., 2005; Becher et al., 2009; Wang et al., 2016; Medina et al., 2018). Workers that develop at the lower end of the optimal range tend to be larger, and have longer adult lifespans (Szentgyörgyi et al., 2018). In addition, workers reared more than 1.5°C away 34.5°C have reduced number of olfactory microglomeruli (Groh et al., 2004) indicating rearing temperature affects adult neural function. Likely due to their sensitivity, honey bees more tightly regulate the temperature of their hives when brood are present (Kronenberg and Heller, 1982) and are more likely to thermoregulate brood comb than other hive areas (Simpson, 1961).

Mechanisms for warming the hive

Unlike most insect species that live in temperate climates, honey bee colonies remain active throughout the cold winter months. To keep warm, honey bees cluster together and use metabolic energy to "shiver" by isometrically contracting their wing muscles to produce endothermic heat (Phillips and Demuth, 1914; Kronenberg and Heller, 1982). In broodless colonies, bees cluster when ambient temperatures fall to 14°C (Phillips and Demuth, 1914), but begin to cluster at 20°C in the presence of brood (Simpson, 1961; Kronenberg and Heller, 1982). The bees in the cluster's core produce most of the endothermic heat, while bees on the mantle function mostly as insulation (Stabentheiner et al., 2003). In ambient temperatures as low as 2.5°C the core of a cluster can sustain temperatures greater than 35°C, high enough to incubate brood, while the mantle remains above 17°C (Simpson, 1961; Heinrich, 1981; Watmough and Camazine, 1995; Stabentheiner et al., 2003). To maintain a constant internal temperature as ambient temperatures decrease, the bees cluster closer together and consume more honey and nectar to facilitate heat production (Simpson, 1961). The tightening of the cluster results in a smaller area being maintained within the optimal temperature range for brood rearing. Even a single abnormally cold night can contract the cluster, which may explain why brood mortality is higher near the periphery of a hive (Fukuda and Sakagami, 1968). Given that rearing temperature can strongly affect an individual bee's adult behavior and physiology, eggs laid in the center of the brood cluster may be more likely to develop into effective adults.

Since clustering begins nearly 15° below the optimal rearing temperature for brood, honey bees use more localized mechanisms to warm brood once temperatures drop below 32°C (Stabentheiner et al., 2021). Individual bees can warm brood using their own endothermic heat, either by pressing their thoraxes on individual capped brood cells (Bujok et al., 2002), or by entering an empty cell to warm brood in surrounding cells (Kleinhenz et al., 2003). Only bees older than about 2 days will actively heat brood, while bees under 2 days are much more likely to seek exothermic heat sources to help complete their own flight muscle development (Stabentheiner et al., 2010).

Honey bee colonies may also behaviorally increase their temperature to induce a "fever" which is hypothesized to be a generalized response to illness, particularly in large colonies (Simone-Finstrom et al., 2014; Bonoan et al., 2020). Colonies with the fungal infection *Ascosphaera apis* behaviorally increase their temperature (Starks et al., 2000), while individuals infected by N*osema ceranae* seek high temperature areas within the colony (Campbell et al., 2010). It is also known that parasitic *Varroa* mites breed optimally at the same temperature as honey bees, with their reproductive success decreasing sharply above 36.5°C (Le Conte et al., 1990) and that colonies with high varroa load elevate their temperatures by 1.5°C (Hou et al., 2016). Artificially heating colonies to above 40°C has also been commonly suggested as an effective treatment for *Varroa* infestations (Hoppe and Ritter, 1987; Rosenkranz, 1987; Harbo, 2000).

Mechanisms for cooling the hive

Honey bees are more able to keep large sections of their colony cool than warm (Stabentheiner et al., 2021). This is because some of the primary behaviors used to cool the hive like fanning, where bees use their wings to drive cool air currents around the hive, paired with evaporative cooling, induced by regurgitating water across the hive, can cool the hive without requiring direct body contact (Heinrich, 1979; Kronenberg and Heller, 1982; Seeley, 1985). The temperature at which individuals begin fanning is genetically influenced, and multi-patriline colonies are more effective at regulating the temperature of their hive because the variation of the temperature at which individuals begin fanning is increased (Jones et al., 2004; Graham et al., 2006). Some bees begin to fan at temperatures as low as 20°C, possibly as a response to high levels of carbon dioxide within the hive (Seeley, 1974), and fanning probability rises quickly as temperatures increase (Cook and Breed, 2013; Cook et al., 2016). Social context also affects the likelihood of fanning, with the likelihood increasing if a bee is surrounded by other fanners (Kaspar et al., 2018).

The mechanism honey bees use to fan is strongly affected by the physical properties of the hive itself. If the hive has a small entrance, bees alternate between fanning warm air high in carbon dioxide out of the hive, and briefly stopping to let oxygen-rich air return by diffusion in what has been described as "slow breathing" of the colony (Seeley, 1974; Southwick and Moritz, 1987). In hives with large entrances, bees stand along one side of the entrance to fan air out, while others fan air in from the other side to create cyclic air currents within the hive (Peters et al., 2019). In cases of extreme heat, large numbers of bees will leave the hive to increase the area available for air circulation, creating a "beard" near the entrance (Winston, 1987).

When heat stress is localized, honey bees engage in a behavior known as "heat shielding" where individual bees position themselves between a heat source and developing brood, protecting brood by absorbing excess heat into their own bodies (Starks and Gilley, 1999; Siegel et al., 2005). Bees between age 12 and 14 are most likely to engage in heat shielding, indicating performance of this task is influenced by a bee's developmental stage (Starks et al., 2005). Once individuals absorb enough heat they move toward the periphery of the nest, rapidly speeding up the diffusion of heat in a manner analogous to mammalian vascular dynamics in thermoregulation (Bonoan et al., 2014).

Rationale

The aforementioned cooling methods are energy intensive making them best suited for managing acute heat stress. As extended heatwaves become more common worldwide (Marx et al., 2021) it is vital we improve our understanding of how honey bee colonies mange chronic heat stress in their hives. Wild honey bees tend to make their nests in insulated spaces like tree hollows that help preserve the temperature within their hives (Seeley and Morse, 1976). They also organize their comb with a central cluster of brood surrounded by thick layers of honey and nectar, which has been hypothesized to have an insulating effect on the brood (Seeley and Morse, 1976; Camazine et al., 1990). It has been speculated that bees may use natural thermal gradients in the hive to determine where they place their stores, which may help them more easily maintain the temperature of their brood (Camazine, 1991; Montovan et al., 2013). Previous work from our lab provides evidence that honey bees are able to alter comb arrangement in response to localized chronic heat stress in a way that thermally protects brood (Weinberg et al., 2022).

In the study presented here, we tested the hypothesis that comb phenotype is affected by chronic hive-wide heat stress in full-sized free-foraging honey bee colonies. We subjected honey bee colonies to chronic heat stress over 6 weeks and measured the phenotype of brood, carbohydrates (honey and nectar), pollen stored within the comb three times over 6 weeks by recording the area on each frame in each hive covered in each store. We hypothesized that exposure to chronic heat stress would cause comb to become arranged in a way that better insulates brood: a more densely clustered brood comb, with thicker stores of honey and nectar on the periphery. The goal of this study was to increase our understanding of how honey bee colonies behaviorally respond to mitigate chronic heat stress in their hives. These insights may allow for the creation of better strategies to mitigate the effect of chronic heat stress on managed honey bee colonies, which already suffer from high yearly mortality (Gregorc, 2020).

Methods

Subjects and experimental setup

Five-frame nucleus colonies ($n = 20$) of *Apis mellifera ligustica* were used as subjects for this experiment. Twelve colonies were installed at the Cummings School of Veterinary Medicine in Grafton, MA (N 42°23′98″, W 71°68′67″) on June 2–21, and eight were installed at the Tufts University campus in Medford, MA (N 42°24′20″, W 71°06′51″) on June 3–21. Half of the colonies at each site were used as control hives, and the other half were outfitted to be experimentally heated. Two control hives from the Medford site, and one control hive and one experimental hive from the Grafton site were excluded due to having no brood stores at the end of the study, indicating the loss of the colony's queen. This left seven control hives and nine experimentally heated hives. The size of each colony size was measured in week 1 and week 4 using a modified Liebenfield method (Imdorf et al., 1987; Dainat et al., 2020). Each frame was removed from each hive, and the proportion of each side of the frame covered in bees on was visually estimated to the nearest 25%. Colony size did not differ between treatments in week 1 (Welches two sample *t*-test $p = 0.4$, average full frames of bees = 3.1 for control and 2.8 for heated) or week 4 (Welches two sample *t*-test, $p = 0.28$, average full frames of bees = 2.67 for control and 3.4 for heated).

Two 15 cm × 20 cm heating pads (Zoo Med ReptiTherm®) were installed in each experimental hive. Each heating pad was fastened onto an empty frame and installed facing inwards on the left and right most side of the hive interior. We elected not to install heating pads on control hives since previous studies have shown that powered down heating pads to not affect honey bee behavior (Starks and Gilley, 1999; Weinberg et al., 2022). The installation of heating frames restricted experimental colonies to building on 8 frames. To maintain size consistency between groups, control colonies were also restricted to 8 frames using exclusion boards. Both heating pads in each experimental hive were powered by a single temperature controller (InkBird ITC-306 T). A probe from the temperature controller was fastened to one heating pad in each hive, which controlled power to both pads based on the temperature of the probe. The probe was set to maintain the temperature of the heating pad at 41 ± 2°C from 8 a.m. to 4 p.m. and 31 ± 2°C from 4 p.m. to 8 a.m. The heating pads were activated on June 17–21 (Week 0) in Grafton and June 15–21 (Week 0) in Medford.

Temperature gradients were measured at three timepoints in two hive of each treatment. Nine temperature probes were placed in each hive from which measurements were taken: one probe was placed between each frame, and one each on the outer edge of the left and right most frame. Probe position was recorded based on distance, in full frames, from the center of the hive. Since there were 9 probes, distances ranged from 0 to 4 frames from the center (Figure 1). To further validate that our method was sufficient to warm the hives, after the end of the field season we used temperature loggers (iButton DS1921H-F5) to take temperature readings in one control and one experimental hive every 15 min for 24 h (Figure 1). These readings were taken in empty colonies which were placed indoors, but were subjected to the same heat regimen as our experimental hives during the field season. A Welch two-sample *t*-test validated that temperatures were significantly elevated within heated hives during this validation ($p < 0.00001$).

Data collection

Data were collected from the hives three times, at approximately three-week intervals. Previous research has shown that 3 weeks is a sufficient period of time for comb alteration to occur (Weinberg et al., 2022). In Grafton, data collection occurred on June 17–21 (Week 0),

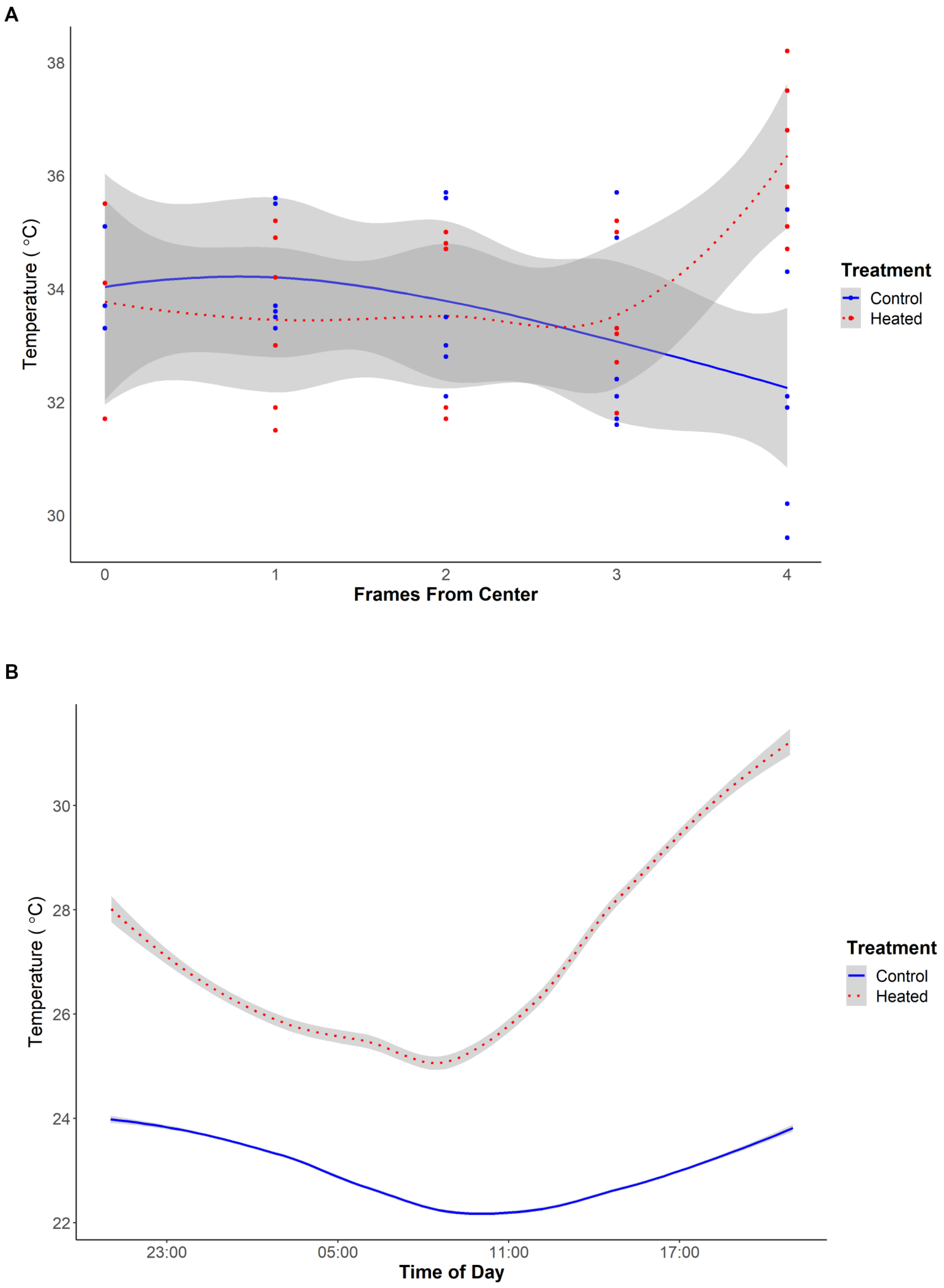


FIGURE 1
(A) Temperature gradient in degrees Celsius from the edge to the center of the hive in control (blue, solid) and heated (red, dotted) honey bee colonies (*N*=3 for both treatments). Temperature was taken between each frame and on the outside of the left and right most frames. Each frame is approximately 38mm wide. Gray lines represent standard error. All figures made using R. **(B)** Average temperature in degrees Celsius of empty hives in either control (blue, solid) or heated (red, dotted) colonies. Temperatures were taken every 15min for 24h from 4:00p.m. to 4p.m. using seven probes distributed in one hive of each treatment. Gray lines represent standard error.

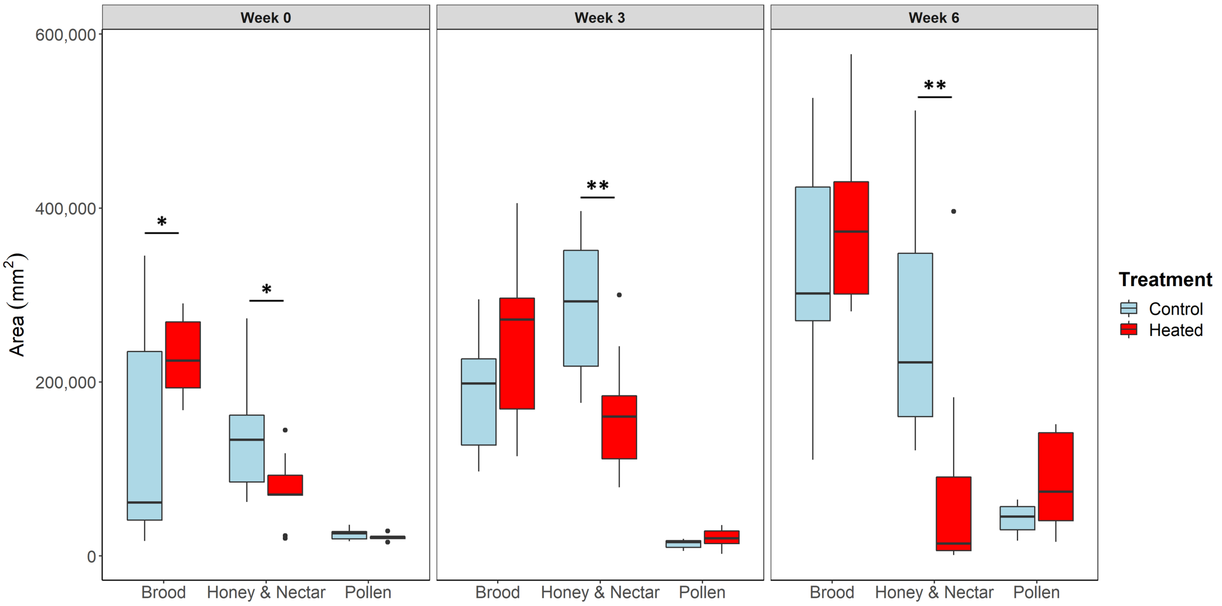


FIGURE 2
Box and whisker plot showing the average total area in mm^2 of brood, honey, and nectar, and pollen in heated (red) and control (blue) hives (*N*=7 control and 9 heated colonies) in weeks 0, 3, and 6 of the study. **p*<0.05; ***p*<0.001.

July 8–21 (Week 3) and August 2–21 (Week 6), and in Medford, data collection occurred on June 15–21 (Week 0), July 10–21 (Week 3), and July 28–21 (Week 6). To analyze comb phenotype, photographs were then taken of both sides of all eight frames in each colony, for a total of 320 comb sides imaged and analyzed each day. Each frame was lifted out of the hive one at a time. Any bees remaining on the frame were lightly brushed back into the hive before a photograph of both sides of the frame were taken. The frame was then inserted back into the hive in its previous position before the next frame was removed. Comb photographs were analyzed using Fiji (Schindelin et al., 2012). Cells containing honey, nectar, pollen, capped brood, and uncapped brood were traced to determine the total area filled by each store. For analysis, comb stores were split into three categories based on type: brood, which consisted of capped and uncapped brood; carbohydrates, which consisted of honey and nectar; and protein, which consisted of pollen (Weinberg et al., 2022).

Statistical methods

Comb phenotype

Generalized linear mixed models created in R using the “glmmTMB” package (Magnusson et al., 2021; R Core Team, 2022) were used to evaluate the effect of heat stress on three separate response variables: Brood, honey and nectar, and pollen. Since comb storage across a colony tends to be symmetrical (Seeley and Morse, 1976), comb sections were grouped based on their distance, in half frames, from the center of the hive. Each side of a frame counted as a half integer, so distance ranged from 0 to 3.5 frames from the center. To ensure our hives were symmetrical, the side of the hive each frame was taken from was included as a predictor variable in our models. To account for the large number of zeros in our data, we used zero inflated models in our analysis (Yang et al., 2017).

To determine the effect of heat stress on comb phenotype, models were created for all three response variables for each week. Models used the interaction between distance from center of the hive and treatment as predictor variables, side as a predictor variable, colony as a random effect, and distance from center of the hive as a zero inflated effect. The most complex model was competed against all nested models using the Akaike information criterion (AIC). The model with the lowest AIC was selected as the best fit model for each week and response variable.

Hive temperature gradients

Generalized linear models were created in R using the base “stats” package (R Core Team, 2022) to evaluate the effect treatment had on internal hive temperature. Separate models were created for both the point readings taken during the experimental season, and the treatment validation performed after the end of the field season on empty hives. To determine the effect of treatment on hive temperature gradients during the field season, a model using temperature as the response variable, and the interaction between treatment and distance from the center of the hive as response variables was used for both response variables. These models were tested against all nested models using AIC, with the best model reported below.

Results

Brood

In week 0 there was significantly more brood in heated colonies ($\chi^2=6$, $p<0.05$) despite efforts to visually balance hives between treatment groups. There was no difference in brood quantity between treatments in week 3 ($\chi^2=0.02, p=0.87$) or week 6 ($\chi^2=0.67, p=0.41$) (Figure 2). Brood quantity was not affected by distance from the

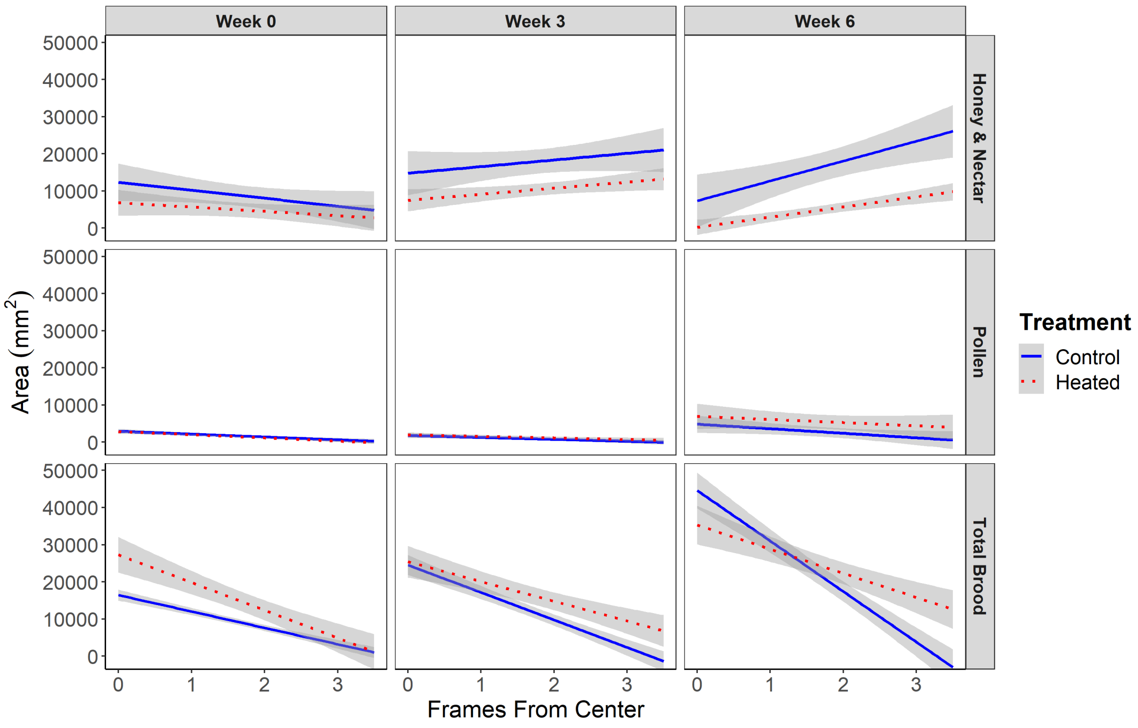


FIGURE 3
Total area in mm^2 of total honey and nectar (top), pollen (middle), and brood (bottom) in control (blue, solid) and heated (red, dashed) honey bee colonies (N=7 control and 9 heated colonies). X axis represents the distance from the center of the hive in frames. Each frame is approximately 38mm wide. Vertical facets represent the week of the study measurements were taken. Gray outlines show standard error. Honey and nectar were located significantly closer to the periphery of the hive in both treatments during weeks 0 (χ^2=13, p<0.001), 3 (χ^2=49, p<0.00001), and 6 (χ^2=136, p<0.00001). Brood was stored closer to the periphery of hives in both treatments in week 3 (χ^2=22, p<0.0001), and 6 (χ^2=30, p<0.00001). Brood comb phenotype significantly differed between treatments in week 6 (χ^2=5, p<0.05), with brood comb more densely clustered in the center of control hives. Pollen storage did not differ between treatments at any point in the study.

center of the hive within the hive in week 0 ($\chi^2 = 2$, $p = 0.12$), but was stored more densely in the center of hives of both treatments in week 3 ($\chi^2 = 22$, $p < 0.0001$), and week 6 ($\chi^2 = 30$, $p < 0.00001$) (Figure 3). This was expected given that honey bee colonies tend rear brood in the center of the colony (Seeley and Morse, 1976). Finally, brood quantity was not affected by the interaction between treatment and distance from the center of the hive in either week 0 ($\chi^2 = 0.6$, $p = 0.42$), or week 3 ($\chi^2 = 0.1$, $p = 0.71$), but was significantly affected by this interaction in week 6 ($\chi^2 = 5$, $p < 0.05$). This indicates differences in brood comb phenotype between heated and unheated colonies arise sometime between weeks 3 and 6, with the brood comb in heated hives being less densely clustered in the center than in control hives. This timeframe aligns well with natural brood development, which takes around 3 weeks (Winston, 1987). Side was never included in the winning model for brood quantity, indicating brood comb was symmetric across the hive at all timepoints in the study.

Honey and nectar

There was significantly more honey and nectar in unheated colonies during weeks 0 ($\chi^2 = 6$, p < 0.05), 3 ($\chi^2 = 11$, $p < 0.001$), and 6 ($\chi^2 = 8$, $p < 0.001$) (Figure 2) of our study. Initial differences occurred despite attempts to visually balance treatment groups, however the differences between treatments continued to increase throughout the study, even as brood quantity converged, indicating starting differences alone were likely not responsible for differences at the end of the study. Honey and nectar were stored significantly closer to the hive periphery in weeks 0 ($\chi^2 = 13$, p < 0.001), 3 ($\chi^2 = 49$, $p < 0.00001$), and 6 ($\chi^2 = 136$, $p < 0.00001$) (Figure 3) which was expected (Seeley and Morse, 1976). Honey and nectar quantity was not affected by the interaction between treatment and distance from the center of the hive in weeks 0 ($\chi^2 = 0.8$, $p = 0.369$), 3 ($\chi^2 = 0.3$, $p = 0.55$), or 6 ($\chi^2 = 42$, $p < 0.51$), indicating honey and nectar phenotype was similar not different between treatments throughout the study. Side did have a significant effect on honey and nectar storage during week 0 ($\chi^2 = 9$, $p = 0.01$), indicating that carbohydrate storage was not symmetric across the hive at the beginning of the study. Side was not included in winning models in either 6, indicating carbohydrate storage did become symmetric as the study continued.

Pollen

There was no difference in pollen quantity in weeks 0 ($\chi^2 = 0.56$, $p = 0.45$), 3 ($\chi^2 = 0.16$, $p = 0.68$), or 6 ($\chi^2 = 2.4$, $p = 0.11$) of the study. Pollen was stored closer to the center of the hive in weeks 0 ($\chi^2 = 5.7$, $p < 0.05$), and 3 ($\chi^2 = 11$, $p < 0.001$), but was not affected by hive location in week 6 ($\chi^2 = 2.5$, $p = 0.098$). Finally, the interaction between treatment and location was not significant in weeks 0 ($\chi^2 = 0.11$,

$p = 0.73$), 3 ($\chi^2 = 1.9$, $p = 0.016$) or 6 ($\chi^2 = 0.74$, $p = 0.38$). Pollen storage was significantly affected by side in week 3 of the study ($\chi^2 = 8.5$, $p < 0.01$), indicating pollen storage was not symmetric across the hive. Side was not included in winning models in either week 0 or week 6, indicating pollen storage was symmetric across the hive at these timepoints.

Temperature gradients

Temperature gradient within the hive was not significantly predicted by treatment ($\chi^2 = 3$, $p = 0.08$) or distance from the center of the hive ($\chi^2 = 0.09$, $p = 0.76$) but was significantly predicted by the interaction of treatment and distance from the center of the hive ($\chi^2 = 12$, $p < 0.001$) (Figure 1). This indicates that bees were able to keep the temperature relatively stable within their hives, except on the very edges which were significantly hotter in heated colonies.

Discussion

We found that after 6 weeks of treatment, brood comb became less clustered in the center of hives exposed to chronic heat stress (Figure 3) but overall brood quantity did not differ in overall quantity between treatments (Figure 2). These results support the hypothesis that honey bee comb phenotype is affected by chronic hive-wide heat stress, but are the opposite of our expectation that chronic heat stress would cause the brood cluster to become concentrated more tightly in the center of the hive, which would necessitate the thermoregulation of a smaller hive section. We also found that, while heated colonies began with smaller stores of honey and nectar, this difference consistently widened throughout the study (Figure 3), indicating that heated colonies were less able to build up carbohydrate reserves than unheated colonies (Figure 2). This likely indicates that hives exposed to chronic heat stress had higher energy requirements, and thus needed to consume more honey and nectar.

Our expectation was that in response to heat stress, brood would become more centrally clustered in order to improve the insulation provided by honey and nectar. Instead, we found brood comb became significantly less clustered and spread more toward the hive periphery (Figure 3). These results suggest that it may be the coldest temperatures colonies experience, not the hottest, which drive brood comb phenotype. Since honey bees possess effective diffuse cooling behaviors (Heinrich, 1979; Kronenberg and Heller, 1982; Peters et al., 2019), experimentally heated colonies were able to maintain a stable temperature throughout most of the hive during the day and prevent widespread brood death (Figure 1). However, all warming behaviors are localized and require endothermic heat transfer through direct contact (Simpson, 1961; Kronenberg and Heller, 1982; Watmough and Camazine, 1995). This means the ability of bees to keep their brood warm at night is likely a limiting factor for overall brood comb area. The minimum size of the nightly warming cluster, which gets smaller as ambient temperatures decrease (Simpson, 1961), is likely the maximum possible size of the brood cluster. This could explain why heated colonies, which were exposed to increased night time temperatures, had an increased amount of brood closer to the hive periphery. We found no significant difference in brood quantity between treatment groups, which indicates that heat did not affect rate of ovipositing, and that differences in brood quantity between treatment groups were not driving our results.

After 6 weeks, hives exposed to chronic heat stress contained significantly fewer carbohydrate stores than control hives, even after accounting for initial differences in carbohydrate quantity (Figure 2). It is likely that this decrease in carbohydrate stores is a direct result of an increased level of active thermoregulatory behaviors, like fanning, used by the bees to keep heated hives cool. These behaviors are energetically expensive (Peters et al., 2017) and increase the amount of honey and nectar a colony needs to eat in order to maintain temperature homeostasis, which would directly reduce carbohydrate store quantity. It is also possible that more workers were recruited to thermoregulate, leaving fewer available to forage for nectar (Ostwald et al., 2016), which would have resulted in fewer carbohydrate stores entering the colony. Finally, it is possible that a lack of honey and nectar stores contributed to the more diffuse layout of the brood comb, since areas typically occupied by honey and nectar became vacant, allowing space for the queen to oviposit. We believe it unlikely, however, that lack of carbohydrate stores alone would result in a more diffuse brood comb, since brood would still freeze to death in the hive periphery if left outside of the warming cluster. Interestingly, there was no difference in pollen quantity between treatments throughout the study. Since pollen is primarily used as a protein source to facilitate the growth of brood (Winston, 1987), lack of differences in pollen storage between treatments can be seen as a further indicator that brood rearing effort did not differ between treatments.

The most striking result of this study is the apparent tradeoff honey bee colonies make regarding brood survival and carbohydrate storage in response to chronic heat stress. Exposure to chronic heat did not affect brood quantity, but did significantly decrease the quantity of honey and nectar stored in the hive. This implies that in response to extreme temperatures, honey bees do not decrease investment in brood rearing and therefore must increase the energetic investment required to actively thermoregulate their brood. High summer temperatures have been found to increase winter mortality of honey bee colonies (Schweiger et al., 2010; Switanek et al., 2017; Calovi et al., 2021), and the tradeoff presented here may offer a mechanism that explains this occurrence. Decreases in carbohydrate stores likely result in decreased winter reservoirs, and therefore decreased winter survival for colonies exposed to chronic heat stress during the summer. Interestingly, this tradeoff may not be as detrimental for honey bee colonies kept at lower latitudes, where year round forage is more likely to be available. In these locations, colonies would be able to manage high temperatures without reducing the size of their brood comb, while not being as detrimentally affected by low quantities of carbohydrate stores. These results may, therefore, be seen as a positive for honey bee colonies kept in the tropics, or other areas where nectar can be collected year round.

This study suggests multiple avenues for future research, namely experiments that further elucidate the mechanism by which comb rearrangement is induced, and experiments that determine the physiological consequences of comb rearrangement on individual bees. Mechanistically, it would be interesting to test the hypothesis that the coolest temperatures a hive experiences determines the degree to which brood comb is clustered. This could be done by decreasing internal hive temperatures during the coldest parts of the night. If brood comb were affected primarily by the thermal minimum, we would expect cooled colonies to have a more clustered brood comb than control colonies. Physiologically, it would be interesting to determine if bees in colonies with a less clustered brood comb are more susceptible to defects associated with abnormal rearing temperatures. Brood reared on the outer edges the brood cluster likely have their temperature less well regulated and are therefore more susceptible to temperature fluctuations. This should be exacerbated in colonies with less clustered brood comb, since the heating cluster will likely encase less brood. Since even a single night below 20°C can seriously impact brood development (Wang et al., 2016) it is likely that abnormal comb phenotype would increase susceptibility to cold stress and therefore result in more abnormal adults. Finally, it would be valuable to determine the mechanism by which heated colonies were unable to build robust carbohydrate stores. Active measurements of colony energetic expenditure could confirm heated colonies had higher energetic expense and therefore consumed more honey and nectar. Additionally, measuring foraging effort by colonies could determine whether heated colonies were simply collecting less nectar resources as more bees were engaged in active thermoregulation.

As the climate crisis continues, studies that record the natural suite of behaviors animals use in response to environmental stressors are invaluable for developing effective conservation strategies. We found that while chronic heat stress may not reduce overall brood quantity, it does result in alterations to brood arrangement within the colony. Additionally, chronic heat stress results in a significant decrease in carbohydrate reserves, which likely negatively impacts winter survival. Our findings indicate that ensuring colonies have access to rich sources of carbohydrates may be particularly important during periods of abnormal heat. Studies that investigate the behavioral response of honey bees to heat will allow for the implementation of new effective management strategies as we attempt to prevent honey bee declines and ensure the services they provide to our food systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

IW was the primary contributor to manuscript preparation and data analysis, and was a contributor to experimental design and data collection. JW contributed to the manuscript preparation, data collection, and data analysis. EK, AK, RG, and JZ were contributors to data collection and data analysis. PS contributed to the experimental design and manuscript preparation. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Science Foundation Research Experience for Undergraduates (NSF DBI 1852438).

Acknowledgments

The authors would also like to thank Jessie Thuma, Helen McCrery, and Angelle Antoun for help in editing early versions of this manuscript.

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

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1119452/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Total change in area in mm^2 of total honey and nectar **(top)**, pollen **(middle)**, and brood **(bottom)** in control (blue, solid) and heated (red, dashed) honey bee colonies (*N*=7 control and 9 heated colonies). X axis represents the distance from the center of the hive in frames. The left column represents change in area between weeks 0 and 3, and the right column represents change in area between weeks 3 and 6.

SUPPLEMENTARY FIGURE 2
Box and whisker plots displaying total area of brood, honey and nectar, and pollen in the left (green) and right (orange) side of hives in weeks 0, 3, and 6 of the study. $^*P<0.01$.

References

Becher, M. A., Scharpenberg, H., and Moritz, R. F. A. (2009). Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.). *J. Comp. Physiol. A.* 195, 673–679. doi: 10.1007/s00359-009-0442-7

Bonoan, R. E., Iglesias Feliciano, P. M., Chang, J., and Starks, P. T. (2020). Social benefits require a community: the influence of colony size on behavioral immunity in honey bees. *Apidologie* 51, 701–709. doi: 10.1007/s13592-020-00754-5

Bonoan, R., Goldman, R., RWong, P., and TStarks, P. (2014). Vasculature of the hive: heat dissipation in the honey bee (*Apis mellifera*) hive. *Naturwissenschaften* 101, 459–465. doi: 10.1007/s00114-014-1174-2

Bujok, B., Kleinhenz, M., Fuchs, S., and Tautz, J. (2002). Hot spots in the bee hive. *Naturwissenschaften* 89, 299–301. doi: 10.1007/s00114-002-0338-7

Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. *PLoS One* 7:e37235. doi: 10.1371/journal.pone.0037235

Calovi, M., Grozinger, C. M., Miller, D. A., and Goslee, S. C. (2021). Summer weather conditions influence winter survival of honey bees (*Apis mellifera*) in the northeastern United States. *Sci. Rep.* 11:1553. doi: 10.1038/s41598-021-81051-8

Camazine, S. (1991). Self-organizing pattern formation on the combs of honey bee colonies. *Behav. Ecol. Sociobiol.* 28, 61–76.

Camazine, S., Sneyd, J., Jenkins, M. J., and Murray, J. D. (1990). A mathematical model of self-organized pattern formation on the combs of honeybee colonies. *J. Theor. Biol.* 147, 553–571. doi: 10.1016/S0022-5193(05)80264-4

Campbell, J., Kessler, B., Mayack, C., and Naug, D. (2010). Behavioural fever in infected honeybees: parasitic manipulation or coincidental benefit? *Parasitology* 137, 1487–1491. doi: 10.1017/S0031182010000235

Chacon-Almeida, V. M. L., Soares, A. E. E., and Malheiros, E. B. (1999). Induction of the Split sting trait in Africanized *Apis mellifera* (Hymenoptera: Apidae) by cold treatment of pupae. *Ann. Entomol. Soc. Am.* 92, 549–555. doi: 10.1093/aesa/92.4.549

Coelho, J. (1991). Heat transfer and body temperature in honey bee (Hymenoptera: Apidae) drones and workers. *Environ. Entomol.* 20, 1627–1635. doi: 10.1093/ee/20.6.1627

Cook, C. N., and Breed, M. D. (2013). Social context influences the initiation and threshold of thermoregulatory behaviour in honeybees. *Anim. Behav.* 86, 323–329. doi: 10.1016/j.anbehav.2013.05.021

Cook, C. N., Kaspar, R. E., Flaxman, S. M., and Breed, M. D. (2016). Rapidly changing environment modulates the thermoregulatory fanning response in honeybee groups. *Anim. Behav.* 115, 237–243. doi: 10.1016/j.anbehav.2016.03.014

Dainat, B., Dietemann, V., Imdorf, A., and Charrière, J.-D. (2020). A scientific note on the 'Liebefeld method' to estimate honey bee colony strength: its history, use, and translation. *Apidologie* 51, 422–427. doi: 10.1007/s13592-019-00728-2

Fukuda, H., and Sakagami, S. F. (1968). Worker brood survival in honeybees. *Popul. Ecol.* 10, 31–39. doi: 10.1007/BF02514731

Gallai, N., Salles, J.-M., Settele, J., and Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* 68, 810–821. doi: 10.1016/j.ecolecon.2008.06.014

Gonzalez, V. H., Oyen, K., Ávila, O., and Ospina, R. (2022). Thermal limits of Africanized honey bees are influenced by temperature ramping rate but not by other experimental conditions. *J. Therm. Biol.* 110:103369. doi: 10.1016/j.jtherbio.2022.103369

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347:1255957. doi: 10.1126/science.1255957

Graham, S., Myerscough, M. R., Jones, J. C., and Oldroyd, B. P. (2006). Modelling the role of intracolonial genetic diversity on regulation of brood temperature in honey bee (*Apis mellifera* L.) colonies. *Insect. Soc.* 53, 226–232. doi: 10.1007/s00040-005-0862-5

Gregorc, A. (2020). Monitoring of honey bee Colony losses: a special issue. *Diversity* 12:403. doi: 10.3390/d12100403

Groh, C., Tautz, J., and Rössler, W. (2004). Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4268–4273. doi: 10.1073/pnas.0400773101

Harbo, J. R. (2000). Heating adult honey bees to remove *Varroa Jacobsoni*. *J. Apic. Res.* 39, 181–182. doi: 10.1080/00218839.2000.11101041

Heinrich, B. (1979). Keeping a cool head: honeybee thermoregulation. *Science* 205, 1269–1271. doi: 10.1126/science.205.4412.1269

Heinrich, B. (1980). Mechanisms of body-temperature regulation in honeybees, *Apis mellifera* I. regulation of head temperature. *J. Exp. Biol.* 85, 61–72. doi: 10.1242/jeb.85.1.61

Heinrich, B. (1981). The regulation of temperature in the honeybee swarm. *Sci. Am.* 244, 146–160. doi: 10.1038/scientificamerican0681-146

Himmer, A. (1932). Die Temperaturverhältnisse bei den sozialen Hymenopteren. *Biol. Rev.* 7, 224–253. doi: 10.1111/j.1469-185X.1962.tb01042.x

Hoppe, H., and Ritter, W. (1987). Experiments using combined heat therapy to control Varroa disease. *Apidologie* 18, 383–385.

Hou, C. S., Li, B. B., Deng, S., and Diao, Q. Y. (2016). Effects of *Varroa destructor* on temperature and humidity conditions and expression of energy metabolism genes in infested honeybee colonies. *Genet. Mol. Res.* 15:gmr8997. doi: 10.4238/gmr.15038997

Hristov, P., Shumkova, R., Palova, N., and Neov, B. (2020). Factors associated with honey bee colony losses: a mini-review. *Vet. Sci.* 7:166. doi: 10.3390/vetsci7040166

Hung, K.-L. J., Kingston, J. M., Albrecht, M., Holway, D. A., and Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proc. R. Soc. B Biol. Sci.* 285:20172140. doi: 10.1098/rspb.2017.2140

Imdorf, A., Buehlmann, G., Gerig, L., Kilchenmann, V., and Wille, H. (1987). Überprufung der schatzmethode zur ermittlung der brutflache und der anzhal arbeiterinnen in freifliegenden bienenvolkern. *Apidologie* 18, 137–146. doi: 10.1051/apido:19870204

Jones, J. C., Helliwell, P., Beekman, M., Maleszka, R., and Oldroyd, B. P. (2005). The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. *J. Comp. Physiol. A.* 191, 1121–1129. doi: 10.1007/s00359-005-0035-z

Jones, J. C., Myerscough, M. R., Graham, S., and Oldroyd, B. P. (2004). Honey bee nest thermoregulation: diversity promotes stability. *Science* 305, 402–404. doi: 10.1126/science.1096340

Jones, J., and Oldroyd, B. (2006). Nest thermoregulation in social insects. *Adv. Insect Physiol.* 33, 153–191. doi: 10.1016/S0065-2806(06)33003-2

Kaspar, R. E., Cook, C. N., and Breed, M. D. (2018). Experienced individuals influence the thermoregulatory fanning behaviour in honey bee colonies. *Anim. Behav.* 142, 69–76. doi: 10.1016/j.anbehav.2018.06.004

Kleinhenz, M., Bujok, B., Fuchs, S., and Tautz, J. (2003). Hot bees in empty broodnest cells: heating from within. *J. Exp. Biol.* 206, 4217–4231. doi: 10.1242/jeb.00680

Kronenberg, F., and Heller, H. C. (1982). Colonial thermoregulation in honey bees (*Apis mellifera*). *J. Comp. Physiol. B.* 148, 65–76. doi: 10.1007/BF00688889

Le Conte, Y., Arnold, G., and Desenfant, P. (1990). Influence of brood temperature and hygrometry variations on the development of the honey bee ectoparasite *Varroa jacobsoni* (Mesostigmata: Varroidae). *Environ. Entomol.* 19, 1780–1785. doi: 10.1093/ee/19.6.1780

Lin, H., and Winston, M. L. (1998). The role of nutrition and temperature in the ovarian development of the worker honey bee (*Apis mellifera*). *Can. Entomol.* 130, 883–891. doi: 10.4039/Ent130883-6

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., et al. (2021). *glmm TMB: generalized linear mixed models using template model builder (1.1.2.3)*. Available at: https://CRAN.R-project.org/package=glmmTMB

Marx, W., Haunschild, R., and Bornmann, L. (2021). Heat waves: a hot topic in climate change research. *Theor. Appl. Climatol.* 146, 781–800. doi: 10.1007/s00704-021-03758-y

Medina, R. G., Paxton, R. J., De Luna, E., Fleites-Ayil, F. A., Medina Medina, L. A., and Quezada-Euán, J. J. G. (2018). Developmental stability, age at onset of foraging and longevity of Africanized honey bees (*Apis mellifera* L.) under heat stress (Hymenoptera: Apidae). *J. Therm. Biol.* 74, 214–225. doi: 10.1016/j.jtherbio.2018.04.003

Montovan, K. J., Karst, N., Jones, L. E., and Seeley, T. D. (2013). Local behavioral rules sustain the cell allocation pattern in the combs of honey bee colonies (*Apis mellifera*). *J. Theor. Biol.* 336, 75–86. doi: 10.1016/j.jtbi.2013.07.010

Ostwald, M. M., Smith, M. L., and Seeley, T. D. (2016). The behavioral regulation of thirst, water collection and water storage in honey bee colonies. *J. Exp. Biol.* 219, 2156–2165. doi: 10.1242/jeb.139824

Panziera, D., Requier, F., Chantawannakul, P., Pirk, C. W. W., and Blacquière, T. (2022). The diversity decline in wild and managed honey bee populations urges for an integrated conservation approach. *Front. Ecol. Evol.* 10:767950. doi: 10.3389/fevo.2022.767950

Perez, R., and Aron, S. (2020). Adaptations to thermal stress in social insects: recent advances and future directions. *Biol. Rev.* 95, 1535–1553. doi: 10.1111/brv.12628

Peters, J. M., Gravish, N., and Combes, S. A. (2017). Wings as impellers: honey bees co-opt flight system to induce nest ventilation and disperse pheromones. *J. Exp. Biol.* 220, 2203–2209. doi: 10.1242/jeb.149476

Peters, J. M., Peleg, O., and Mahadevan, L. (2019). Collective ventilation in honeybee nests. *J. R. Soc. Interface* 16:20180561. doi: 10.1098/rsif.2018.0561

Petz, M., Stabentheiner, A., and Crailsheim, K. (2004). Respiration of individual honeybee larvae in relation to age and ambient temperature. *J. Comp. Physiol. B* 174, 511–518. doi: 10.1007/s00360-004-0439-z

Phillips, E. F., and Demuth, G. S. (1914). *The temperature of the honeybee cluster in winter* Washington, D.C: U.S. Department of Agriculture.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353. doi: 10.1016/j.tree.2010.01.007

R Core Team. (2022). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Reilly, J. R., Artz, D. R., Biddinger, D., Bobiwash, K., Boyle, N. K., Brittain, C., et al. (2020). Crop production in the USA is frequently limited by a lack of pollinators. *Proc. R. Soc. B Biol. Sci.* 287:20200922. doi: 10.1098/rspb.2020.0922

Rosenkranz, P. (1987). Temperature treatment of sealed worker brood as a method of controlling varroatosis. *Apidologie* 18, 385–388.

Sánchez-Bayo, F., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676–682. doi: 10.1038/nmeth.2019

Schweiger, O., Biesmeijer, J. C., Bommarco, R., Hickler, T., Hulme, P. E., Klotz, S., et al. (2010). Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination. *Biol. Rev.* 85, 777–795. doi: 10.1111/j.1469-185X.2010.00125.x

Seeley, T. D. (1974). Atmospheric carbon dioxide regulation in honey-bee (*Apis mellifera*) colonies. *J. Insect Physiol.* 20, 2301–2305. doi: 10.1016/0022-1910(74)90052-3

Seeley, T. D. (1985). *Honeybee ecology: a study of adaptation in social life*, New Jersey: Princeton University Press.

Seeley, T. D., and Morse, R. A. (1976). The nest of the honey bee (*Apis mellifera* L.). *Insect. Soc.* 23, 495–512. doi: 10.1007/BF02223477

Siegel, A. J., Hui, J., Johnson, R. N., and Starks, P. T. (2005). Honey bee workers as mobile insulating units. *Insect. Soc.* 52, 242–246. doi: 10.1007/s00040-005-0805-1

Simone-Finstrom, M., Foo, B., Tarpy, D. R., and Starks, P. T. (2014). Impact of food availability, pathogen exposure, and genetic diversity on thermoregulation in honey bees (*Apis mellifera*). *J. Insect Behav.* 27, 527–539. doi: 10.1007/s10905-014-9447-3

Simpson, J. (1961). Nest climate regulation in honey bee colonies. *Science* 133, 1327–1333.

Southwick, E. E., and Moritz, R. F. A. (1987). Social control of air ventilation in colonies of honey bees, *Apis mellifera*. *J. Insect Physiol.* 33, 623–626. doi: 10.1016/0022-1910(87)90130-2

Stabentheiner, A., Kovac, H., and Brodschneider, R. (2010). Honeybee colony thermoregulation--regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. *PLoS One* 5:e8967. doi: 10.1371/journal.pone.0008967

Stabentheiner, A., Kovac, H., Mandl, M., and Käfer, H. (2021). Coping with the cold and fighting the heat: thermal homeostasis of a superorganism, the honeybee colony. *J. Comp. Physiol. A.* 207, 337–351. doi: 10.1007/s00359-021-01464-8

Stabentheiner, A., Pressl, H., Papst, T., Hrassnigg, N., and Crailsheim, K. (2003). Endothermic heat production in honeybee winter clusters. *J. Exp. Biol.* 206, 353–358. doi: 10.1242/jeb.00082

Starks, P. T., Blackie, C. A., and Seeley, T. D. (2000). Fever in honeybee colonies. *Naturwissenschaften* 87, 229–231. doi: 10.1007/s001140050709

Starks, P. T., and Gilley, D. C. (1999). Heat shielding: a novel method of colonial thermoregulation in honey bees. *Naturwissenschaften* 86, 438–440. doi: 10.1007/s001140050648

Starks, P. T., Johnson, R. N., Siegel, A. J., and Decelle, M. M. (2005). Heat shielding: a task for youngsters. *Behav. Ecol.* 16, 128–132. doi: 10.1093/beheco/arh124

Switanek, M., Crailsheim, K., Truhetz, H., and Brodschneider, R. (2017). Modelling seasonal effects of temperature and precipitation on honey bee winter mortality in a temperate climate. *Sci. Total Environ.* 579, 1581–1587. doi: 10.1016/j.scitotenv.2016.11.178

Szentgyörgyi, H., Czekońska, K., and Tofilski, A. (2018). Honey bees are larger and live longer after developing at low temperature. *J. Therm. Biol.* 78, 219–226. doi: 10.1016/j.jtherbio.2018.09.007

Tautz, J., Maier, S., Groh, C., Rössler, W., and Brockmann, A. (2003). Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. *Proc. Natl. Acad. Sci. U. S. A.* 100, 7343–7347. doi: 10.1073/pnas.1232346100

Wang, Q., Xu, X., Zhu, X., Chen, L., Zhou, S., Huang, Z. Y., et al. (2016). Low-temperature stress during capped brood stage increases pupal mortality, misorientation and adult mortality in honey bees. *PLoS One* 11:e0154547. doi: 10.1371/journal.pone.0154547

Watmough, J., and Camazine, S. (1995). Self-organized thermoregulation of honeybee clusters. *J. Theor. Biol.* 176, 391–402. doi: 10.1006/jtbi.1995.0207

Weinberg, I. P., Kuttner, I. L., Tyminski, A. E., Kalfus, R. L., and Starks, P. T. (2022). Comb phenotype of *Apis mellifera ligustica* is altered after exposure to chronic-localized heat stress. *Insect. Soc.* 69, 177–183. doi: 10.1007/s00040-022-00858-4

Winston, M. L. (1987). *The biology of the honey bee* Cambridge, Massachusetes: Harvard University Press.

Wood, T. J., and Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environ. Sci. Pollut. Res.* 24, 17285–17325. doi: 10.1007/s11356-017-9240-x

Yang, S., Harlow, L. L., Puggioni, G., and Redding, C. A. (2017). A comparison of different methods of zero-inflated data analysis and an application in health surveys. *J. Mod. Appl. Stat. Methods* 16, 518–543. doi: 10.22237/jmasm/1493598600

Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 06 September 2023
DOI 10.3389/fevo.2023.1166057

OPEN ACCESS

EDITED BY
Rachael E. Bonoan,
Providence College, United States

REVIEWED BY
Eduardo Périco,
Universidade do Vale do Taquari – Univates, Brazil
Charl Deacon,
Stellenbosch University, South Africa

*CORRESPONDENCE
Tainã Silva Rocha
tainasilva.tr@gmail.com
Lenize Batista Calvão
lenizecalvao@gmail.com

RECEIVED 14 February 2023
ACCEPTED 15 August 2023
PUBLISHED 06 September 2023

CITATION
Rocha TS, Calvão LB, Juen L and Oliveira-Junior JMB (2023) Effect of environmental integrity on the functional composition of the Odonata (Insecta) community in streams in the Eastern Amazon. *Front. Ecol. Evol.* 11:1166057. doi: 10.3389/fevo.2023.1166057

Effect of environmental integrity on the functional composition of the Odonata (Insecta) community in streams in the Eastern Amazon

Tainã Silva Rocha[1]*, Lenize Batista Calvão[2]*, Leandro Juen[1] and José Max Barbosa Oliveira-Junior[1,3]

[1]Programa de Pós-Graduação em Ecologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Brazil, [2]Programa de Pós-Graduação em Ciências Ambientais, Universidade Federal do Amapá, Macapá, Brazil, [3]Laboratório de Estudos de Impacto Ambiental, Instituto de Ciências e Tecnologia das Águas, Universidade Federal do Oeste do Pará, Santarém, Brazil

Anthropic activities affect the dynamics of aquatic communities and can influence the reproductive behavior of many species. In addition, functional diversity is expected to be influenced by the environment. In this context, we evaluated how the biological functional characteristics of the Odonata adult community respond to impacts caused by human action on streams in the Eastern Amazon, using bionomic characteristics as response variables. Concomitantly, we analyzed which characteristics are responsible for the presence of species in the environment. We sampled adults of Odonata in 98 preserved and altered streams in the Eastern Amazon. We used as functional characteristics: oviposition, thermoregulation and body size, and as morphological characteristics: width of the thorax, width of the wing at the base, length of the abdomen and length of the thorax. We recorded 80 species, distributed in 16 functional groups and three categories: present in all environments, present only in preserved environments, and present only in altered environments. There was variation in the functional characteristics studied between the environments (PerMANOVA; $F = 15,655$; $P < 0.01$), with a significant difference in the composition of attributes between the environments studied. Although PCoA did not find a strong relationship between the functional attributes and the level of integrity, the individuals found in altered areas are heliothermic, exophytic oviposition, with a wider wing width at the base and larger size. Individuals with smaller body size and endophytic and epiphytic oviposition, and thermal and endothermic conformators are found in preserved areas. Our study provides evidence that functional attributes are determining factors for the occurrence of species in the environment. The high quality of environment has a significant effect on the composition of functional groups. Exophytic and heliothermic species are favored by altered environments, while in preserved environments, the species that are best adapted are those that present epiphytic and endophytic oviposition and ectothermic thermoregulation (thermal conformers). As for morphology, altered environments favor medium to large individuals, with greater thorax length and abdomen size, preserved environments may favor the smaller and/or specialized species.

KEYWORDS

Amazon, dragonflies, oviposition, thermoregulation, environmental change

1 Introduction

Currently, studies indicate a worrying decline in the diversity of invertebrates, especially insects (Sánchez-Bayo and Wyckhuys, 2019). The main factors causing biodiversity loss are related to human activities such as logging, the expansion and intensification of agriculture, and urbanization (Ceballos et al., 2017). Such human intervention causes a significant change in the riparian vegetation of a waterbody, thus affecting the richness and abundance of species less tolerant to these changes (Ferreira and Petrere, 2007). Factors such as canopy openness (facilitating light entry) and the amount of vegetation around the water body can affect the distribution of aquatic insects (Harabis and Dolný, 2010; Monteiro-Júnior et al., 2013; Suhonen et al., 2013; Rodrigues et al., 2016), the reproductive behavior of many species (Rodrigues et al., 2016), and their functional diversity (Pereira et al., 2019).

The morphological structures of an organism and its dimensions are directly related to the functions performed for its survival (Arnold, 1983). When we evaluate species within the same taxonomic group, it is possible to notice that some individuals are more resistant to environmental changes while others disappear, thus becoming a major challenge for conservation ecology (Powney et al., 2015). Although taxonomic measures are efficient, they alone cannot detect variations in the functional structure of communities (Colzani et al., 2013). There are more recent approaches and diversity metrics that aim to understand the patterns that have reached the distribution of species (Dalzochio et al., 2015; Dalzochio et al., 2018). Among them is the functional diversity approach, whose objective is to group species by function and not by taxonomic group (Poff et al., 2006; Pereira et al., 2019; Resende et al., 2021). This approach is based on biological, morphological and behavioral characteristics that are connected with ecosystem functions, these characteristics are called functional traits (Violle et al., 2007; Dalzochio et al., 2015; Dalzochio et al., 2018). Therefore, the use of airways in functional traits can help identify the characteristics that allow some species of aquatic insects to resist anthropogenic pressures (Firmiano et al., 2021).

Among a variety of aquatic groups, dragonflies (Odonata) stand out due to their great habitat specificity and environmental sensitivity (Banks-Leite et al., 2012; Oliveira-Junior et al., 2022). They are widely used to detect environmental disturbance because their distribution, richness, and composition are highly associated with variations in the conditions that comprise a physical habitat (Williams et al., 2004; Silva-Pinto et al., 2012). Furthermore, they possess a relatively long-life cycle (Miguel et al., 2017), the dragonflies can live up to a year in the tropics (Stoks and Córdoba-Aguilar, 2012), have a broad distribution in aquatic systems (Corbet, 1980), and are affected by environmental change (Monteiro-Júnior et al., 2015; Mendoza-Penagos et al., 2021). Features like these make Odonata especially important for conservation because they provide a greater understanding of the relationship between physical variables, biological variables, and biological communities (Juen and De Marco, 2012).

Morphological variation has implications that need to be considered to increase our understanding of the distribution of species. For example, the width of the wing base is directly related to the ability to glide (Nilsson-Ortman et al., 2012) and inversely related to the maneuverability of the wings, since individuals with a wider wing base cannot perform more elaborate maneuvers (Johansson et al., 2009). The abdomen is important for the thermoregulation, reproduction, and territorialism of these individuals (May, 1976; Michiels and André, 1990). In some species, an increase in wing size is related to the ability to fly (Conrad et al., 2002). The width and length of the hind wing are important for maneuverability, and flight duration (De Marco et al., 2015) and are related to the ability to glide (Corbet, 1962).

The thorax, in contrast, is important for flight because it houses the musculature, and like the abdomen, it also has an important role in thermoregulation because the cooling coefficient is related to small body size (May, 1976). The speed of the group is generally related to body size and thermoregulation (Corbet, 1980). Bigger dragonflies tend to be endothermic and have a high dispersal ability (May, 1991; Corbet and May, 2008). In general, the Anisoptera have more robust bodies and are more capable of dispersal than the Zygoptera, which have slender bodies (Corbet, 1999; Heiser and Schmitt, 2010). Some Odonata species have elaborate territorial and competitive behavior (Corbet, 1999), where the adult male fights against competing males, to restrict access to the territory in which these organisms reproduce (Corbet, 1999).

Another important behavior in the distribution of dragonflies is oviposition. The oviposition environment is essential in the distribution of species because female dragonflies need places with adequate substrate for oviposition (Corbet, 1999; Suhonen et al., 2013). Environmental factors can act as filters for these characteristics (Pereira et al., 2019). Environmental stressors (such as the degradation of freshwater ecosystems) play an important role in shaping the feature composition of the aquatic insect community (Ding et al., 2017). The presence of different microhabitats provides good locations for oviposition and roosting sites (Clark and Samways, 1996). This idea also elucidates the theory of habitat models (Southwood, 1977), which proposes that habitat properties are determined by the composition and diversity of biotic communities (Townsend and Hildrew, 1994; Statzner et al., 2001).

The thermoregulation hypothesis shows that although all thermoregulatory groups are present throughout the entire length of the water body, their proportion in the assemblage composition changes with the width of the stream because of the increase in river order, causing a greater incidence of sunlight (De Marco et al., 2015). This ecophysiological hypothesis is widespread among odonatologists, however, it does not clearly explain the causes of the distribution of species within each of the thermoregulatory groups, especially if one considers the hypothesis that all thermoregulatory groups are found throughout the watercourse, differing only in the proportion of species distribution (Pereira et al., 2019).

The aim of this study was to investigate how the functional characteristics of Odonata respond to impacts caused by human activity in the Eastern Amazon, using bionomic traits as response variables. The hypotheses of the study were: i) species with greater proportions of wing base width, due to reduced maneuverability (Mcauley, 2013), are better related to altered environments, due to the reduction of riparian forest, while for species with more petiolate wings, maneuverability is essential for more preserved riparian forests due to the greater number of obstacles (Hedenström and Møller, 1992). individuals with size and length of the abdomen and larger body size are closely linked to altered environments, dragonflies with such characteristics must perform thermoregulation more efficiently and in places with high solar irradiation, while smaller dragonflies are dependent on the temperature of the environment (De Marco et al., 2005); ii) degraded environments, with low forest cover and altered riparian vegetation favor heliothermic individuals, due to their thermoregulation specificity. While thermal and endothermic conforming individuals will be more frequent in preserved environments (De Marco and Resende, 2002; De Marco et al., 2015). Thus, we expect to have a separation of functional groups according to thermoregulation between the studied areas; iii) oviposition will also be a determining factor in the occurrence of species among the studied areas. Since Odonata species need suitable substrates to lay their eggs (Corbet, 1999; Suhonen et al., 2013), it also acts as a filter that determines the location of occurrence (Pereira et al., 2019). For this reason, we expect a high occurrence of species with endophytic and epiphytic oviposition in preserved environments, once this trait demands the presence of wood and riparian vegetation. In contrast, we expect the occurrence of exophytic species to be higher in altered environments because oviposition of these species is performed directly in the water column.

2 Materials and methods

2.1 Study area

Two drainage basins of Eastern Amazonia were analyzed, one in the municipalities of Santarém, Belterra, and Mojuí dos Campos, and the other in the municipality of Paragominas, located in the State of Pará, Brazil (Figure 1). The municipalities of Santarém (02°26’22”S, 54°41’55”W), Belterra (02°4’54”S, 54°53’18”W), and Mojuí dos Campos (02°40’53”S, 54°38’33”W), are located west of the state. In other words, it is characterized as a rainy tropical climate with a well-defined short dry season, with rainfall of less than 60 mm. The region’s rainfall is, on average 2,000 to 2,300 mm/

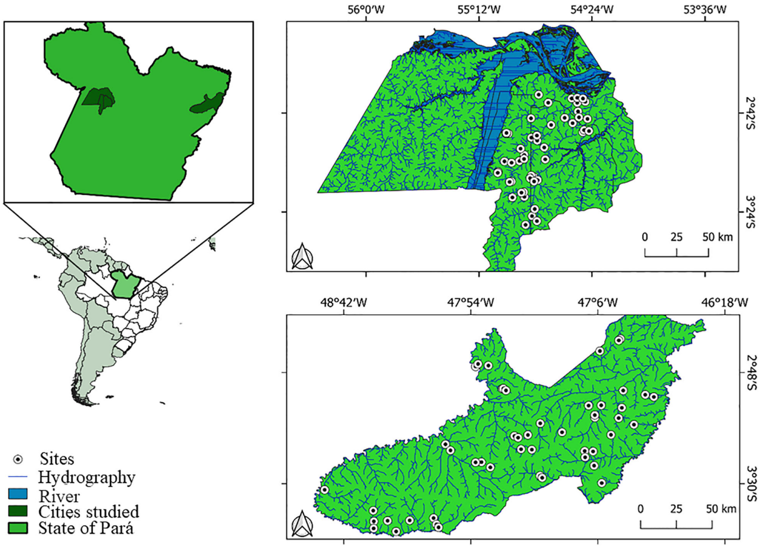


FIGURE 1
Drainage basins and streams sampled in the regions of Paragominas and Santarém, Belterra and Mojuí dos Campos, in Eastern Amazon, Pará, Brazil.

year, the maximum temperature varies from 25°C and the relative humidity is 86%.

The rainiest period occurs between December and May, with about 80% of rainfall, and between June and November, the rest of the rainfall is recorded in the region (Nepstad et al., 2002). Paragominas, in turn, is in northeastern Pará (02°26’22”S, 54°41’55”W) with average annual precipitation of 1,766 mm, an average annual temperature of 27°C, and relative humidity of 81% (Watrin and Rocha, 1992). This type of climate corresponds to tropical rainy climates with a short and well-defined dry season.

Santarém, Belterra and Mojuí dos Campos in the Amazon biome present dense rainforest and relief diversification, with regions varying from plateau to floodplains. The predominant vegetation in the region is tropical forest, except Amazon savannahs found in northwestern Santarém (Berenguer et al., 2014). Small reforestation areas are found in the central portion of the municipality, and reference sites can be found in the Tapajós National Forest (Belterra), adjacent to the region (Feitosa et al., 2012). The vegetation of the Paragominas region is classified as a dense tropical forest (Veloso et al., 1991).

Both sampled regions have a land use gradient (Moura et al., 2013), which comprises altered areas that go from secondary forests formed after the complete destruction of the native forest (Putz and Redford, 2010), and areas of cattle grazing and mechanized agriculture and soybean plantation (Gardner et al., 2013; Moura et al., 2013; Oliveira-Junior et al., 2015). However, largely preserved remnants can still be observed consisting of primary forest, with original climax physiognomy that has clearly never been logged (Gardner et al., 2013; Moura et al., 2013).

2.2 Data sampling

2.2.1 Biological sampling

A total of 98 first- to third-order streams were sampled (an average of 2 to 5 m wide, according to Strahler (1957) classification), distributed over a gradient of environmental conditions ranging from fully preserved areas to those heavily modified by cattle ranching and agriculture. The collections were performed in both areas during the dry season, with 48 sampling sites in Santarém, Belterra, and Mojuí dos Campos (July to August 2010) and 50 in Paragominas (July to August 2011). The dry season was selected due to: (i) the ecophysiological requirements of Odonata (May, 1976; May, 1991; Corbet, 1999); (ii) sampling in a single seasonal period can reduce “noise” in statistical analyses (see Heino and Peckarsky, 2014); iii) several studies show that the highest diversity of Odonata can be found during the dry season (Fulan and Henry, 2007); and iv) the low depth of the water column during this season concentrates these insects in smaller areas, which allows us to find and capture them more easily (Oliveira-Junior and Juen, 2019).

At each stream, a 150 m stretch was delimited and subdivided into ten longitudinal sections of 15 m each, separated by bank-to-bank transects (see Oliveira-Junior and Juen, 2019). The 15 m longitudinal sections were subdivided into three segments of five meters each, and only the first two segments of each section were sampled, totaling 20 segments of 5 m in each stream marked with biodegradable strips from A (downstream) to K (upstream) (Figure 2). The fixed area scanning methodology was used successfully by other researchers (Juen and De Marco, 2011; Silva-Pinto et al., 2012; Oliveira-Junior et al., 2013; Calvão et al., 2016; Batista et al., 2021; Cezário et al., 2021). Using an entomological net (40 cm diameter, 65 cm depth, and 90 cm long aluminum handle), the spotted Odonata adults were collected, following the collection protocol by Oliveira-Junior et al. (2015) and conditioned according to Lencioni (2006).

Collections were performed between 10 AM and 2 PM, when sunlight reaches the main stream channel, following De Marco and Resende (2002) and Batista et al. (2021), in order to ensure favorable conditions for all Odonata groups, both thermal, heliothermic, and endothermic conformers to be active (May, 1976; May, 1991; Corbet, 1999; De Marco and Resende, 2002; Batista et al., 2021).

To identify the collected specimens, we used specialized taxonomic keys (Garrison, 1990; Lencioni, 2005; Garrison et al., 2006; Lencioni, 2006; Garrison et al., 2010), and compared with the

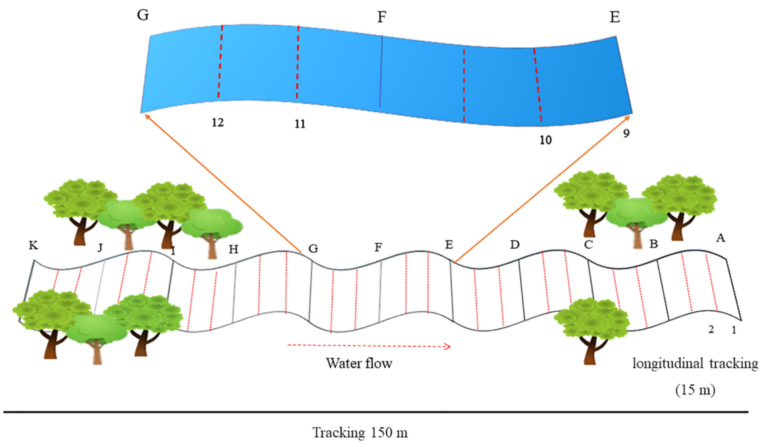


FIGURE 2
Schematic drawing of the transect established in each stream for Odonata sampling. Each transect was named (with letters from A to K) from downstream to upstream.

testimony material of the collection from the Zoology Museum of the Universidade Federal do Pará. After identification, the specimens were deposited as testimonial material in the Collection of the Zoology Museum of the Universidade Federal do Pará, Pará state, Brazil.

2.2.2 Morphological traits

With the aid of a digital caliper (precision of 0.01 mm), we measured four morphological characteristics: a) width of the posterior wing at base height (WPWBH); b) abdomen length (AL); c) thorax length (TL); and d) thorax width (TW). Measurements were taken in at least 10 male individuals of each species, taking care to take measurements only in the left side of the specimen, measuring every trait three times, to standardize and minimize errors. The average values of the specimens measured were used as the final measurement for the species in each morphological character.

Body size (BS) was measured as the total length of adults (from the top of the head at the end of the appendices). We measured an average of 10 individuals of each species, using these measurements to obtain the average length of each species. Posteriorly, the species were divided into three size classes: (1) small, individuals measuring 18 to 29 mm; (2) mid-sized, individuals ranging from 30 to 43 mm; and (3) large, individuals over 44 mm (Table 1). To determine the size classes, we followed Dalzochio et al. (2018).

2.2.3 Type of oviposition

For Odonata, there are three basic types of oviposition: (i) endophytic – where the eggs are laid inside a plant tissue (living or dead), however, these individuals prefer living tissue; (ii) epiphytic – the eggs are laid at the surface of rocks, trunks, leaves and other substrates, both over and under the water column; (iii) exophytic – eggs are released directly into the water (Fincke, 1986; Corbet, 1999) (Table 1). To determine the type of oviposition of each species, we used the literature as a basis, by means of a direct search of the description of the type of oviposition of each species made in articles or books. In case information about the species was unavailable, we used a category described for the genus level, and in case this was also unavailable, the most frequent oviposition category in the family was used.

TABLE 1 Functional traits of Odonata (Insecta) adults, sampled in streams of Eastern Amazon, Pará, Brazil.

Traits	Category	Estate	Code	Reference
Body size	Adult	18–29,99 mm	1	Personal observation; Dalzochio et al. (2018)
		30–43,99 mm	2	
		Above 44 mm	3	
Oviposition	Endophytic	Present	1	Resende and De Marco (2010); Hamada et al. (2014); Vilela et al. (2016); Rodrigues et al. (2019); Pereira et al. (2019)
		Absent	0	
	Epiphytic	Present	1	
		Absent	0	
	Exophytic	Present	1	
		Absent	0	
Thermoregulation	Conformers	Present Absent	1 0	May (1976); May (1991); De Marco and Resende (2002); Paulson (2004); De Marco et al. (2005); De Marco et al. (2015)
	Heliotherms	Present Absent	1 0	
	Endotherms	Present Absent	1 0	

2.2.4 Types of thermoregulation

Due to the ecophysiological requirements of adult individuals regarding their ability to thermoregulate, Odonata species can be divided into (i) thermal conformers, of small body size, show increased conductance and the body temperature varies with the environment, mainly due to heat exchange by convection (Corbet, 1999); (ii) heliotherms, have a larger body and, consequently, lower conductance, and their activities are determined mainly by solar irradiation; (iii) endotherms, they produce heat by controlling the circulation of the hemolymph (May, 1976) (Table 1). The strategies and ecophysiological patterns of Odonata also associate thermoregulatory capacity with body size (Corbet, 1999).

The type of thermoregulation for each species was determined based on the literature, through a direct search of the description of the type of oviposition of each species in articles or books. In cases where information about the species was not found, a category already described for the genus level was used; if this was not available, the most frequent category of oviposition in the family was used.

2.2.5 Evaluation of the physical condition of the studied streams

Six variables were used to measure the environmental quality in the 98 studied streams: average canopy cover over the channel, percentage of forest in the surrounding landscape, and four physical and chemical descriptors of the water: water temperature (°C), electrical conductivity (mg/L), hydrogen potential (pH) and dissolved oxygen (mg/L), obtained from a Horiba probe, Model U51. Several previous studies have demonstrated the importance of these physicochemical variables for the structure of aquatic insect communities, including water temperature and electric conductivity (Oliveira-Junior et al., 2013; Oliveira-Junior et al., 2017), dissolved oxygen (Jacob et al., 1984), and pH (Courtney and Clements, 1998), therefore these variables were selected for our study.

To estimate the integrity of the environment in each stream, we used the habitat integrity index (HII) of Nessimian et al. (2008). This index consists of 12 items that describe the environmental conditions of the streams, assessing characteristics such as the pattern of land use adjacent to the riparian vegetation; width of the riparian forest and its state of preservation; state of the riparian forest within a 10 m band; description of the channel condition regarding the type of sediment and presence of retention devices; structure and wear of the marginal ravines of the stream; characterization of the stream bed regarding substrate, aquatic vegetation, detritus, and arrangement of the areas of rapids, pools, and meanders. Each item comprises four to six alternatives that are ordered to represent increasingly more pristine systems, with the index value varying between 0 (least pristine) and 1 (most pristine). This index is directly related to the level of environmental conservation and has been successfully used in many studies assessing the integrity of aquatic systems (Carvalho et al., 2013; Giehl et al., 2014; Juen et al., 2014; Monteiro-Júnior et al., 2015; Bastos et al., 2021), and in a recent meta-analysis, it was shown to be one of the most important metrics for explaining community variation (Brasil et al., 2021).

To estimate mean canopy cover, we used a convex densiometer positioned at the central point of the channel, where we made four measurements: upstream, downstream, and at the left and right margins. Canopy cover is frequently considered one of the main characteristics of the physical habitat that influences Odonata distribution patterns in tropical streams (Oliveira-Junior et al., 2017). For the forest percent cover, we defined forest cover within 100 m buffers, which we used to delineate the landscape within which the percent cover was estimated. The proportion of habitats in preserved environments or natural cover is among the key variables that explain species distribution and community structure in natural environments (Fahrig, 2003).

2.3 Data analyses

2.3.1 Physical and chemical condition of streams

To assess the variation in the studied environments, we summarized the values of six environmental variables (canopy cover, percentage of forest, water temperature, electric conductivity, pH, and dissolved oxygen) through a Principal Component Analysis (PCA), with a correlation matrix to determine the pattern of variation in environmental and structural parameters among samples. To reduce multicollinearity, we assessed the degree of correlation between variables. Since the environmental variables were measured in units of varying orders of magnitude, the values (except pH) were standardized using a Euclidean distance matrix. A random Broken-stick model was used to determine which principal components would be retained for analysis, retaining only those components that describe the main gradients of habitat variation (Jackson, 1993). The HII score (in the PCA ordination) was used to identify the categories of stream integrity (altered and preserved). All analyses were performed in the vegan package (Oksanen et al., 2022), in R software, version 4.0.4 (R Core Team, 2021).

2.3.2 Determination of functional groups

To determine the functional groups (FG), a trait matrix produced from the coding of all species with respect to these features in their respective states was used. After this, we created a dendrogram of Odonata species grouped by functional characteristics, using Ward's linkage method based on Gower's distance (Casanoves et al., 2011), using the FD package (Laliberté et al., 2014) in R, version 4.0.4 (R Core Team, 2021). The dendrogram was cut at point "5", to determine the functional groups (Casanoves et al., 2011; Dalzochio et al., 2018).

2.3.3 Weighted average values of traits at the community level

To assess the variation in the composition of bionomic traits between altered and preserved streams, we first calculated a community-weighted mean (CWM) index, using the FD package (Laliberté et al., 2014), in R (R Core Team, 2021) version 4.0.4. This index represents the relative abundance of each trait inside each studied community, correlating the species-by-trait matrix with the species relative abundance matrix, according to Violle et al. (2007). Thus, after calculating the CWM, a matrix of the studied communities by functional traits was generated. Relative abundance (~ n) was obtained by dividing the number of individuals collected from each species by the total number of individuals in each sample.

2.3.4 Comparison of Odonata communities among the studied locations

With the Functional Groups (FG) and CWM matrices, a Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to test for variation in trait composition among the sites studied, with Gower dissimilarity. To visualize these differences, we proceeded with a Principal Coordinate Analysis (PCoA), also with Gower dissimilarity. The PCoA function calculates the principal coordinate decomposition (also called classical scale) of a distance matrix D (Gower, 1966).

3 Results

3.1 Physical condition of streams and water quality

The studied streams presented considerable variation in physical conditions and physical-chemical properties of water, reflecting environmental change due to anthropogenic activities. The values of the variables were: habitat integrity index – HII (0.83–0.50), average canopy cover on the channel (71.14–84.53%), percentage of primary forest (0.00–100.00%), water temperature (22.50–29.90°C), electrical conductivity (44.64–74.70 µS/cm), dissolved oxygen (5.20–5.33 mg/L), and pH (4.88–4.77).

The HII shows a variation in stream integrity, classifying them into two distinct categories of integrity: 56 altered streams (HII = 0.15 to 0.69) and 42 preserved streams (HII = 0.70 to 0.99). The means and variances of the values of the environmental variables are presented by category of stream integrity in Table 2.

The association between the two PCA axes accounted for 55.92% of the environmental variation (axis 1 = 30.27%; axis 2 = 25.65%). Only the first axis component was analyzed, since the value of the second component was lower than that estimated from the Broken-stick model. The first component alone accounted for 30.27% of the variance in the results (eigenvalue = 1.82). This analysis showed that there was a clear separation of streams into two distinct integrity categories. The streams with the highest HII values (HII ≥ 0.70; preserved) were grouped with the highest canopy cover, percentage of primary forest, and dissolved oxygen.

TABLE 2 Mean and variance (in parentheses) of environmental variables recorded in each type of stream integrity in two regions of the Eastern Amazon, Pará, Brazil.

Type of integrity	Environmental variables						
	HII	WT	EC	DO	pH	CC	PF
Altered	0.50 (0.02)	25.66 (2.02)	24.32 (118.63)	5.20 (2.18)	4.88 (1.71)	71.14 (840.30)	44.64 (734)
Preserved	0.83 (0.01)	25.18 (1.78)	25.08 (195.73)	5.33 (1.71)	4.77 (1.50)	84.53 (560.92)	74.70 (979.37)

HII, Habitat Integrity Index; WT, Water temperature; EC, electrical conductivity; DO, dissolved oxygen; pH, hydrogen potential; CC, average canopy cover; PF, % primary forest in the riparian forest zone of a 100m buffer.

Streams with lower HII values (HII ≤ 0.69; altered) had a loss or significant change in these same variables (Table 3; Figure 3).

The variables that contributed most to the formation of the first component were related to the physical structure of the riparian forest and water quality, explaining the observed clustering patterns. Canopy cover (CC) and dissolved oxygen (DO) were positively related to the level of stream integrity, while pH was negatively related to integrity levels (Table 3).

3.2 Functional groups

We evaluated 80 species, 48 collected in preserved environments and 70 in altered environments, of these, 44 species belonged to the suborder Zygoptera and 36 belonged to the suborder Anisoptera. The dendrogram based on the designated characteristics resulted in 16 functional groups (Figure 4; Table 4).

Of the total functional groups 12 (FG 1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 14, and 16) were present in both altered and preserved streams (Figure 4; Table 4). Most of these groups include species with diverse functional characteristics. Individuals belonging to these groups have the following average morphological traits: width of the posterior wing at base height – WPWBH (4.49 mm), abdomen length – AL (22.66 mm), thorax length – TL (5.72), and thorax width – TW (2.16). For the behavioral traits, the vast majority of the species are small (1) and medium-sized (2), with exophytic oviposition and heliothermic thermoregulation (Appendix 1). The most abundant functional group was GF 9 (n = 20.3), followed by GF 6 (n = 12.3), and the other groups showed low abundance. compared to the presented groups.

TABLE 3 The six environmental variables used to describe the environmental conditions of sampled streams in two regions of the Eastern Amazon, Pará, Brazil, and their correlation with the principal components (axes 1 and 2) of the Principal Component Analysis (PCA).

Environmental variable	*Loading*	
	Axis 1	Axis 2
% primary forest in the riparian forest zone of a 100m buffer (PF)	0,282	0,527
Canopy cover (CC)	**0,645**	0,445
Electrical conductivity (EC)	−0,588	0,359
Hydrogen potential (pH)	**−0,629**	0,374
Water temperature (WT)	−0,462	−0,717
Dissolved oxygen (DO)	**0,605**	−0,530
Eigenvalues	1,816	1,539
Broken-stick	2,450	1,450

Values in bold represent the variables that contributed the most (≥ 0.60) to the formation of the first component (axis 1) of the PCA.

Only two groups were present in altered streams (FG 11 and 13). The species in these groups have the following average morphological traits: width of the posterior wing at base height – WPWBH (10.89 mm), abdomen length – AL (16.44 mm), thorax length – TL (5.79 mm), thorax width – TW (2.32 mm). As for behavioral attributes, they have size 2 (medium) species, exophytic oviposition, and heliotropic thermoregulation. Groups GF 8 and 15 were the only ones exclusively present in preserved areas, with species showing the following average morphological traits: width of the posterior wing at base height – WPWBH (8.14 mm), abdomen length – AL (42.43 mm), thorax width – TW (9.09 mm), thorax length TL (5.81) and behavioral traits, epiphytic oviposition, and endothermic thermoregulation, regarding size, we had small (1) and large (3) species.

The composition of biological characteristics was represented by two axes of principal coordinate analysis (PCoA), the first axis explained 73.94% and the second, 24.39% of the functional variation in the community (Figure 5). It can be seen that individuals found in altered areas are heliothermic, with exophytic oviposition and wider width of the posterior wing at base height WPWBH. While individuals with smaller body sizes and endophytic oviposition, and thermal conformers are commonly found in preserved areas. Although some sampling sites were overlapped, PERMANOVA showed that there is a pattern of separation between the studied environments (Pseudo-F = 15.655; $P < 0.01$) (Figure 5).

4 Discussion

The functional characteristics of the Odonata community respond to the impacts caused by human activities in Amazonian streams, which corroborates the hypothesis proposed by this study. Environmental integrity has a negative relationship with some morphological traits such as larger body size and larger thorax width and length, and favor species with exophytic oviposition (Remsburg and Turner, 2009), therefore, there is an increase in the abundance of species that possess these characteristics in altered environments. It is possible to observe that there was a separation

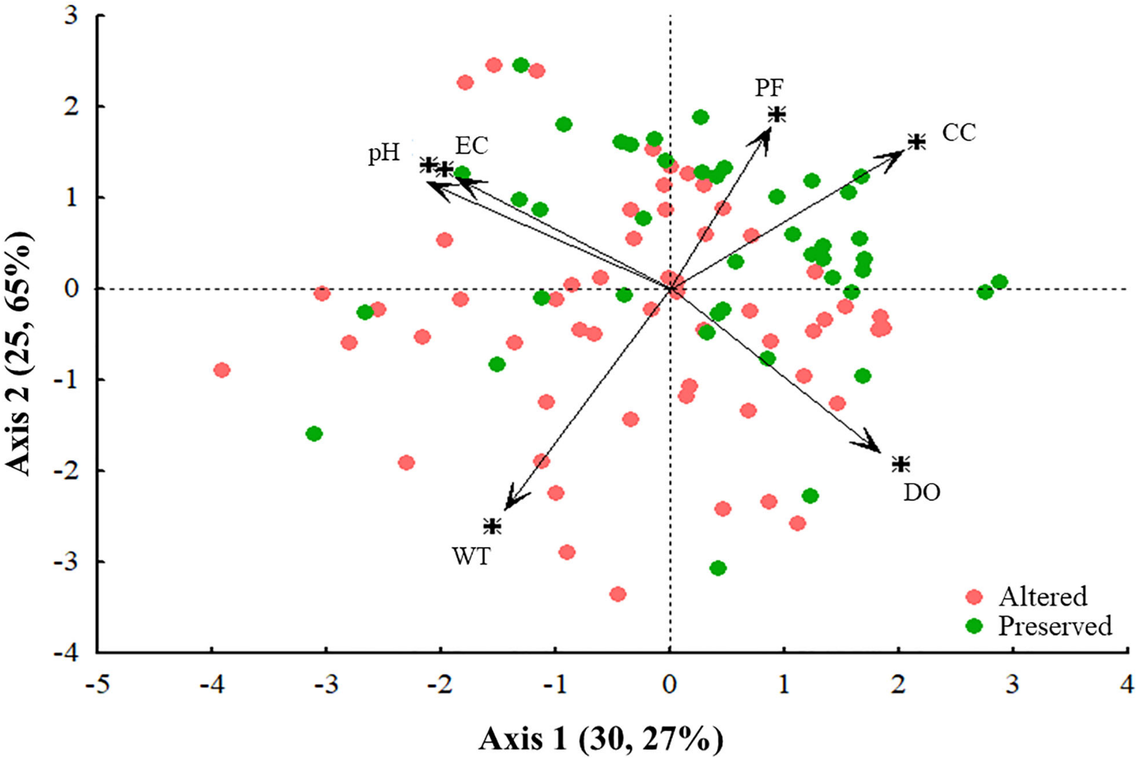


FIGURE 3
Ordination of the six environmental variables of the sampled streams in two regions of the Eastern Amazon, Pará, Brazil, based on principal component analysis (PCA) (PF, % of primary forest in the riparian network within a 100 m buffer; CC, average canopy cover; EC, electrical conductivity; pH, hydrogen potential; WT, water temperature; DO, dissolved oxygen). The "+" symbol is just a marker for each variable in the figure.

of groups between the environments (altered and preserved). Factors such as environmental integrity, canopy cover, as well as the presence or absence of vegetation in the streambeds, affect the distribution of Odonata species (Monteiro-Júnior et al., 2013). The transformation of the terrestrial landscape significantly affects the diversity of dragonflies in terms of taxonomic composition and richness, especially in relation to the loss of vegetation and physical and chemical changes in aquatic environments (Stoks and Córdoba-Aguilar, 2012; Bried and Samways,

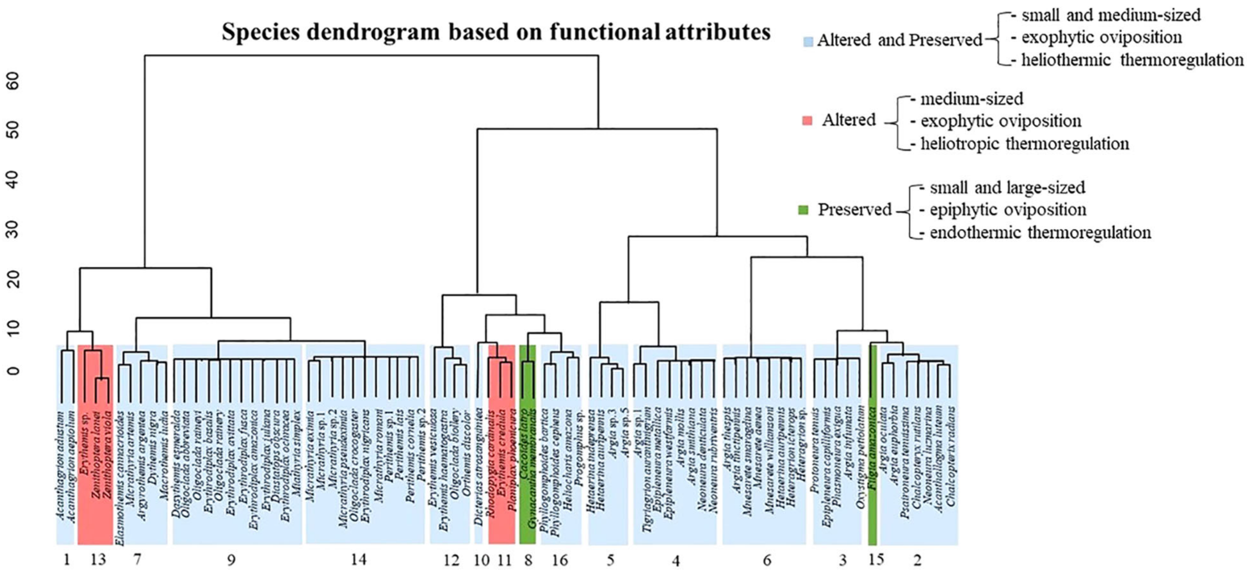


FIGURE 4
Dendrogram based on functional traits of Odonata species, sampled in streams with different conservation categories (altered and preserved), in the Eastern Amazon, Pará, Brazil.

TABLE 4 Abundance of functional groups of Odonata (Insecta) adults sampled in altered and preserved streams in the Eastern Amazon, Pará, Brazil.

Integrity type	Functional groups															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Altered	1.0	4.8	3.5	4.2	2.9	12.3	1.4	**0.0**	20.3	0.2	0,2	2.9	1.0	2.8	**0.0**	0.4
Preserved	0.1	6.2	7.5	2.7	1.6	15.4	0.3	0.2	3.9	0.8	**0.0**	0.1	**0.0**	0.5	0.1	0.5

Values in bold represent functional groups with abundance < 0.1.

2015; De Marco et al., 2015; Monteiro-Júnior et al., 2015; Rodrigues et al., 2016; Rodrigues et al., 2019).

Oviposition is a trait that can influence the distribution of Odonata species and is important in determining the separation of functional groups between environments (Calvão et al., 2022). Although a habitat may appear suitable for many aspects of dragonfly life history, crucial oviposition sites are often of poor quality or absent (Grönroos et al., 2013; Heino and Peckarsky, 2014). Oviposition behavior is an important tool to assess the behavior of Odonata adults in the face of environmental changes caused by different land uses (Rodrigues et al., 2019). Suitable egg-laying characteristics are necessary for successful oviposition and consequently for the population establishment and dynamics of dragonfly larvae (MacCreadie and Adler, 2012; Heino et al., 2014; Tavares et al., 2017).

Studies have shown that characteristics such as size and thermoregulation are key to understanding how anthropogenic environmental changes may affect dragonfly diversity (Juen and De Marco, 2011; Nóbrega and De Marco, 2011; De Marco et al., 2015). Species size is important for thermoregulation (De Marco et al., 2015), while wing base width is related to gliding ability, this same characteristic is inversely related to flight maneuverability (Corbet, 1962; Johansson et al., 2009). Sites with a higher integrity index present greater obstacles, due to the presence of vegetation, so they generate a selectivity in favor of more petiolate wings (Hedenström and Møller, 1992). However, wider wings benefit long flights and are related to open sites with few obstacles (Pereira et al., 2019). Odonata body size influences group competition because larger animals (most Anisoptera), are more attached to open areas (Bastos et al., 2021; Oliveira-Junior et al., 2021). In our study, species that have larger size and heliothermal thermoregulation are better associated with altered environments, as is the case of individuals of *Rhodopygia cardinalis*, which have exophytic oviposition. On the other hand, *Fylgia amazonica* had a greater relationship with preserved environments, and these species have a small body size, possibly as an adaptation to shady environments of primary forests (Samways and Taylor, 2004).

Heliotherms were more closely related to altered environments. They are mostly larger in size, and may include some Zygoptera and

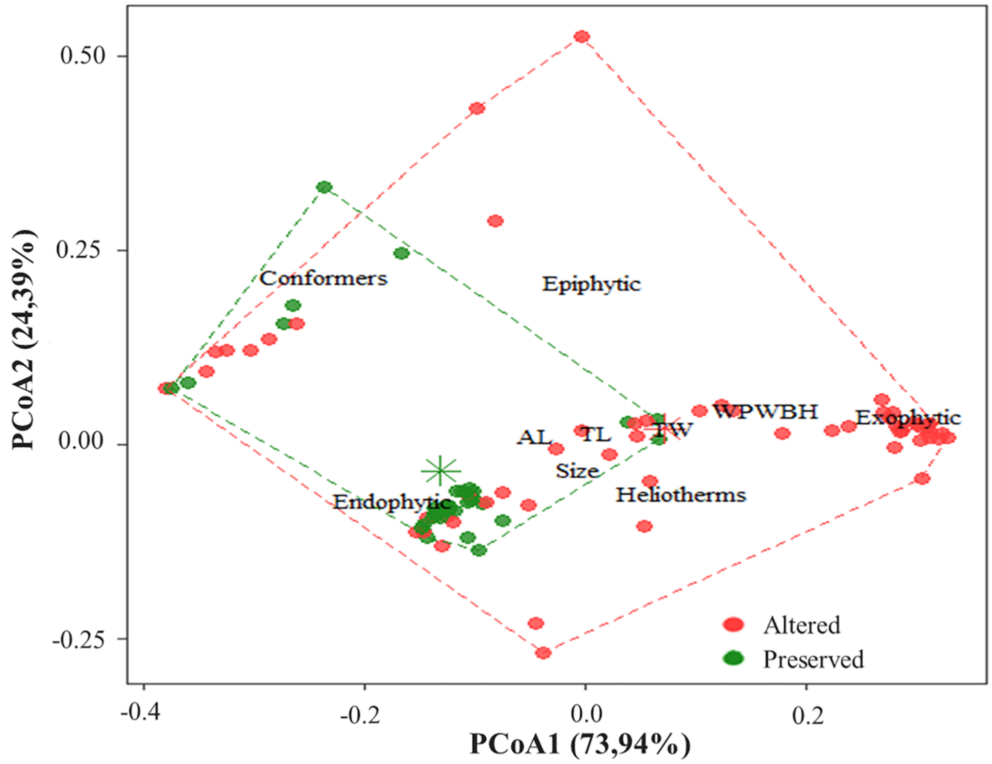


FIGURE 5
Ordination of the functional composition of Odonata species, sampled in altered and preserved streams, Eastern Amazon, Pará, Brazil.

most Anisoptera, have lower conductance, and their activity is determined mainly by solar irradiance (Corbet and May, 2008). Thermal conformers were better related to preserved environments because this group needs shaded areas (De Marco et al., 2015) and the alteration of habitats ends up excluding species with specific characteristics. Species with large body sizes such as Aeshnidae are considered endothermic, as they regulate their temperature through flight, determining heat production (May, 1991).

The functional groups found in both areas were composed of both Anisoptera and Zygoptera. The most frequent Zygoptera families were Coenagrionidae and Calopterygidae. Dalzochio et al. (2018) obtained similar results, where they state that species from the Calopterygidae family are more generalist and are found in many habitats. The high abundance of species of the genus *Argia* Rambur may suggest the presence of many individuals of Zygoptera with sizes ranging from small to medium. The Anisopterans found in the groups present in the two categories of integrity were composed almost exclusively of the family Libellulidae, mostly by species of the genera *Erythrodiplax* Brauer and *Micrathyria* Kirby. The genus *Erythrodiplax* Brauer is one of the most diverse genera of Libelullidae (Garrison et al., 2006; Del Palacio and Muzón, 2019). Its species inhabit different types of wetlands, and many of them can be abundant in temporary ponds, swamps, and stream pools (Del Palacio et al., 2020). These characteristics make the species less affected by disturbance events as they support more solar incidence along the stream channel (Ball-Damerow et al., 2014; De Marco et al., 2015; Powney et al., 2015) and consequently allow them to explore more broadly a variety of environments. This explains the occurrence of these groups in both environments.

The groups that were exclusively present in altered streams showed exophytic oviposition and heliothermic thermoregulation, similar to the species of the genus *Erythemis* that in this study present heliothermic thermoregulation. The relationship of these species with altered areas may be due to the fact that modifications in the stream channels increase the number of backwaters along the water body and the incidence of sunlight due to clearings from human activities (Oliveira-Junior et al., 2015; Rodrigues et al., 2019). In streams with compromised integrity, the removal of riparian vegetation can raise solar incidence and consequently reduce the availability of resources for a wide variety of species. This process may generate homogenization of Odonata species, with the replacement of specialized species by more generalist species (Remsburg and Turner, 2009; Couceiro et al., 2012; Oliveira-Junior et al., 2013).

Altered environments are used by many exophytic species as oviposition sites. This factor contributes to the increase in species richness and abundance in sites with little or no forest cover surrounding streams (Corbet, 1999; Dutra and De Marco, 2015). The exophytic oviposition behavior of Odonata may be related to the alteration of the riparian vegetation, which opens space for greater insolation; these environments also favor species with heliothermic thermoregulation, since heliothermic individuals need sun rays falling directly on their bodies for their metabolic activities (De Marco et al., 2005).

The most abundant family in groups exclusive to altered areas was Libellulidae, the size ranged from medium to large, and morphological characteristics were similar among the species of this family, especially in the abdomen length (AL), which is associated with several ecological behaviors of these individuals such as thermoregulation, oviposition/reproduction, and territorialism (May, 1976). Another factor is the width of the posterior wing at base height (WPWBH), which in the species of this group is wider. The interaction between wing width and integrity index also demonstrates its influence on maneuverability factors (Pereira et al., 2019).

The absence of riparian forest in altered streams, can increase the entry of sediments in the channel, change lotic channels, form puddles, and/or reduce the streamflow, favoring species that lay eggs directly on the water surface (exophytic oviposition) (Rodrigues et al., 2016). The most representative genera in the groups were *Erythemis* and *Erythrodiplax*, these genera are often found in studies of Odonata fauna (Souza and Costa, 2006; Dalzochio et al., 2011). The thermoregulatory behaviors (endothermic and hypothermic) and exophytic oviposition in species of *Erythemis* and *Erythrodiplax* may influence their species diversity in environments considered lentic (De Marco et al., 2015; Rodrigues et al., 2018).

The most abundant groups in preserved environments were mostly composed of individuals from the Zygoptera suborder, with endophytic oviposition and largely thermal conformers, all medium-sized. Thermal conformer species have bodies with high conductance, varying according to air temperature (Heinrich, 1974). However, only two groups were unique to pristine areas, which in turn comprised Anisoptera species with epiphytic oviposition, endothermic, and large size. The morphological characteristics were quite similar among the species. One of the groups (GF 8) had only two species*: Cacoides latro* and *Gynacantha membranalis*, both from the Gomphidae family. Adults from the Gomphidae family are usually found in areas of denser vegetation, and the nymphs are usually associated with streams or rivers with clear water and currents with sandy sediments (Garrison, 2009), are described as territorialists, being very common in lake environments (Moore and Machado, 1992).

Species from the *Gynacantha* Rambur genus inhabit mud-bottomed pools and/or temporary ponds within tropical forests (Garrison et al., 2006). They also have a phytotelmata habit, meaning they rely primarily on tree trunks or plants (such as bromeliads) capable of accumulating water for egg laying, this represents a distinctive aquatic habitat and supports a fauna that can be highly specific (Frank, 1983). These habits may explain the association of *Gynacantha* individuals with preserved environments. Larvae of the genus *Cacoides* Cowley inhabit vegetated ponds, sandy bank ditches, and lakes in the forest (Garrison et al., 2006), characteristics that are most associated with preserved environments. GF 15 was composed of a single species: *Fylgia amazonica*, this species is associated with pristine environments (Monteiro-Júnior et al., 2015), although it is considered a common species, individuals of the genus *Fylgia* Kirby, inhabit lotic and forest environments (Machado, 1954; Garrison et al., 2006).

5 Conclusion

This study provides evidence that functional traits are determining factors for the occurrence of species in the environment. A complex picture was observed where the species evaluated have a set of characteristics that limit them to specific habitats. Environmental quality, measured by the habitat integrity index, has a significant effect on the composition of functional groups. Altered environments favor species with exophytic oviposition and heliothermic thermoregulation, while preserved environments favor individuals with endophytic and epiphytic oviposition and ectothermic thermoregulation (thermal conformers). Species with these characteristics are mainly represented by Zygoptera, although some individuals of this suborder are resistant to changes, the lack of habitat for the oviposition of these individuals ends up causing the exclusion of many species from the environment. As for morphology, altered environments favor medium to large individuals, with greater posterior wing width at base height, thorax length, and abdomen size, while preserved environments may favor smaller and/or specialized species. The effects caused by altered aquatic environments such as landscape changes (e.g., loss of native vegetation) can affect the level of reproductive success of aquatic insects, altering local species composition and richness. The analyses performed show that functional diversity measures can provide additional information on the effects of habitat integrity on biodiversity. From a conservation perspective, the results of this study can be used to incorporate species preservation into the sustainable development of the Eastern Amazon.

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 authors.

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local legislation and institutional requirements. Ethical approval for research on invertebrates is under permanent license to collect zoological material (Sisbio number 11841-6).

Author contributions

All authors contributed to the writing of the text: TR, LC, LJ, and JO-J. The collected species data used in the study were made available by the authors: JO-J and LJ. LC provided data and Oviposition. TR, LC, and JO-J performed the statistical analyses. All authors contributed to the article and approved the submitted version.

Funding

This project was financed by the Coodenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting a master's scholarship for two years. This work also received financial support from PROPESP/UFPA through Edital 01/2023 (Programa de Apoio à Publicação Qualificada – PAPQ). JO-J is grateful to CNPq for his productivity grant to (process 307808/2022-0).

Acknowledgments

Laboratory of Ecology and Conservation (LABECO) of the Federal University of Pará (UFPA), for the availability of the database used in this study. The Environmental Impact Studies Laboratory (LEIA) of the Federal University of Western Pará (UFOPA), for academic and logistical support.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1166057/full#supplementary-material

References

Arnold, S. J. (1983). Morphology, performance and fitness. *Am. Zool.* 2, 347–361. doi: 10.1093/icb/23.2.347

Ball-Damerow, J. E., M'Gonigle, L. K., and Resh, V. H. (2014). Changes in occurrence, richness, and biological traits of dragonflies and damselflies (Odonata) in California and Nevada over the past century. *Biodivers. Conserv* 23, 2107–2126. doi: 10.1007/s10531-014-0707-5

Banks-Leite, C., Ewers, R. M., and Metzger, J. P. (2012). Unraveling the drivers of community dissimilarity and species extinction in fragmented landscapes. *Ecology* 93, 2560–2569. doi: 10.1890/11-2054.1

Bastos, R. C., Brasil, L. S., Oliveira-Junior, J. M. B., Carvalho, F. G., Lennox, G. D., Barlow, J., et al. (2021). Morphological and phylogenetic factors structure the distribution of damselfly and dragonfly species (Odonata) along an environmental gradient in Amazonian streams. *Ecol. Indic.* 122, 107257. doi: 10.1016/j.ecolind.2020.107257

Batista, J. D., Ferreira, V. R. S., Cabette, H. S. R., De Castro, L. A., De Marco, P., and Juen, L. (2021). Sampling efficiency of a protocol to measure Odonata diversity in tropical streams. *PloS One* 16 (3), e0248216. doi: 10.1371/journal.pone.0248216

Berenguer, E., Ferreira, J., Gardner, T. A., Aragão, L. E. O. C., Camargo, P. B., Cerri, C. E., et al. (2014). A large-scale field assessment of carbon stocks in human-modified tropical forests. *Global Change Biol.* 20, 3713–3726. doi: 10.1111/gcb.12627

Brasil, L. S., Lima, E. L., Spigoloni, Z. A., Ribeiro-Brasil, D. R. G., and Juen, L. (2021). The habitat integrity index and aquatic insect communities in tropical streams: A meta-analysis. *Ecol. Indic.* 116, 1470–160X. doi: 10.1016/j.ecolind.2020.106495

Bried, J. T., and Samways, M. J. (2015). A review of odonatology in freshwater applied ecology and conservation science. *Freshw. Sci.* 34 (3), 1023–1031. doi: 10.1086/682174

Calvão, L. B., Nogueira, D. S., Montag, L. F. A., Lopes, M. A., and Juen, L. (2016). Are Odonata communities impacted by conventional or reduced impact logging? *For. Ecol. Manage.* 382, 143–150. doi: 10.1016/j.foreco.2016.10.013

Calvão, L. B., Siqueira, T., Faria, A. P. J., Paiva, C. K. S., and Juen, L. (2022). Correlates of Odonata species composition in Amazonian streams depend on dissimilarity coefficient and oviposition strategy. *Ecol. Entomol.* 47 (6), 998–1010. doi: 10.1111/een.13188

Carvalho, F. G., Pinto, N. S., Oliveira-Junior, J. M. B., and Juen, L. (2013). Effects of marginal vegetation removal on Odonata communities. *Acta Limnol. Bras* 25 (1), 10–18. doi: 10.1590/S2179-975X2013005000013

Casanoves, F., Pla, L., Di Rienzo, J. A., and Díaz, S. (2011). Diversity: a software package for the integrated analysis of functional diversity. *Methods Ecol. Evol.* 2, 233–237. doi: 10.1111/j.2041-210X.2010.00082.x. F.

Ceballos, G., Ehrlich, P. R., and Dirzo, R. (2017). Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proc. Natl. Acad. Sci.* 114 (30), 6089–6096. doi: 10.1073/pnas.1704949114

Cezário, R. R., Pena-Firme, P., Pestana, G. C., Vilela, D. S., Juen, L., Cordero-Rivera, A, et al. (2021). In: J. C. Santos and G. W. Fernandes (eds) *Measuring Arthropod Biodiversity* Springer, Cham. doi: 10.1007/978-3-030-53226-0_9

Clark, T. E., and Samways, M. J. (1996). Dragonflies (Odonata) as indicators of biotope quality in the Kruger National Park, South Africa. *J. Appl. Ecol.* 33, 1001–1012. doi: 10.2307/2404681

Colzani, E., Siqueira, T., Suriano, M. T., and Roque, F. O. (2013). Responses of aquatic insect functional diversity to landscape changes in Atlantic Forest. *Biotropica* 45, 343–350. doi: 10.1111/btp.12022

Conrad, K. F., Wilson, K. H., Wilthifield, K., Harvey, I. F., Thomas, C. J., and Sherratt, T. N. (2002). Characteristc of dispersing *Ischnura elegans* and *Coenagrion puella* (Odonata): age, sex, size, morph and ectoparasitism. *Ecography* 25, 339–445. doi: 10.1034/j.1600-0587.2002.250406.x

Corbet, P. S. (1962). *A biology of Dragonfliers, witheby* (Witherby, London), 247.

Corbet, P. S. (1980). Biology of odonata. *Annu. Rev. Entomol.* 25, 189–217. doi: 10.1146/annurev.en.25.010180.001201

Corbet, P. S. (1999). *Dragonflies: behaviour and ecology of odonata* (London: Comstock Pub Assoc), 802.

Corbet, P. S., and May, M. L. (2008). Fliers and perchers among Odonata: dichotomy or multidimensional continuum? A provisional reappraisal. *Int. J. Odona-tology* 11, 155–171. doi: 10.1080/13887890.2008.9748320

Couceiro, S., Hamada, N., Forberg, B. R., and Pimentel, T. P. (2012). A macroinvertebrate multimetric index to evaluate the biological condition of streams in the Central Amazon region of Brazil. *Ecol. Indic.* 18, 118–125. doi: 10.1016/j.ecolind.2011.11.001

Courtney, L. A., and Clements, W. H. (1998). Effects of acidic pH on benthic macroinvertebrate communities in stream microcosms. *Hydrobiologia* 379, 135–145. doi: 10.1023/A:1003442013650

Dalzochio, M. S., Baldin, R., Stenert, C., and Malchik, L. (2015). How does the management of rice in natural ponds alter aquatic insect Community functional structure? *Mar. Freshw. Res.* 67, 1644–1654. doi: 10.1071/MF14246

Dalzochio, M. S., Costa, J. M., and Uchôa, M. A. (2011). Diversity of Odonata (Insecta) in lotic systems from Serra da Bodoquena, Mato Grosso do Sul State, Brazil. *Rev. Bras. Entomologia* 5 (1), 88–94. doi: 10.1590/S0085-56262011000100014

Dalzochio, M. S., Périco, E., Renner, S., and Sahlén, G. (2018). Effect of tree plantations on the functional composition of Odonata species in the highlands of southern Brazil. *Hydrobiologia* 808, 283–300. doi: 10.1007/s10750-017-3431-9

Del Palacio, A., and Muzón, J. (2019). Redescription of Erythrodiplax chromoptera Borror 1942 with comments on the morphology of the vesica sperMalis (Odonata: Libellulidae). *Zool. Anz.* 278, 90–94. doi: 10.1016/j.jcz.2018.12.004

Del Palacio, A. D., Muzón, J., Juen, L., Ferreira, V. R. S., and Batista, J. D. (2020). Erythrodiplax nataliae sp. nov., a new species for the state of Mato Grosso, Brazil. |. *Ann. Braz. Acad. Sci.* 92 (2), 3–7. doi: 10.1590/0001-3765202020181149

De Marco, P., Latini, A. O., and Resende, D. C. (2005). Thermoregulatory constraints on behavior: patterns in a Neotropical dragonfly assemblage. *Neotrop. Entomol.* 34, 155–162. doi: 10.1590/S1519-566X2005000200002

De Marco, P., Nóbrega, C. C., Souza, R. A., and Neiss, U. G. (2015). Modeling the distribution of a rare Amazonian odonate in relation to future deforestation. *Freshw. Sci.* 34 (3), 1123–1132. doi: 10.1086/682707

De Marco, P. Jr., and Resende, D. C. (2002). Activity patterns and thermoregulation in a tropical dragonfly assemblage. *Odonatologica* 31 (2), 129–138.

Ding, N., Yang, W., Zhou, Y., GonzaLez-Bergonzoni, I., Zhang, J., Chen, K., et al. (2017). Different responses of functional traits and diversity of stream macroinvertebrates to environmental and spatial factors in the Xishuangbanna watershed of the upper Mekong River Basin, China. *Sci. Total Environ.* 574, 288–299. doi: 10.1016/j.scitotenv.2016.09.053

Dutra, S., and De Marco, P. Jr. (2015). Bionomic differences in odonates and their influence on the efficiency of indicator species of environmental quality. *Ecol. Indic.* 49, 132–142. doi: 10.1016/j.ecolind.2014.09.016

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 34, 487–515. doi: 10.1146/annurev.ecolsys.34.011802.132419

Feitosa, M. A. C., Julião, G. M., Costa, M. D. P., Belém, B., and Pessoa, F. A. C. (2012). Diversity of sand flies in domiciliary environment of Santarém, state of Pará, Brazil: species composition and abundance patterns in rural and urban areas. *Acta Amazonica* 42 (4), 507–514. doi: 10.1590/S0044-59672012000400008

Ferreira, F. C., and Petrere, J. M. (2007). Anthropic effects on the fish community of Ribeirão Claro, Rio Claro, SP, Brazil. *Braz. J. Biol.* 67, 23–32. doi: 10.1590/S1519-69842007000100004

Fincke, O. (1986). Lifetime reproductive success and the opportunity for selection in a nonterritorial damselfly (Odonata: Coenagrionidae). *Evolution* 40, 791–803. doi: 10.1111/j.1558-5646.1986.tb00539.x

Firmiano, K. R., Castro, D. M. P., Linares, M. S., and Callisto, M. (2021). Functional responses of aquatic invertebrates to anthropogenic stressors in riparian zones of Neotropical savanna streams. *Sci. Total Environ.* 753, 141865. doi: 10.1016/j.scitotenv.2020.141865bb

Frank, J. H. (1983). "Bromeliad phytotelmata and their biota, especially mosquitoes," in *Phytotelmata: terrestrial plants as host for aquatic insect communities*. Eds. J. H. Frank and L. P. Lounibos (Marlton, New Jersey, EUA: Plexus), 101–128.

Fulan, J. A., and Henry, R. (2007). Distribuição temporal de imaturos de Odonata (Insecta) associada a *Eichhornia azurea* (Kunth) na Lagoa do Camargo, Rio Paranapanema, São Paulo. *Rev. Bras. Entomologia* 51 (2), 224–227. doi: 10.1590/S0085-56262007000200013

Gardner, T. A., Ferreira, J., Barlow, J., Lees, A. C., Parry, L., Vieira, I. C. G., et al. (2013). A social and ecological assessment of tropical land-uses at multiple scales: The Sustainable Amazon Network. *Philos. Trans. R. Soc. Ser. B* 368 (1619), 20120166. doi: 10.1098/rstb.2012.0166

Garrison, R. W. (1990). A synopsis of the genus *Hetaerina* with descriptions of four new species (Odonata: Calopterigidae). *Trans. Am. Entomological Soc.* 116 (1), 175–259.

Garrison, R. W. (2009). A synopsis of the genus Telebasis (Odonata: Coenagrionidae). *Int. J. Odonatol.* 12 (1), 1–121. doi: 10.1080/13887890.2009.9748331

Garrison, R. W., Von Ellenrieder, N., and Louton, J. A. (2006). *Dragonfly genera of the new world: an illustrated and annotated key to the anisoptera* (Baltimore: The Johns Hopkins University Press), 368.

Garrison, R. W., Von Ellenrieder, N., and Louton, J. A. (2010). *Damselfly genera of the new world: an illustrated and annotated key to the zygoptera* (Baltimore: The Johns Hopkins University Press), 490.

Giehl, N. F. S., Dias-Silva, K., Juen, L., Batista, J. D., and Cabette, H. S. R. (2014). Taxonomic and numerical resolutions of Nepomorpha (Insecta: Heteroptera). Cerrado streams. *PloS One* 9 (8), e103623. doi: 10.1371/journal.pone.0103623

Gower, J. C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53 (3-4), 325–338. doi: 10.1093/biomet/53.3-4.325

Grönroos, M., Heino, J., Siqueira, T., Landeiro, V. L., Kotanen, J., and Bini, L. M. (2013). Metacommunity structuring in stream networks: roles of dispersal mode, distance type, and regional environmental context. *Ecol. Evol.* 3 (13), 4473–4487. doi: 10.1002/ece3.834

Hamada, N., Nessimian, J. L., and Querino, R. B. (2014). *Insetos aquáticosaquáticos na Amazônia brasileira: taxonomia, biologia e ecologia* (Manaus, Brazil: Editora do INPA).

Harabis, F., and Dolný, A. (2010). Ecological factors determining the density-distribution of Central European dragonflies (Odonata). *Eur. J. Entomol.* 107 (4), 571–577. doi: 10.14411/eje.2010.066

Hedenström, A., and Møller, A. P. (1992). Morphological adaptations to song flight in passerine birds: a comparative study. *Proc. R. Soc. London Ser. B Biol. Sci.* 247, 183–187. doi: 10.1098/rspb.1992.0026

Heino, J., Melo, A. S., Siqueira, T., Soininen, J., Valenko, S., and Bini, M. (2014). Metacommunity organization, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. *Freshw. Biol.* 60 (5), 845–869. doi: 10.1111/fwb.12533

Heino, J., and Peckarsky, B. L. (2014). Integrating behavioral, population and large-scale approaches for understanding stream insect communities. *Insect Sci.* 2, 7–13. doi: 10.1016/j.cois.2014.06.002

Heinrich, B. (1974). Thermoregulation in endothermic insects. *Science* 185, 747–756. doi: 10.1126/science.185.4153.747

Heiser, M., and Schmitt, T. (2010). Do different dispersal capacities influence the biogeography of the western Palearctic dragonflies (Odonata)? *Biol. J. Linn. Soc.* 99 (1), 177–195. doi: 10.1111/j.1095-8312.2009.01349.x

Jackson, D. A. (1993). Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* 74, 2204–2214. doi: 10.2307/1939574

Jacob, U., Walther, H., and and Klenke, R. (1984). Aquatic insect larvae as indicators of limiting minimal contents of dissolved oxygen-part II. *Aquat. Insects* 6 (3), 185–190. doi: 10.1080/01650428409361183

Johansson, F., Söderquist, M., and Bokma, F. (2009). Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. *Biol. Jounal Linn. Soc.* 97, 362–372. doi: 10.1111/j.1095-8312.2009.01211.x

Juen, L., and De Marco, P. Jr. (2011). Odonate biodiversity in terra- firme forest streamlets in Central Amazonia: on the relative effects of neutral and niche drivers at small geographical extents. *Insect Conserv. Diversity* 4 (4), 265–274. doi: 10.1111/j.1752-4598.2010.00130.x

Juen, L., and De Marco, P. Jr. (2012). Dragonfly endemism in the Brazilian Amazon: competing hypotheses for biogeographical patterns. *Biodiversity Conserv.* 21, 3507–3521. doi: 10.1007/s10531-012-0377-0

Juen, L., Oliveira-Junior, J. M. B., Shimano, Y., Mendes, T. P., and Cabette, H. S. R. (2014). Composição e riqueza de Odonata (Insecta) em riachos com diferentes níveis de conservação em um ecótone Cerrado-Floresta Amazônica. *Acta Amazonica* 44, 175–184. doi: 10.1590/S0044-59672014000200008

Laliberté, E., Legendre, P., and Shipley, B. (2014). FD: measuring functional diversity from multiple traits, and other tools for functional ecology. *R Package version* 1, 0–12.

Lencioni, F. A. A. (2005). *The Damselflies of Brazil: An Illustrated Identification Guide 1 – The Noncoenagrionidae families*. All Print Editora, São Paulo, Brazil, 324 p.

Lencioni, F. A. A. (2006). *Damselflies of Brazil, an illustrated identification guide: coenagrionidae families* (São Paulo, Brazil: All Print Editora), 330.

MacCreadie, J. W., and Adler, P. H. (2012). The roles of abiotic factors, dispersal, and species interactions in structuring stream assemblages of black flies (Diptera: Simuliidae). *Aquat. Biosyst.* 8 (14), 1–14. doi: 10.1186/2046-9063-8-14

Machado, A. B. M. (1954). *Elga santosi* sp. n. e redescrição de *Elga leptostyla* Ris 1911 (Odonata, Libellulidae). *Rev. Bras. Biol.* 14 (3), 303–312.

May, M. L. (1976). Thermoregulation and adaptation to temperature in dragonflies (Odonata: Anisoptera). *Ecol. Monogr.* 46, 1–32. doi: 10.2307/1942392

May, M. L. (1991). Thermal adaptations of dragonflies, revisited. *Adv. Odonatology* 5, 71–88.

Mendoza-Penagos, C., Calvão, L. B., and Juen, L. (2021). A new biomonitoring method using taxonomic families as substitutes for the suborders of the Odonata (Insecta) in Amazonian streams. *Ecol. Indic.* 124, 1470–160x. doi: 10.1016/j.ecolind.2021.107388

Mcauley, S. J. (2013). Relationship between morphology dispersal, and habitat distribution in three species of *Libellula* (Odonata: Anisoptera). *Aquat. Insect* 34 (3–4), 195–204. doi: 10.1080/01650424.2013.800557

Michiels, N. K., and André, A. (1990). Costs and benefits associated with oviposition site selection in the dragonfly *Sympetrum danae* (Odonata: Libellulidae). *Anim. Behav.* 40 (4), 668–678. Dhondt. doi: 10.1016/S0003-3472(05)80696-7

Miguel, T. B., Oliveira-Junior, J. M. B., Ligeiro, R., and Juen, L. (2017). Odonata (Insecta) as a tool for the biomonitoring of environmental quality. *Ecol. Indic* 81, 555–566. doi: 10.1016/j.ecolind.2017.06.010

Monteiro-Júnior, C. S., Juen, L., and Hamada, N. (2015). Analysis of urban impacts on aquatic habitats in the central Amazon basin: adult odonates as bioindicators of environmental quality. *Ecol. Indic.* 48, 303–311. doi: 10.1016/j.ecolind.2014.08.021

Monteiro-Júnior, C. S., Couceiro, S. R. M., Hamada, N., and Juen, L. (2013). Effect of vegetation removal for road building on richness and composition of Odonata communities in Amazonia, Brazil. *Int. J. Odonat.* 16 (2), 135–144. doi: 10.1080/13887890.2013.764798

Moore, A. J., and Machado, A. B. M. (1992). A note on *Cacoides latro* (Erichson), a territorial lacustrine gomphid (Anisoptera: Gomphidae). *Odonatologica* 21 (4), 499–503.

Moura, N. G. M., Lees, A. C. L., Andretti, C., Davis, B., Solar, R., Aleixo, A., et al. (2013). Avian biodiversity in multiple-use landscapes of the Brazilian Amazon. *Biol. Conserv.* 167, 339–348. doi: 10.1016/j.biocon.2013.08.023

Nepstad, D. C., Moutinho, P. R. S., Dias-Filho, M. B., Davidson, E., Cardinot, G., Markewitz, D., et al. (2002). The effects of rainfall exclusion on canopy processes and biogeochemistry of an Amazon Forest. *J. Geophysical Res.* 107, 80–85. doi: 10.1029/2001JD000360

Nessimian, J. L., Venticinque, E., Zuanon, J., De Marco, P., Gordo, M., Fidelis, L., et al. (2008). Land use, habitat integrity, and aquatic insect assemblages in Central Amazonian streams. *Hydrobiologia* 614, 117–131. doi: 10.1007/s10750-008-9441-x

Nilsson-Ortman, V., Stoks, R., De Block, M., and Johansson, F. (2012). Generalists and specialists along a latitudinal transect: patterns of thermal adaptation in six species of damselflies. *Ecology* 93, 1340–1352. doi: 10.1890/11-1910.1

Nóbrega, C. C., and De Marco, P. (2011). Unprotecting the rare spe- cies: a niche-based gap analysis for odonates in a core Cerrado area. *Diversity Distributions* 17 (3), 491–505. doi: 10.1111/j.1472-4642.2011.00749.x

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., et al. (2022). *Vegan community ecology package*. R package version 2.6-4. Available at: https://CRAN.R-project.org/package=vegan.

Oliveira-Junior, J. M. B., Cabette, H. S. R., Pinto, N. S., and Juen, L. (2013). As variações na comunidade de Odonata (Insecta) em córregos podem ser preditas pelo Paradoxo do Plâncton? Explicando a riqueza de espécies pela variabilidade ambiental. *EntomoBrasilis* 6, 1–8. doi: 10.12741/ebrasilis.v6i1.250

Oliveira-Junior, J. M. B., De Marco, P., Dias-Silva, K., Leitão, R. P., Leal, C. G., Pompeu, P. S., et al. (2017). Effects of human disturbance and riparian conditions on Odonata (Insecta) assemblages in eastern Amazon basin streams. *Limnologica* 66, 31–39. doi: 10.1016/j.limno.2017.04.007

Oliveira-Junior, J. M. B., and Juen, L. (2019). Structuring of dragonfly communities (Insecta: odonata) in eastern Amazon: effects of environmental and spatial factors in preserved and altered streams. *Insects* 10 (10), 322. doi: 10.3390/insects10100322

Oliveira-Junior, J. M. B., Rocha, T. S., Vinagre, S. F., MIranda-Filho, J. C., Mendoza-Penagos, C. C., Dias-Silva, K., et al. (2022). A bibliometric analysis of the global research in odonata: trends and gaps. *Diversity* 14, 1074. doi: 10.3390/d14121074

Oliveira-Junior, J. M. B., Shimano, Y., Gardner, T. A., Hughes, R. M., De Marco, P. Jr, and Juen., L. (2015). Neotropical dragonflies (Insecta: Odonata) as indicators of ecological condition of small streams in the eastern Amazon. *Austral Ecol.* 40 (6), 733–744. doi: 10.1111/aec.12242

Oliveira-Junior, J. M. B., Teodósio, M. A., and Juen, L. (2021). Patterns of co-occurrence and body size in dragonflies and damselflies (Insecta: Odonata) in preserved and altered Amazonian streams. *Austral Entomol.* 60 (2), 436–450. doi: 10.1111/aen.12535

Paulson, D. R. (2004). Why do some zygopterans (Odonata) perch with open wings? *Int. J. Odonatatology* 7 (3), 505–515. doi: 10.1080/13887890.2004.9748235

Pereira, D. F. G., Oliveira-Junior, J. M. B., and Juen, L. (2019). Environmental changes promote larger species of Odonata (Insecta) in Amazonian streams. *Ecol. Indic.* 98, 179–192. doi: 10.1016/j.ecolind.2018.09.020

Poff, N. L., Olden, J. D., Vieira, N. K. M., Finn, D. S., Simmons, M. P., and Kondratieff, B. C. (2006). Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. *J. North Am. Benthological Soc.* 25, 730–755. doi: 10.1899/0887-3593(2006)025[0730:FTNONA]2.0.CO,2

Powney, G. D., Cham, S. S. A., Smallshire, D., and Isaac, N. J. B. (2015). Trait correlates of distribution trends in the Odonata of Britain and Ireland. *PeerJ* 3, e1410. doi: 10.7717/peerj.1410

Putz, F. E., and Redford, H. K. (2010). The importance of defining 'Forest': Tropical forest degradation, deforestation, long-term phase shifts, and further transitions. *Biotropica* 42 (1), 10–20. doi: 10.1111/j.1744-7429.2009.00567.x

R Core Team (2021). *R: A language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing). Available at: https://www.R-project.org/.

Remsburg, A. J., and Turner, M. C. (2009). Aquatic and terrestrial drivers of dragonfly (Odonata) assemblages within and among north-temperate lakes. *J. North Am. Benthological Soc.* 28 (1), 44–56. doi: 10.1899/08-004.1

Resende, B. O., Ferreira, V., Brasil, L., Calvão, L. B., Mendes, T. P., Carvalho, F. G., et al. (2021). Impact of environmental changes on the behavioral diversity of the Odonata (Insecta) in the Amazon. *Sci. Rep.* 11 (1), 2045–2322. doi: 10.1038/s41598-021-88999-7

Resende, D. C., and De Marco, P. Jr. (2010). First description of reproductive behavior of the Amazonian damselfly *Chalcopteryx rutilans* (Rambur) (Odonata, Polythoridae). *Rev. Bras. Entomologia* 54 (3), 436–440. doi: 10.1590/S0085-56262010000300013

Rodrigues, M. E., Moura, E. B., Koroiva, R., Borges, A. C. P., and Roque, F. O. (2018). Survey of dragonflies (Odonata) in palm swamps of cerrado hotspot. *Entomol. News* 128 (1), 24–38. doi: 10.3157/021.128.0104

Rodrigues, M. E., Roque, F. O., Guillermo-Ferreira, R., Saito, V. S., and Samways, M. J. (2019). Egg-laying traits reflect shifts in dragonfly assemblages in response to different amount of tropical forest cover. *Insect Conserv. Diversity* 12 (3), 231–240. doi: 10.1111/icad.12319

Rodrigues, M. E., Roque, F. O., Quintero, J. M. O., and Castro-Pena, J. C. (2016). Nonlinear responses in damselfly community along a gradient of habitat loss in a savanna landscape. *Biol. Conserv.* 194, 113–120. doi: 10.1016/j.biocon.2015.12.001

Samways, M. J., and Taylor, S. (2004). Impacts of invasive alien plants on Red-Listed South African dragonflies (Odonata). *South Afr. J. Sci.* 100, 78–80.

Sánchez-Bayo, F., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biol. Conserv.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Silva-Pinto, N., Juen, L., Cabette, H. S. R., and De Marco, P. Jr. (2012). Fluctuating asymmetry and wing size of *Argia tinctipennis* Selys (Zygoptera: Coenagrionidae) in relation to Riparian Forest Preservation Status. *Neotrop. Entomol.* 41, 1–9. doi: 10.1007/s13744-012-0029-9

Southwood, T. R. (1977). Habitat, the templet for ecological strategies? *J. Anim. Ecol.* 46, 337–365. doi: 10.2307/3817

Souza, L. O. I., and Costa, J. M. (2006). "Inventário da Odonatofauna no Complexo Aporé-Sucuriú," in *Biodiversidade do Complexo Aporé-Sucuriú. Subsídios à conservação e manejo do bioma Cerrado*. Eds. T. C. S. Pagotto and P. R. Souza (Campo Grande: Editora da UFMS), 81–88, 308. org.

Statzner, B., Bis, B., Dolédec, S., and Usseglio-Polatera, P. (2001). Perspectives for biomonitoring at large spatial scales: a unified measure for the functional composition of invertebrate communities in European running waters. *Basic Appl. Ecol.* 2, 73–85. doi: 10.1078/1439-1791-00039

Stoks, R., and Córdoba-Aguilar, A. (2012). Evolutionary ecology of Odonata: A complex life cycle perspective. *Annu. Rev. Entomol.* 57, 249–265. doi: 10.1146/annurev-ento-120710-100557

Strahler, A. N. (1957). Quantitative analysis of watershed geomorphology. *Trans. Am. Geophysical Union* 38 (6), 913–920. doi: 10.1029/tr038i006p00913

Suhonen, J., Suutari, E., Kaunisto, K. M., and Krams, K. (2013). Patch area of macrophyte *Stratioites aloides* as a critical resource for declining dragonfly *Aeshna viridis*. *J. Insect Conserv.* 2, 393–398. doi: 10.1007/s10841-012-9521-0

Tavares, R. I. S., Mandelli, A. M., Mazão, G. R., and Guillermo-Ferreira, R. (2017). The relationship between habitat complexity and emergence time in damselflies. *Limnologica* 65 (1), 1–3. doi: 10.1016/j.limno.2017.04.009

Townsend, C. R., and Hildrew, A. G. (1994). Species traits in relation to a habitat templet for river systems. *Freshw. Biol.* 31, 265–275. doi: 10.1111/j.1365-2427.1994.tb01740.x

Veloso, H. P., Rangel Filho, A. L. R., and Lima, J. C. A. (1991). *Classificação da vegetação brasileira adaptada a um sistema universal* (Rio de Janeiro, Brazil: IBGE), 123.

Vilela, D. S., Ferreira, R. G., and Del-Claro, K. (2016). The Odonata community of a Brazilian vereda: seasonal patterns, species diversity and rarity in a palm swamp environment. *Biosci. J.* 32 (1), 486–495. doi: 10.14393/BJ-v32n2a2016-30491

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2007). Let the concept of trait be functional. *Oikos* 116, 882–892. doi: 10.1111/j.0030-1299.2007.15559.x

Watrin, O. S., and Rocha, A. M. A. (1992). *Levantamento da Vegetação Natural e do Uso da Terra no Município de Paragominas (PA) Utilizando Imagens TM/Landsat* Vol. p (Belém, Brazil: EMBRAPA/CPATU), Boletim de Pesquisa), 40. EMBRAPA/CPATU.

Williams, P., Whitfield, M., Biggs, J., Bray, S., Fox, G., Nicolet, P., et al. (2004). Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. *Biol. Conserv.* 115, 329–341. doi: 10.1016/S0006-3207(03)00153-8

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 26 October 2023
DOI 10.3389/fevo.2023.1267705

OPEN ACCESS

EDITED BY
Helen McCreery,
Tufts University, United States

REVIEWED BY
Adolfo Cordero-Rivera,
University of Vigo, Spain
Gianandrea La Porta,
University of Perugia, Italy

*CORRESPONDENCE
Rassim Khelifa
✉ rassim.khelifa@concordia.ca

RECEIVED 27 July 2023
ACCEPTED 09 October 2023
PUBLISHED 26 October 2023

CITATION
Mahdjoub H, Zebsa R, Amari H, Bensouilah S, Zouaimia A, Youcefi A and Khelifa R (2023) Potential fitness consequences of roosting spatiotemporal selection in an endangered endemic damselfly: conservation implications. *Front. Ecol. Evol.* 11:1267705. doi: 10.3389/fevo.2023.1267705

Potential fitness consequences of roosting spatiotemporal selection in an endangered endemic damselfly: conservation implications

Hayat Mahdjoub[1], Rabah Zebsa[2], Hichem Amari[3], Soufyane Bensouilah[4], Abdelheq Zouaimia[2], Abdeldjalil Youcefi[5] and Rassim Khelifa[1,6*]

[1]Biology Department, Concordia University, Montreal, QC, Canada, [2]Department of Nature and Life Sciences, Faculty of Nature and Life Sciences and Earth and Universe Sciences, University of 08 May 1945, Guelma, Algeria, [3]Department of Natural Sciences, Ecole normale supérieure de Ouargla, Ouargla, Algeria, [4]Biology Department, Université de Laghouat, Laghouat, Algeria, [5]Biology Department, University of Tamanrasset, Tamanrasset, Algeria, [6]Institute for Resources, Environment and Sustainability, University of British Columbia, Vancouver, BC, Canada

Understanding habitat requirements of species of conservation concern is central for their conservation and management. Although much of the research attention has been focused on reproductive sites, the understanding of roosting behavior and microhabitat selection, and their potential fitness consequences is also crucial. Here, we assess the roosting behavior of an endangered endemic damselfly *Calopteryx exul* Selys in a lotic habitat of Northeast Algeria. Based on marked individuals, we specifically investigated their vertical and horizontal distribution at roosting sites, as well as the timing of roosting and its correlation with lifespan (as a measure of fitness). We found that individuals were philopatric to roosting sites and less so to vertical stratification. Roosting sites were used for both foraging and roosting. Individuals that occupied lower strata in roosting sites had longer lifespans and ceased roosting earlier. Average temperature of the day affected the timing of roosting such that on warm days roosting started later and ended earlier. Individuals with longer lifespans roosted earlier, suggesting potential scramble competition for roosting sites. Our results suggest that *C. exul* individuals show variability in the vertical and horizontal location as well as the timing of roosting, and these choices potentially have fitness consequences. This study highlights the importance of bank vegetation as roosting sites for lotic insects, and emphasizes the benefits of protecting these sites and including them as integral parts of the conservation plans of species.

KEYWORDS

habitat preferences, resting, odonates, dragonflies, insects, *Calopteryx exul*

Introduction

Many threatened species of animals, including insects, are on the brink of extinction due to human activities (Maczulak, 2010; Wagner, 2020). Species of conservation concern need particular research attention because of their sensitivity to environmental disturbance (Maczulak, 2010; Samways et al., 2010; Samways, 2020). Conservationists have sounded the alarm about the rapid decline of insects in different regions worldwide due to various anthropogenic factors such as habitat destruction, pesticides, and climate change (Van Klink et al., 2020; Hallmann et al., 2021; Raven and Wagner, 2021; Uhler et al., 2021; Outhwaite et al., 2022). Thus, there is an increasing concern for threatened species because of their high sensitivity to habitat degradation and other anthropogenic stressors (New, 2009). Endemic species, in particular, are among the most threatened groups because their typical small range size limits their ability to cope with rapid changes in environmental conditions (Burlakova et al., 2011; Carmona et al., 2019). Because some anthropogenic factors are unavoidable in a human-dominated world, it is imperative to understand fundamental aspects of species' ecological requirements to increase their resilience and promote their persistence in their natural habitat.

There is a need to develop a holistic understanding of the habitat requirements of threatened species to better protect them and manage their habitat (Deacon et al., 2020; Samways et al., 2020; Kietzka et al., 2021). This involves a better comprehension of the spatial and temporal dimensions that species occupy to perform their ecological functions and fulfill their biological needs (Samways et al., 2020). While conservationists have paid particular attention to foraging and reproductive sites as vital habitat components for the conservation of species (Foster and Soluk, 2006; Majewska and Altizer, 2020; Shipley et al., 2023), other aspects of habitat preferences such as roosting sites have been understudied despite their crucial role in the species' life history (Grether and Switzer, 2000; Grether and Donaldson, 2007; Teng et al., 2012). Roosting sites may provide various services to individuals, including shelter, safety, and social information. Many species of animals, including mammals, birds, fish, and insects roost in communal aggregations of a dozen to millions of individuals (Krause and D., 2002). Compared to vertebrates (Laughlin et al., 2014; Deng et al., 2023), communal roosting behavior in insects has received far less research attention.

The evolution of communal aggregation behavior has been highly debated during the last centuries leading to various theories (Dwyer et al., 2018). Many of those theories such as thermal benefits (Vulinec, 1990), information center (Bijleveld et al., 2010), and aposematism (Turner, 1975) do not apply to a large number of insect groups (Grether and Switzer, 2000). The predator dilution theory as an antipredator defense mechanism; however, is one of the most applicable hypotheses to a wide range of taxa (Lack, 1968), including within insects (Vulinec, 1990). Studies have shown that species have specific preferences for the timing and location of communal roosting (Miller, 1989; Teng et al., 2012; Finkbeiner, 2014; Laughlin et al., 2014), often exhibiting high levels of philopatry in both vertebrates (Lewis, 1995; Beauchamp, 1999) and invertebrates (Miller, 1989; Grether and Donaldson, 2007; Finkbeiner, 2014). However, our understanding of the implication of microhabitat selection within roosting sites for the survival and fitness of individuals has not attracted comparable research attention.

Odonates are integral components of freshwater systems (Corbet, 1999; Martín and Maynou, 2016), occupying both terrestrial and aquatic habitats, and interacting with a wide range of taxa (Kaunisto et al., 2020). As adults, both dragonflies and damselflies spend the daytime foraging and reproducing near the water, however, at night, they occupy their roosting sites which are either near or far from the water (Corbet, 1999). Odonates are suitable organisms for the study of roosting behavior because they can be easily marked, recaptured, measured, and surveyed in the field throughout their entire lifespan (Cordero-Rivera and Stoks, 2008). Damselflies in particular perform mostly short-range movements and do not disperse frequently. There have been several records of communal roosting behavior in odonates (Neubauer and Rehfeldt, 1995; Grether and Switzer, 2000; Switzer and Grether, 2000; Rouquette and Thompson, 2007; Hykel et al., 2018), but only a few studies have explored microhabitat choice, site fidelity, and the potential fitness implications of roosting site selection.

Calopteryx exul is an endemic damselfly listed as Endangered on the IUCN Red list (Boudot, 2018). The species has a relatively patchy distribution with populations spanning from Morocco in the west to Tunisia in the East. In Algeria, the species had not been recorded for almost a century, from 1910 to 2007 (Khelifa et al., 2011). In later years, multiple subpopulations have been discovered in the Seybouse river, but several of them have perished following habitat degradation (Khelifa and Mellal, 2017). In more recent years, new sites have been recently discovered in the central North and east of Algeria (Chelli et al., 2019; Elafri, 2022), improving our understanding of its geographic distribution in the region. The historical sites of the country have encountered severe changes due to habitat destruction, exploitation (water pumping for irrigation), pollution (pesticides and fertilizers for agriculture), and climate change (severe drought and extreme heat) (Khelifa et al., 2021). Studies on the life history, reproductive behavior, habitat requirements, and geographic range dynamics have been carried out during the last decade (Khelifa, 2017; Mellal et al., 2018; Khelifa, 2019), furthering our understanding of the species ecology and behavior. However, there has not been any study on the roosting behavior in *C. exul*, restricting our holistic understanding of habitat preferences of the species. Filling in this gap of knowledge is crucial for the better management of natural habitats and maintenance of populations in their natural environment.

In this study, we investigate the roosting behavior of *C. exul* using capture-mark-recapture in the Seybouse river, Northeast Algeria. We marked individuals across a 100 m transect of the watercourse and surveyed their vertical stratification, horizontal distribution, timing of occupancy of roosting sites, and lifespan. We aim to test whether: 1) vertical and horizontal distribution differed between sexes, 2) vertical stratification and timing of roosting is correlated with fitness components (lifespan); and 3) temperature influences the timing of roosting. We hypothesize that: 1) there is sexual segregation in space and time due to behavioral differences between sexes; 2) vertical stratification and the timing of roosting are correlated with lifespan because better spatiotemporal choices have fitness consequences; and 3) timing of roosting depends on temperature such that individuals roost later on warm days.

Materials and methods

Roosting behavior

Mature adults of *C. exul* spend their daytime mating in patches of vegetation floating on the water (territories). At the close of the day, the damselflies gather in specific areas along the watercourse, both males and females joining together in a communal roosting site. These areas are often nestled amidst the bank vegetation, providing foraging, resting, and roosting sites. Prior to roosting, damselflies perched on stems or leaves to forage using a sit-and-wait tactic and seize the passing insects that fly during the dawn. When it becomes dark, damselflies take their roosting position and posture (Figure 1), spreading their wings open and remaining in the same posture until sunrise. Thus, this behavioral display allows us to identify roosting individuals and investigate intraspecific variation in spatiotemporal distributions of roosting behavior.

Study site

The research was conducted in the Seybouse river in the northeastern region of Algeria. The local climate is Mediterranean with hot and dry summers and cool and wet winters. In the Seybouse river, the mean annual rainfall varies between 350 mm upstream and 608 mm downstream (ABHCSM, 2009). The hydrology displays a wet season that encompasses the period from October to May, followed by a dry season that extends from June to September. The behavioral study was carried out upstream of the Seybouse River, approximately 5 km west of Guelma city (36° 28023.16″N and 7°22032.73″E; 210 m elevation). The watercourse was a stream with a shallow depth and a 2-4 m width. The vegetation along the banks predominantly comprises *Typha angustifolia* L., *Cyperus longus* L., *Juncus maritimus* Lam., and *Paspalum distichum* L. In the same site, odonate assemblage is dominated by *C. exul*, *C. haemorrhoidalis*, *P. subdilatata*, and *G. lucasii*.

Capture-mark-recapture

We took advantage of the daily capture-mark-recapture scheme that took place between 9:30 AM and 4:00 PM between late April and mid-July 2011 (total individuals marked 1417) as described in Khelifa et al. (2016). Six researchers carried out captures of adults with a hand net across a transect of 2 km. Adults were marked on the hindwing using permanent markers (Edding paint marker 780). Thus, a large proportion of individuals were marked throughout the flight season, which facilitated behavioral surveys of roosting behavior. Using repeated resightings, we were able to estimate the lifespan of individuals as the number of days between the first capture and the last resight.

Behavioral survey

To assess the roosting behavior of *C. exul*, we selected a transect of 100 m where the largest number of individuals occurred. A prior behavioral survey on the reproduction of the species took place in the same location (Khelifa, 2019). Two observers performed all behavioral surveys (each observer surveyed 50 m). To locate individuals within the studied area, we segmented the transect into 10 m sections using flags.

FIGURE 1
A roosting mature male (left) and female (right) of *Calopteryx exul* adult early in the morning in the Seybouse river, Northeast Algeria. Wing spreading occurs only when individuals are roosting. The typical wing posture when individuals are perched is joint wings.

The location within each 10 m-section was estimated visually to the nearest meter. The spatial and temporal distribution of roosting behavior was surveyed in the early morning between 5:00 am and 09:00 am and in the late afternoon between 05:30 pm and 09:00 pm for seven days (18-27 May 2023). We carried out scans every 10 minutes across the 100 m transect where we recorded the time of the day, individual ID, sex, location within the transect, and vertical stratification on the perching site. To estimate the vertical height of perching and roosting sites, we provided gridded sticks at different parts of the transect which allowed an estimation to the nearest 1-5 cm. The perching height of unmarked individuals was also estimated to determine the vertical stratification (involving a total number of observations of marked and unmarked individuals of 2357 and 589, respectively). Uniquely marked individuals included 122 adults, involving 70 females and 52 males.

Statistical analyses

We used R 4.2.2 to perform our statistical analyses (R Development Core Team, 2023). All mixed effects models (LME) were carried out using lme4 (Bates et al., 2015). To assess sexual differences in the vertical and horizontal distribution of individuals across the watercourse, we carried out a two-sample Kolmogorov-Smirnov test. To determine the temporal change in vertical stratification in the morning and the evening, we used a repeated measure correlation using the rmcorr package (Bakdash and Marusich, 2017). To assess philopatry to roosting sites and vertical stratification, we also analyzed the repeatability of location across days using the rpt function of the rptR package (Stoffel et al., 2017) with an LME including only the random effects of individual ID and sampling date. To determine whether there is a difference in the vertical stratification of individuals at communal roosting sites between sexes, marked and unmarked individuals, and time, we conducted an LME with sex, marking (marked or unmarked), and time of the day as explanatory variables, the height of roosting individuals as a response variable, and individual ID and sampling date as random effects. To test whether lifespan was correlated with vertical stratification, we used a generalized mixed-effects model (with negative binomial errors) that includes lifespan as a response variable, average roosting height (mean across days) and sex as explanatory variables, and individual ID as a random effect. To determine whether the temporal pattern of roosting differed between sexes and temperatures, we conducted a generalized LME (with binomial errors) for each period (morning and evening) with the presence/absence of roosting behavior as a response variable, time, sex, and average daily temperature as explanatory variables, and individual ID and sampling date as random effects. We used DHARMa package (Hartig, 2022) to perform residual diagnostics and check for the assumptions of the mixed-effects models (Supplementary material). Values shown in the text are mean ± SD.

Results

Vertical stratification

During the sampling period, adults occupied an average height of 65.6 ± 40.6 cm (n= 2946; range= 1-200 cm) strata. On average, males occupied significantly higher strata (70.0 ± 42.4 cm) than females (62.5 ± 39.0 cm) (two-sample Kolmogorov-Smirnov test: D = 0.09, $P<0.0001$) (Figure 2). Vertical stratification showed a temporal decline in the morning (from dawn to early morning) ($r = -0.20$ [95% -0.24 – -0.15], $P < 0.0001$) whereas a temporal increase in the evening (from late afternoon to dusk) ($r = 0.45$ [95% 0.40 –0.50], $P < 0.0001$).

In roosting sites, when we consider only marked individuals, we found that there was no significant difference in vertical stratification between sexes (LME: $\chi^2 = 2.25$, $P=0.13$) and across days (LME: $\chi^2 = 1.58$, $P=0.20$). In fact, vertical stratification of roosting behavior showed a low but significant level of repeatability across days ($R=0.13$ [0-0.38], $P = 0.008$). Interestingly, there was a significant negative correlation between the average height of roosting and the lifespan of individuals (GLME: $\chi^2 = 4.54$, $P=0.03$), revealing that individuals that had longer lifespans occupied lower strata (Figure 3).

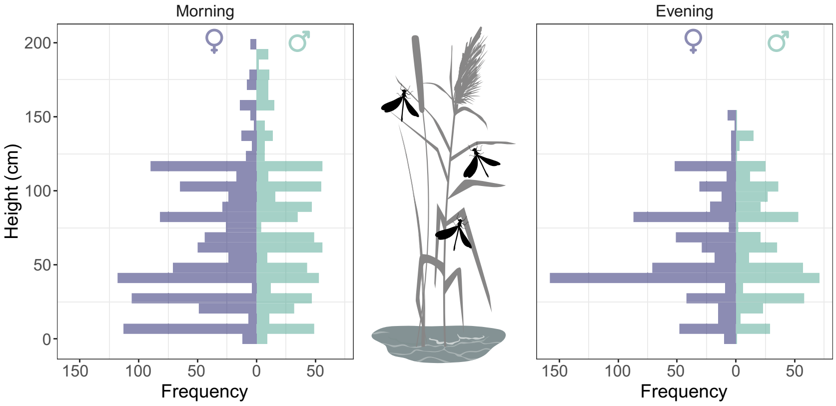


FIGURE 2
Vertical stratification of *Calopteryx exul* adult in roosting sites in early morning and evening in the Seybouse river, Northeast Algeria.

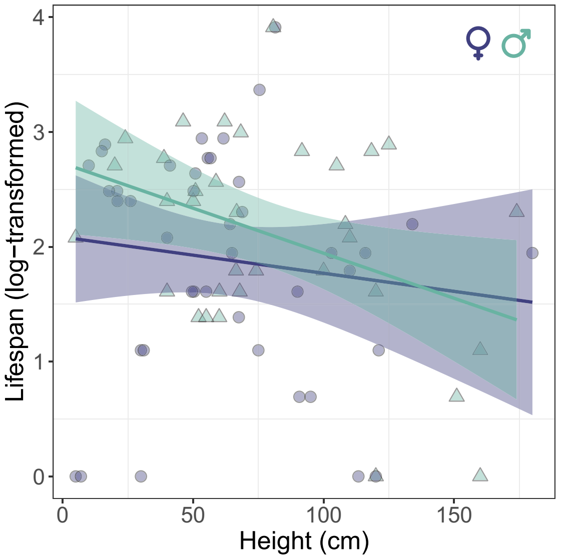


FIGURE 3
Relationship between the average height of vertical stratification at roosting sites and lifespan of *Calopteryx exul* adult in the Seybouse river, Northeast Algeria.

Horizontal distribution

Roosting sites had a patchy horizontal distribution across the vegetated areas of the watercourse. Overall, males and females had a similar horizontal distribution of roosting sites in the evening (two-sample Kolmogorov-Smirnov test: D = 0.21, P=0.06) (Figure 4). Roosting site selection across the watercourse was significantly repeatable from one day to another (R=0.49 [0.03-0.77], P < 0.0001). Almost all individuals (94%) were recorded near the water with one individual (<1%) recorded roosting at 10 m away from the water.

Timing of roosting behavior

There was intraspecific variation in the timing of the beginning (in the evening) and ending (in the morning) of roosting. The proportion of roosting individuals increased gradually with time in the evening (LME: χ^2 = 58.8, P < 0.0001), and decreased in the morning (χ^2 = 88.4, P < 0.0001) (Figure 5), revealing a gradual chronological shift in the proportion of roosting individuals. The absence of time-by-sex interaction in the morning indicates that males and females had similar timing of roosting (χ^2 = 0.03, P=0.84), but in the evening the rate of roosting was slightly but not significantly faster in females than males (χ^2 = 0.71, P=0.39). Timing of roosting interacted significantly with the average temperature of the day in the morning (χ^2 = 58.1, P < 0.0001) and the evening (χ^2 = 32.6, P< 0.0001), revealing that individuals ceased the roosting posture earlier in the morning and started the roosting posture later in the evening in warmer days (Figure 5). There was a marginal negative correlation between the timing of roosting and lifespan in males but not in females, as revealed by the marginal interaction of lifespan and sex (χ^2 = 3.19, P = 0.08) (Figure 6).

Discussion

Although communal roosting behavior in odonates has been documented in both damselflies (Grether and Switzer, 2000; Rouquette and Thompson, 2007) and dragonflies (Miller, 1989), the study of its vertical stratification and timing and its potential fitness consequences has not attracted much research attention. In this study, we investigated the roosting behavior of *C. exul*, an endangered endemic damselfly that lives in lotic habitats of North Africa. Our behavioral survey showed that 1) roosting sites were restricted to small areas in the watercourse; 2) adults were philopatric to roosting sites but less so to vertical stratification; 3) vertical stratification and timing (marginal) of roosting were

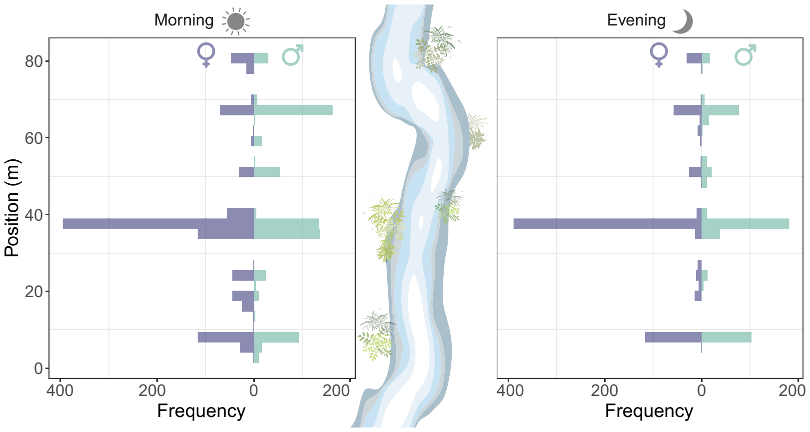


FIGURE 4
Horizontal distribution of the roosting sites of *Calopteryx exul* adult in early morning and evening in the studied stream in the Seybouse river, Northeast Algeria.

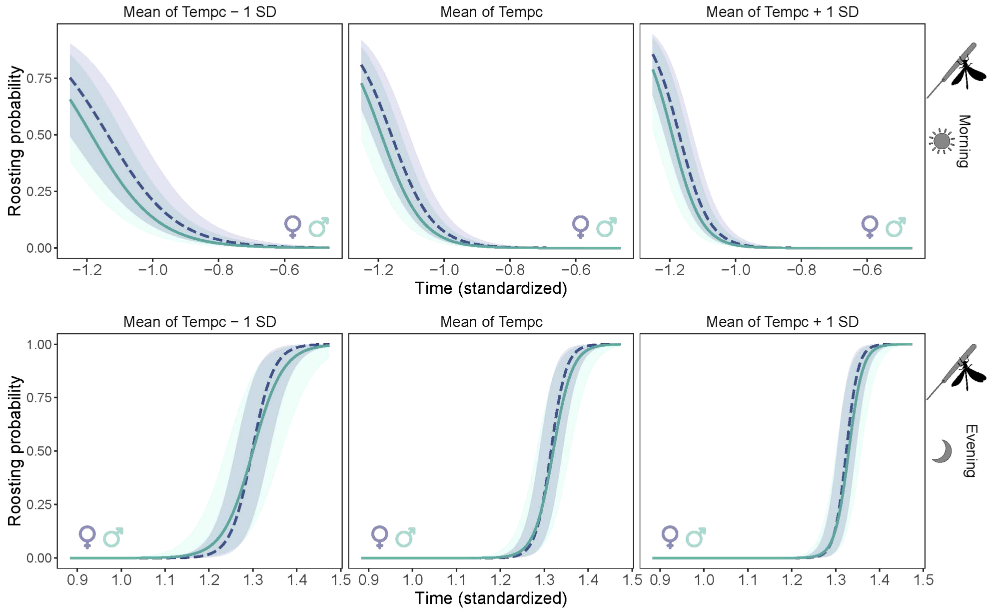


FIGURE 5
Temporal pattern of roosting (wing spreading posture) of *Calopteryx exul* adult at three levels of average daily temperature in early morning and evening in the Seybouse river, Northeast Algeria.

negatively correlated with lifespan; and 4) temperature of the day influenced the timing of roosting. This is the first study that investigates the beginning of roosting behavior was marginally correlated with individual lifespan.

In our study species, the non-random horizontal distribution of individuals suggests that the species exhibits site preferences for communal roosting. These roosting areas were relatively dense vegetated areas on the banks of the watercourse, which likely provided shelter against predators and resilience to withstand adverse nocturnal weather conditions (wind and rain) (Rouquette and Thompson, 2007; Hykel et al., 2018). Concordant with our hypothesis of site fidelity, we found that individuals were philopatric to roosting sites, which is similar to other species of Calopterygidae displaying comparable communal roosting behavior (Grether and Switzer, 2000). Grether and Switzer (2000) suggest that the location of the roosts is, at least partly, socially learned (i.e., traditional rather than habitat-related). In an experimental study on philopatry to roosting sites in a harvestman, Grether and Donaldson (2007) found that past communal usage was the best predictor of roosting site selection despite the occurrence of other similar sites (Grether and Donaldson, 2007). In many insects and other animal groups, philopatry is suggested to have evolved because of its fitness benefits (Stacey and Ligon, 1991; Hendry et al., 2004), which include familiarity with the environmental conditions including competition and predation risks, and minimization of the time and energy allocated to searching new locations (Switzer, 1993). Those benefits could also explain the occurrence of philopatry of odonate adults not only to roosting sites, but also to reproductive and emergence sites (Dolný et al., 2013).

In addition to selecting a particular location of roosting sites, adults of *C. exul* were also selective of vertical strata. The similarity in the vertical stratification between males and females (absence of sexual segregation) and the absence of mating attempts strengthens the hypothesis that vertical stratification is not directly related to mating (Grether and Switzer, 2000). There are many studies that show intraspecific and interspecific variation in the vertical distribution of adults during the active period of the day (Worthen and Jones, 2006; Worthen and Morrow, 2016), as well as larvae during emergence (Cordero, 1995; Hadjoudj et al., 2014).

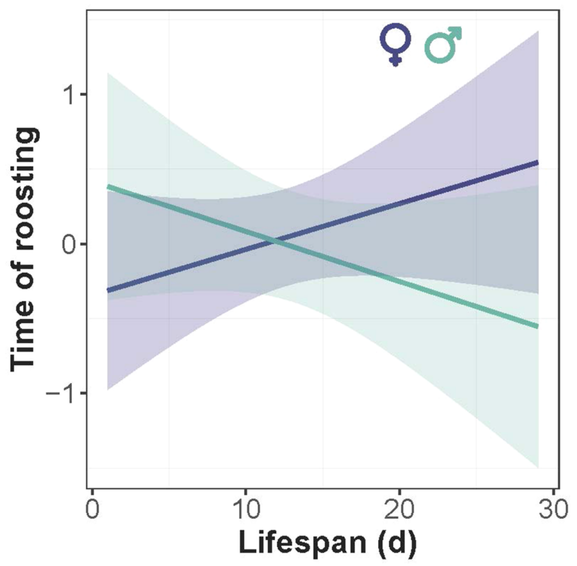


FIGURE 6
Relationship between the timing of roosting and lifespan of *Calopteryx exul* in the Seybouse river, Northeast Algeria. The fitted lines are predicted values from the linear mixed-effects model.

These studies suggest that the selection of heights has many fitness implications, including avoidance of predators, coping with harsh weather conditions, reducing competition, and exposure to sunlight (Switzer and Grether, 2000; Khelifa et al., 2013). Some of these benefits may apply to the vertical stratification of *C. exul* at roosting sites. In particular, roosting in specific strata may allow individuals to receive the first sunlight in the morning (Switzer and Grether, 2000), which could prolongate their hunting period. Importantly, our results support the hypothesis that lower strata likely provided better ecological conditions to roosting adults. In fact, we found a negative correlation between lifespan and vertical stratification of individuals at roosting sites, suggesting survival costs related to the selection of higher strata. It is likely that individuals that roosted in higher strata were more conspicuous to predators and thus more exposed to higher rates of mortality. This hypothesis goes in line with studies showing that more conspicuous individuals of Calopterygids are more often predated by birds (Svensson and Friberg, 2007). Further studies need to be conducted to assess whether the benefits of vertical stratification is condition-dependent, that is, lower strata could be less beneficial in some sites compared to others due to differences in predation or interspecific competition.

Unlike other species of Calopterygidae (Grether and Switzer, 2000), where foraging and roosting sites are distinct patches of vegetation, we found that roosting sites were often used as foraging sites for both males and females of *C. exul*. In the same roosting area, foraging was carried out prior to roosting in the evening and after becoming active in early morning, similar to other Calopterygidae (Switzer and Grether, 2000). However, the vertical strata used for both activities were quite different. As indicated by the temporal decline of vertical stratification in the evening and its gradual increase in the morning, the roosting strata were often lower than the foraging strata in *C. exul.* This might be a behavioral strategy to not only maximize foraging success at higher strata prior to or after roosting, but also reduce detectability and avoid predation at night during roosting. Interestingly, a high proportion of individuals concentrated near the water when roosting although there were vegetated areas farther from the water. Unlike other odonate species that roost away from the water (Rouquette and Thompson, 2007; Hykel et al., 2018), the nocturnal aggregation of individuals near the water renders the bank aquatic plants vital resources for feeding, resting, roosting, and emergence for *C. exul*.

Another finding in our study was that the average temperature of the day influenced the timing of roosting. On warmer days, both males and females started roosting later in the evening and became active earlier in the morning. As an ectotherm, damselfly activity is highly dependent on temperature (Angilletta, 2009), such that as body temperature increases earlier in the morning, individuals become active earlier (May, 1979). On warmer days, individuals also roosted later in the day probably because they spend more time mating and foraging. It is important to note that, since our survey was carried out in the spring, warmer days did not involve extreme heat. A similar influence of weather conditions on the timing of communal roosting behavior was recorded in a social bird (cattle egret) (Youcefi et al., 2019). This finding suggests that climate change could influence the diel pattern of activity and spatial distribution of the studied species (Yang et al., 2021).

Our results showed a marginal negative correlation between the timing of roosting and lifespan in males but not in females. It is likely that the temporal variation in roosting suggests competition for space at roosting sites. It is well known that high-quality males arrive earlier in breeding sites in birds and other taxa (Kokko, 1999; Morrison et al., 2019). If this hypothesis is true, we believe that such competition is more scramble than interference competition due to the rare intraspecific interactions between individuals at roosting sites. This is unlike the interspecific competition for space in perching sites usually recorded in different odonate assemblages where larger species tend to exclude smaller species from preferred habitats (Worthen and Jones, 2006; Worthen and Morrow, 2016). An alternative explanation is that, since foraging often precedes roosting, it is likely that individuals who are more fit (better at surviving longer) are more effective at foraging, thus roosting earlier than other individuals. Further studies are needed to shed light on the mechanisms underlying the negative relationship between the timing of roosting and fitness.

Our study presented some limitations due to the technical difficulty of performing field observations early in the morning and in the evening. Due to the rarity of the species and security issues, we could not perform the behavioral survey on multiple sites and throughout the entire season. We did not measure morphological traits such as body size and territoriality to determine their implication in vertical stratification and roosting behavior. Nevertheless, we believe that our fitness measure (lifespan) is a good proxy for quality because it captures the ability of individuals to survive and live longer. Further studies on roosting behavior need to explore potential behavioral variations of roosting across space and time.

Conservation implications

Our results on the potential fitness consequences of roosting microhabitat selection highlight the importance of investigating roosting behavior and ecology. The dual function of roosting sites as both foraging and roosting areas makes them vital habitat elements and likely key criteria in habitat selection for *C. exul*, and other odonates. Studies have highlighted the pivotal role that bank vegetation and hydrophytes provide for the reproduction of damselflies (Oliveira-Junior et al., 2017), including *C. exul* (Khelifa, 2013; Mellal et al., 2018). Our results, together with the existing literature, highlight the importance of understanding the function of the bank vegetation of aquatic habitats (Guillermo-Ferreira and Del-Claro, 2011; Vilenica et al., 2022) and the ecological implications of its degradation on the aquatic community and ecosystem functioning (Da Silva Monteiro Júnior et al., 2013). The conservation plan for threatened species such as *C. exul* should not only focus on reproductive sites but also consider roosting sites as integral parts of the habitat requirements for the species (Rouquette and Thompson, 2007; Hykel et al., 2018). The conservation of these habitats probably has an umbrella effect on a diversity of terrestrial and aquatic taxa, thus promoting biodiversity and protecting the integrity of ecosystems. Future studies need to investigate the interplay between reproductive behavior, habitat preferences, foraging success, intrinsic traits, and lifetime mating success.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://figshare.com/s/b49f4f90592f2628def9.

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local legislation and institutional requirements.

Author contributions

HM: Data curation, Writing – original draft, Writing – review & editing, Formal Analysis, Visualization. RZ: Data curation, Writing – review & editing, Conceptualization, Investigation. HA: Data curation, Investigation, Writing – review & editing. SB: Data curation, Investigation, Writing – review & editing. AZ: Investigation, Writing – review & editing. AY: Data curation, Investigation, Writing – review & editing. RK: Writing – review & editing, Conceptualization, Data curation, Supervision, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was conducted in the capacity of a Postdoctoral Research Fellow (project: P400PB_191139) awarded by the Swiss National Science Foundation (SNSF).

Acknowledgments

Thanks to the reviewers for their helpful comments and suggestions. We thank many students from the University of Guelma who helped us with the marking of damselflies. We are grateful to Nasr-Eddine Sakrane for providing transportation to and from the field site.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1267705/full#supplementary-material

References

ABHCSM (2009). Qualité des eaux superficielles dans les bassins du Kebir-Rhumel, de la Seybouse et de la Medjerda-Mellegue 2004–2007. *Agence bassin hydrographique Constantinois-Seybouse-Mellegue*. N° 12.

Angilletta, M. J. (2009). *Thermal adaptation: a theoretical and empirical synthesis* (Oxford: Oxford University Press).

Bakdash, J. Z., and Marusich, L. R. (2017). Repeated measures correlation. *Front. Psychol.* 8, 456. doi: 10.3389/fpsyg.2017.00456

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Beauchamp, G. (1999). The evolution of communal roosting in birds: origin and secondary losses. *Behav. Ecol.* 10, 675–687. doi: 10.1093/beheco/10.6.675

Bijleveld, A. I., Egas, M., Van Gils, J. A., and Piersma, T. (2010). Beyond the information centre hypothesis: communal roosting for information on food, predators, travel companions and mates? *Oikos* 119, 277–285. doi: 10.1111/j.1600-0706.2009.17892.x

Boudot, J.-P. (2018). *Calopteryx exul*. The IUCN red list of threatened species 2018, e.T60287A72725790. doi: 10.2305/IUCN.UK.2018-2301.RLTS.T60287A72725790.en

Burlakova, L. E., Karatayev, A. Y., Karatayev, V. A., May, M. E., Bennett, D. L., and Cook, M. J. (2011). Endemic species: contribution to community uniqueness, effect of habitat alteration, and conservation priorities. *Biol. Conserv.* 144, 155–165. doi: 10.1016/j.biocon.2010.08.010

Carmona, E. C., Musarella, C. M., and Ortiz, A. C. (2019). *Endemic Species* (London, United Kingdom: BoD–Books on Demand).

Chelli, A., Zebsa, R., and Khelifa, R. (2019). Discovery of a new population of the endangered *Calopteryx exul* in central North Algeria (Odonata: Calopterygidae). *Notulae Odonatol.* 9, 150–154. doi: 10.5281/zenodo.3539748

Corbet, P. S. (1999). *Dragonflies: behaviour and ecology of Odonata* (Colchester: Harley Books).

Cordero, A. (1995). Vertical stratification during emergence in odonates. *Notulae Odonatol.* 4, 103–105. Available at: https://natuurtijdschriften.nl/pub/593562/NOIOS1995004006004.pdf.

Cordero-Rivera, A., and Stoks, R. (2008). "Mark-recapture studies and demography," in *Dragonflies and damselflies: Model organisms for ecological and evolutionary research*. Ed. A. Córdoba-Aguilar (Oxford: Oxford University Press), 7–20.

Da Silva Monteiro Júnior, C., Couceiro, S. R. M., Hamada, N., and Juen, L. (2013). Effect of vegetation removal for road building on richness and composition of Odonata communities in Amazonia, Brazil. *Int. J. Odonatol.* 16, 135–144. doi: 10.1080/13887890.2013.764798

Deacon, C., Samways, M. J., and Pryke, J. S. (2020). Determining drivers of dragonfly diversity patterns and the implications for conservation in South Africa. *Biol. Conserv.* 245, 108548. doi: 10.1016/j.biocon.2020.108548

Deng, Y., Belotti, M. C. T., Zhao, W., Cheng, Z., Perez, G., Tielens, E., et al. (2023). Quantifying long-term phenological patterns of aerial insectivores roosting in the Great Lakes region using weather surveillance radar. *Global Change Biol.* 29, 1407–1419. doi: 10.1111/gcb.16509

Dolný, A., Mižičová, H., and Harabiš, F. (2013). Natal philopatry in four European species of dragonflies (Odonata: Sympetrinae) and possible implications for conservation management. *J. Insect Conserv.* 17, 821–829. doi: 10.1007/s10841-013-9564-x

Dwyer, J. F., Fraser, J. D., and Morrison, J. L. (2018). Evolution of communal roosting: A social refuge–territory prospecting hypothesis. *J. Raptor Res.* 52, 407–419. doi: 10.3356/JRR-17-101.1

Elafri, A. (2022). New records of the endangered *Calopteryx exul* in a semi-arid territory of north-eastern Algeria (Odonata: Calopterygidae). *Notulae Odonatol.* 9, 451–454. doi: 10.60024/nodo.v9i9.a8

Finkbeiner, S. D. (2014). Communal roosting in Heliconius butterflies (Nymphalidae): Roost recruitment, establishment, fidelity, and resource use trends based on age and sex. *J. Lepidopterists' Soc.* 68, 10–16. doi: 10.18473/lepi.v68i1.a2

Foster, S., and Soluk, D. (2006). Protecting more than the wetland: the importance of biased sex ratios and habitat segregation for conservation of the Hine's emerald dragonfly, Somatochlora hineana Williamson. *Biol. Conserv.* 127, 158–166. doi: 10.1016/j.biocon.2005.08.006

Grether, G. F., and Donaldson, Z. R. (2007). Communal roost site selection in a neotropical harvestman: habitat limitation vs. tradition. *Ethology* 113, 290–300. doi: 10.1111/j.1439-0310.2006.01328.x

Grether, G. F., and Switzer, P. V. (2000). Mechanisms for the formation and maintenance of traditional night roost aggregations in a territorial damselfly. *Anim. Behav.* 60, 569–579. doi: 10.1006/anbe.2000.1511

Guillermo-Ferreira, R., and Del-Claro, K. (2011). Oviposition site selection in *Oxyagrion microstigma* Selys 1876 (Odonata: Coenagrionidae) is related to aquatic vegetation structure. *Int. J. Odonatol.* 14, 275–279. doi: 10.1080/13887890.2011.621109

Hadjoudj, S., Khelifa, R., Guebailia, A., Amari, H., Hadjadji, S., Zebsa, R., et al. (2014). Emergence ecology of *Orthetrum cancellatum*: temporal pattern and microhabitat selection (Odonata: Libellulidae). *Annales la Société Entomologique France (NS)* 50, 343–349. doi: 10.1080/00379271.2014.938941

Hallmann, C. A., Ssymank, A., Sorg, M., De Kroon, H., and Jongejans, E. (2021). Insect biomass decline scaled to species diversity: General patterns derived from a hoverfly community. *Proc. Natl. Acad. Sci.* 118, e2002554117. doi: 10.1073/pnas.2002554117

Hartig, F. (2022). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models* (R package version 0.4.6). Available at: https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html.

Hendry, A. P., Castric, V., Kinnison, M. T., Quinn, T. P., Hendry, A., and Stearns, S. (2004). "The evolution of philopatry and dispersal," in *Evolution illuminated: salmon and their relatives* (New York: Oxford University Press), 52–91.

Hykel, M., Harabiš, F., and Dolný, A. (2018). Diel changes in habitat use by dragonflies: Nocturnal roosting site selection by the threatened dragonfly Sympetrum depressiusculum (Odonata: Libellulidae). *Entomol. Sci.* 21, 154–163. doi: 10.1111/ens.12292

Kaunisto, K. M., Roslin, T., Forbes, M. R., Morrill, A., Sääksjärvi, I. E., Puisto, A. I., et al. (2020). Threats from the air: Damselfly predation on diverse prey taxa. *J. Anim. Ecol.* 89, 1365–1374. doi: 10.1111/1365-2656.13184

Khelifa, R. (2013). Flight period, apparent sex ratio and habitat preferences of the Maghribian endemic *Calopteryx exul* Selys 1853 (Odonata: Zygoptera). *Rev. d'Ecologie (La Terre La Vie)* 68, 37–45. doi: 10.3406/revec.2013.1674

Khelifa, R. (2017). Partial bivoltinism and emergence patterns in the North African endemic damselfly *Calopteryx exul*: conservation implications. *Afr. J. Ecol.* 55, 145–151. doi: 10.1111/aje.12332

Khelifa, R. (2019). Females A'ssist' sneaker males to dupe dominant males in a rare endemic damselfly: sexual conflict at its finest. *Ecology* 100, e02811. doi: 10.1002/ecy.2811

Khelifa, R., Mahdjoub, H., Baaloudj, A., Cannings, R. A., and Samways, M. J. (2021). Effects of both climate change and human water demand on a highly threatened damselfly. *Sci. Rep.* 11, 7725. doi: 10.1038/s41598-021-86383-z

Khelifa, R., and Mellal, M. K. (2017). Host-plant-based restoration as a potential tool to improve conservation status of odonate specialists. *Insect Conserv. Diversity* 10.2, 151–160. doi: 10.1111/icad.12212

Khelifa, R., R. Zebsa, H. Amari, M. Houhamdi, M. Khalil Mellal, H. Mahdjoub and A. Kahalerras (2016). A hotspot for threatened Mediterranean odonates in the Seybouse River (Northeast Algeria): are IUCN population sizes drastically underestimated? *Int. J. Odonatol.* 19, 1–11.

Khelifa, R., Youcefi, A., Kahlerras, A., Al Farhan, A., Al-Rasheid, K. A., and Samraoui, B. (2011). L'odonatofaune (Insecta: Odonata) du bassin de la Seybouse en Algérie: intérêt pour la biodiversité du Maghreb. *Rev. d'écologie* 66, 55–66. Available at: https://hal.science/hal-03530643.

Khelifa, R., Zebsa, R., Amari, H., and Mellal, M. K. (2013). Does wind affect emergence site selection in Odonata? *Afr. Entomol.* 21, 383–387. Available at: https://hdl.handle.net/10520/EJC141291

Kietzka, G. J., Pryke, J. S., Gaigher, R., and Samways, M. J. (2021). Webs of well-designed conservation corridors maintain river ecosystem integrity and biodiversity in plantation mosaics. *Biol. Conserv.* 254, 108965. doi: 10.1016/j.biocon.2021.108965

Kokko, H. (1999). Competition for early arrival in migratory birds. *J. Anim. Ecol.* 68, 940–950. doi: 10.1046/j.1365-2656.1999.00343.x

Krause, J., and D., R. G. (2002). *Living in groups* (Oxford, UK: Oxford University Press).

Lack, D. (1968). *Ecological adaptations for breeding in birds* (London: Methuen).

Laughlin, A. J., Sheldon, D. R., Winkler, D. W., and Taylor, C. M. (2014). Behavioral drivers of communal roosting in a songbird: a combined theoretical and empirical approach. *Behav. Ecol.* 25, 734–743. doi: 10.1093/beheco/aru044

Lewis, S. E. (1995). Roost fidelity of bats: a review. *J. Mammal.* 76, 481–496. doi: 10.2307/1382357

Maczulak, A. E. (2010). *Biodiversity: conserving endangered species* (New York: Infobase Publishing).

Majewska, A. A., and Altizer, S. (2020). Planting gardens to support insect pollinators. *Conserv. Biol.* 34, 15–25. doi: 10.1111/cobi.13271

Martín, R., and Maynou, X. (2016). Dragonflies (Insecta: Odonata) as indicators of habitat quality in Mediterranean streams and rivers in the province of Barcelona (Catalonia, Iberian Peninsula). *Int. J. Odonatol.* 19, 107–124. doi: 10.1080/13887890.2016.1172991

May, M. L. (1979). Insect thermoregulation. *Annu. Rev. Entomol.* 24, 313–349. doi: 10.1146/annurev.en.24.010179.001525

Mellal, M. K., Bensouilah, M., Houhamd, M., and Khelifa, R. (2018). Reproductive habitat provisioning promotes survival and reproduction of the endangered endemic damselfly *Calopteryx exul*. *J. Insect Conserv.* 22, 563–570. doi: 10.1007/s10841-018-0085-5

Miller, P. (1989). Communal roosting in *Potamarcha congener* (Rambur) and its possible functions (Anisoptera: Libellulidae). *Odonatologica* 18, 179–194.

Morrison, C. A., Alves, J. A., Gunnarsson, T. G., Þórisson, B., and Gill, J. A. (2019). Why do earlier-arriving migratory birds have better breeding success? *Ecol. Evol.* 9, 8856–8864. doi: 10.1002/ece3.5441

Neubauer, K., and Rehfeldt, G. (1995). Roosting site selection in the damselfly species *Calopteryx haemorrhoidalis* (Odonata: Calopterygidae). *Entomol. Generalis* 19, 291–302. doi: 10.1127/entom.gen/19/1995/291

New, T. R. (2009). *Insect species conservation* (Cambridge: Cambridge University Press).

Oliveira-Junior, J. M. B. D., De Marco, P., Dias-Silva, K., Leitão, R. P., Leal, C. G., Pompeu, P. S., et al. (2017). Effects of human disturbance and riparian conditions on Odonata (Insecta) assemblages in eastern Amazon basin streams. *Limnologica* 66, 31–39. doi: 10.1016/j.limno.2017.04.007

Outhwaite, C. L., Mccann, P., and Newbold, T. (2022). Agriculture and climate change are reshaping insect biodiversity worldwide. *Nature* 605, 97–102. doi: 10.1038/s41586-022-04644-x

Raven, P. H., and Wagner, D. L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proc. Natl. Acad. Sci.* 118. doi: 10.1073/pnas.2002548117

R Development Core Team (2023). *R: A Language and Environment for Statistical Computing* (Vienna, Austria: R Foundation for Statistical Computing).

Rouquette, J. R., and Thompson, D. J. (2007). Roosting site selection in the endangered damselfly, *Coenagrion mercuriale*, and implications for habitat design. *J. Insect Conserv.* 11, 187–193. doi: 10.1007/s10841-006-9030-0

Samways, M. J. (2020). *Insect conservation: a global synthesis* (Boston, MA: CABI).

Samways, M. J., Barton, P. S., Birkhofer, K., Chichorro, F., Deacon, C., Fartmann, T., et al. (2020). Solutions for humanity on how to conserve insects. *Biol. Conserv.* 242, 108427. doi: 10.1016/j.biocon.2020.108427

Samways, M. J., Mcgeoch, M. A., and New, T. R. (2010). *Insect conservation: a handbook of approaches and methods* (Oxford: Oxford University Press).

Shipley, J. R., Gossner, M. M., Rigling, A., and Krumm, F. (2023). Conserving forest insect biodiversity requires the protection of key habitat features. *Trends Ecol. Evol.* 38, 788–791. doi: 10.1016/j.tree.2023.05.015

Stacey, P. B., and Ligon, J. D. (1991). The benefits-of-philopatry hypothesis for the evolution of cooperative breeding: variation in territory quality and group size effects. *Am. Nat.* 137, 831–846. doi: 10.1086/285196

Stoffel, M. A., Nakagawa, S., and Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol. Evol.* 8, 1639–1644. doi: 10.1111/2041-210X.12797

Svensson, E. I., and Friberg, M. (2007). Selective predation on wing morphology in sympatric damselflies. *Am. Nat.* 170, 101–112. doi: 10.1086/518181

Switzer, P. V. (1993). Site fidelity in predictable and unpredictable habitats. *Evol. Ecol.* 7, 533–555. doi: 10.1007/BF01237820

Switzer, P. V., and Grether, G. F. (2000). Characteristics and possible functions of traditional night roosting aggregations in rubyspot damselflies. *Behaviour* 137, 401–416. doi: 10.1163/156853900502141

Teng, B., Dao, S., Donaldson, Z. R., and Grether, G. F. (2012). New communal roosting tradition established through experimental translocation in a Neotropical harvestman. *Anim. Behav.* 84, 1183–1190. doi: 10.1016/j.anbehav.2012.08.022

Turner, J. (1975). Communal roosting in relation to warning colour in two heliconiine butterflies (Nymphalidae). *J. Lepidopterists Soc.* 29, 221–226.

Uhler, J., Redlich, S., Zhang, J., Hothorn, T., Tobisch, C., Ewald, J., et al. (2021). Relationship of insect biomass and richness with land use along a climate gradient. *Nat. Commun.* 12, 1–9. doi: 10.1038/s41467-021-26181-3

Van Klink, R., Bowler, D. E., Gongalsky, K. B., Swengel, A. B., Gentile, A., and Chase, J. M. (2020). Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science* 368, 417–420. doi: 10.1126/science.aax9931

Vilenica, M., Rebrina, F., Matoničkin Kepčija, R., Šegota, V., Rumišek, M., Ružanović, L., et al. (2022). Aquatic macrophyte vegetation promotes taxonomic and functional diversity of odonata assemblages in intermittent karst rivers in the Mediterranean. *Diversity* 14, 31. doi: 10.3390/d14010031

Vulinec, K. (1990). "Collective security: aggregation by insects as a defense," in *Insect defenses* (Albany, NY: State University of New York Press), 251–288.

Wagner, D. L. (2020). Insect declines in the Anthropocene. *Annu. Rev. Entomol.* 65, 457–480. doi: 10.1146/annurev-ento-011019-025151

Worthen, W. B., and Jones, C. M. (2006). Relationships between body size, wing morphology, and perch height selection in a guild of Libellulidae species (Odonata). *Int. J. Odonatol.* 9, 235–250. doi: 10.1080/13887890.2006.9748281

Worthen, W. B., and Morrow, P. H. (2016). Perch selection by three Cooccurring species of *Celithemis* (Odonata: Libellulidae): testing for a competitive hierarchy among similar species. *Psyche: A J. Entomol.* 2016, 9028105. doi: 10.1155/2016/9028105

Yang, L. H., Postema, E. G., Hayes, T. E., Lippey, M. K., and Macarthur-Waltz, D. J. (2021). The complexity of global change and its effects on insects. *Curr. Opin. Insect Sci.* 47, 90–102. doi: 10.1016/j.cois.2021.05.001

Youcefi, A., Baaloudj, A., Zebsa, R., Mahdjoub, H., Bensakhri, Z., Bensouilah, S., et al. (2019). Weather conditions affect the collective roosting behaviour of the Cattle Egret *Bubulcus ibis*. *Bird Study* 66, 413–419. doi: 10.1080/00063657.2019.1694487

Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 12 December 2023
DOI 10.3389/fevo.2023.1162060

OPEN ACCESS

EDITED BY
Piotr Nowicki,
Jagiellonian University, Poland

REVIEWED BY
Jason Harmon,
North Dakota State University,
United States
Adam Kőrösi,
Büro Geyer und Dolek, Germany

*CORRESPONDENCE
Samantha K. Bussan
samantha.bussan@wsu.edu

RECEIVED 09 February 2023
ACCEPTED 23 November 2023
PUBLISHED 12 December 2023

CITATION
Bussan SK and Schultz CB (2023) Can cattle grazing contribute to butterfly habitat? Using butterfly behavior as an index of habitat quality in an agroecosystem. *Front. Ecol. Evol.* 11:1162060. doi: 10.3389/fevo.2023.1162060

Can cattle grazing contribute to butterfly habitat? Using butterfly behavior as an index of habitat quality in an agroecosystem

Samantha K. Bussan* and Cheryl B. Schultz

School of Biological Sciences, Washington State University Vancouver, Vancouver, WA, United States

The provision of habitat on private agricultural land is vital to the persistence of native species. This study aimed to understand how cattle grazing management strategies influence butterfly behavior. We conducted behavioral experiments with two species of common native butterflies, silvery blue butterflies (*Glaucopsyche lygdamus columbia*) and ochre ringlet butterflies (*Coenonympha california eunomia*), at two native prairies with no grazing; two pastures grazed according to "conventional" management; and two pastures grazed according to "conservation" management. We mapped butterfly flight paths to quantify behavioral response to grazing management to test the hypothesis that butterflies have lower diffusion rates in ungrazed and conservation grazed prairies. We used turning angles, step lengths, and flight time to calculate diffusion rates for each individual. We assessed butterfly movement parameters as a function of sex, management type, and their interaction using generalized linear mixed models and used partial least squares regression to assess the effects of resource availability (host plant volume and nectar inflorescence count) on butterfly diffusion rates. We observed the highest silvery blue female step lengths and diffusion rates in conventional grazing and lowest in native prairie. There was moderate evidence that female silvery blue diffusion rates were higher in conservation grazing than native prairie. Neither silvery blue nor ochre ringlet males differed in their movement parameters between management types. Silvery blue diffusion rates were closely associated with their primary host plants (*Lupinus* spp.). We conclude that there is potential for conservation grazing to contribute to butterfly habitat in the landscape if grazing management practices focus on supporting a diverse plant community with host plants for focal species.

KEYWORDS

butterflies, cattle grazing, conservation, movement, diffusion rates, agriculture, prairie

Introduction

In December 2022, in the fifteenth meeting of the Conference of the Parties, the International Union for the Conservation of Nature called for at least 30% of global ecosystems to be conserved by 2030 (IUCN, 2022). However, protected conservation areas alone are inadequate to preserve native species due to poor management, lack of funding, spatial isolation, and containing only a small proportion of total biodiversity (Watson et al., 2014; Butchart et al., 2015; Kamal et al., 2015; Cortés Capano et al., 2019). The provision of habitat outside of reserves is vital, as approximately as only 21.7% of IUCN red list species have adequate protection in reserves worldwide (Maxwell et al., 2020).

The IUCN also called for the recognition of the importance of agriculture in contributions to biodiversity and habitat connectivity in the face of massive habitat loss and degradation (IUCN, 2022). For example, while the United States has lost 86% of its grassland habitat since European colonization due to fire suppression, urbanization, invasive species introduction, and conversion to agriculture (Samson et al., 2004), approximately 85% of remaining grassland and pastureland is privately owned (Bigelow and Borchers, 2017). In addition, 90% percent of listed species are found solely or in part on private land (Brook et al., 2003).

Livestock grazing occupies more than a third of all land in the US (Bigelow and Borchers, 2017), and it is often considered to be one of the largest threats to grassland habitat (Fleischner, 1994; Noss, 1994). Grazing can influence grassland systems in both positive and negative ways through herbivory, trampling, and defecation, depending how the livestock are managed (Fleischner, 1994). Grazing may cause water pollution, soil compaction, erosion, reduced plant and animal diversity, local extirpation of sensitive species, and invasion of nonnative species (Fleischner, 1994; Manley et al., 1997). Conversely, livestock grazing may also maintain plant community diversity, reduce competitive dominance of invasive grasses, lower vegetation height, and create heterogeneity on a pasture and regional scale, which benefits many different taxa (Weiss, 1999; Pöyry et al., 2004; Jerrentrup et al., 2014; Zakkak et al., 2014; Beck et al., 2015; WallisDeVries et al., 2016; Neilly et al., 2018; Davis et al., 2020). Studies attempting to quantify the effects of grazing on various taxa often use occupancy or abundance of the focal species as an indicator of habitat quality.

However, higher occupancy or abundance of the focal species is not always sufficient to indicate higher habitat preference or higher habitat quality (Van Horne, 1983; Bock and Jones, 2004), though it is a common assumption. Studies using movement or behavior data investigate habitat from the perspective of the focal species (Schultz et al., 2019). A review by Crone et al. (2019) of 78 studies across taxa (n = 18 vertebrates, 29 non-lepidopteran arthropods, 23 lepidopterans, and 8 "others") that categorized movement behavior paired with an independent measure of habitat quality, such as resource density or abundance, showed that animals reduce their rate of movement through high quality habitat compared to low quality. This is because animals exhibit "area restricted search" when encountering high quality habitat, meaning that they will either take shorter steps between turns or larger turning angles, or both (Kareiva and Odell, 1987; Korösi et al., 2008; Crone et al., 2019; Dorfman et al., 2022). This will effectively slow their rate of movement, or diffusion rate (Kuefler et al., 2010; Brown et al., 2017; Schultz et al., 2017; Pugesek and Crone, 2022). For example, Kuefler et al. (2012) showed that rotifer diffusion rates were lower with increased food availability and higher with increased rotifer population density. Many studies have quantified movement behavior to describe some aspect of habitat, land cover, or resources animals encounter (e.g. Revilla et al., 2004; Stevens et al., 2004; de Knegt et al., 2007; Kuefler et al., 2010; Lebeau et al., 2015; Murphy and Boone, 2022). An animal's movement parameters (i.e. step lengths, turning angles, and diffusion rates) in different habitats can indicate perceived habitat quality and provide more information about habitat and management effects on a species than abundance or occupancy estimates alone (Crone et al., 2019; Schultz et al., 2019).

Butterflies are good indicators of ecosystem health because they use different parts of the ecosystem in adult and larval stages and are sensitive to changes in their environment (Kerr et al., 2000). Prominent studies on the effects of grazing on grassland butterflies in Europe have found positive effects of low-intensity cattle grazing over large areas (often referred to as extensive grazing) for butterfly communities and species (Pöyry et al., 2004; Thomas et al., 2009; Jerrentrup et al., 2014; WallisDeVries et al., 2016; but see Kruess and Tscharntke, 2002). There is a consensus within the European literature that historical megafaunal grazing and current extensive cattle grazing has created shifting mosaics of semi-natural grassland. This shifting mosaic increases heterogeneity and niches available for butterflies on a pasture and regional scale, while preventing forest encroachment (Balmer and Erhardt, 2000; Nilsson et al., 2008; Konvička et al., 2021).

There is comparatively little research on butterfly responses to grazing in North America (Bussan, 2022). Most of the existing research is concentrated in the Midwest, with mixed results (Vogel et al., 2007; Debinski et al., 2011; Smith and Cherry, 2014; Delaney et al., 2016). In addition, the North American literature has less of a focus than the European literature on both satisfying conservation goals and providing a livelihood for the farmer or rancher (Bussan, 2022). The relative paucity of information on North American butterfly responses to grazing makes management recommendations and predictions difficult because differing evolutionary histories of grazing and grassland types mean that direct comparisons to European butterflies are not always appropriate.

We conducted an experiment using two common native butterfly species in western Washington prairies in a landscape with working cattle and dairy farms. We compared butterfly diffusion rates as an index of habitat quality in grazed pastures and in native prairie to determine factors that influence their movement through these environments. We quantified aspects of the habitat in which butterflies have lower diffusion rates, assuming that perceived higher quality habitat results in lower diffusion rates. Using butterfly movement parameters as an indicator, we tested how cattle grazing management influenced behavioral responses to grazed habitat. We expected that butterfly diffusion rates would be highest in conventional grazing, intermediate in conservation grazing, and lowest in native upland prairie. We also explored how nectar and host plants and vegetation structure are associated with butterfly movement, given different habitat management. We expected that butterfly diffusion rates would differ depending on the resources available to them along their flight paths. Diffusion rates would be lower when nectar and host plant density was high and diffusion rates would be higher when nectar and host plant density was low.

Materials and methods

Study area

Prior to European colonization, western Washington prairies covered approximately 72,843 ha, but 97% of the original extent of the prairies have been lost due to fire suppression, urbanization, and conversion to agriculture (Washington Department of Fish and Wildlife, 2022). Remnant prairies are restricted to a few isolated reserves, a military base (Joint Base Lewis-McChord), or located on working cattle ranches and dairy farms (Washington Department of Fish and Wildlife, 2022). Remnants are often heavily degraded and invaded by nonnative plants. Western Washington prairies are botanically distinct from other North American prairies, characterized by short bunchgrasses such as Roemer's fescue (*Festuca roemeri*) (Bowcutt and Hamman, 2016, pg. 21). First peoples, including Nisqually, Puyallup, Duwamish, Steilacoom, Squaxin Island, Chehalis, Clatsop, Cowlitz, Chinook, and many others maintained the prairies through cultural burning and traditional harvesting practices for thousands of years (Leopold and Boyd, 1999; Noland and Carver, 2011, pg. 2; Hamman et al., 2011; Velasco, 2021). Unlike the Great Plains and tallgrass prairies of the Midwest, bison (*Bison bison*) were historically not present in western Washington (Zontek, 2007, pg. 29); thus the prairies were primarily grazed by elk (*Cervus canidensis*) (Noland and Carver, 2011, pg. 2). The climate is Mediterranean, with warm, dry summers and wet, cool winters (Western Regional Climate Center, 2022).

Focal species and study sites

From April to September 2019, we observed two common native butterfly species in different grazing management treatments, which allowed us to account for differing habitat needs and phenology (Figure 1). The first species, the silvery blue butterfly (*Glaucopsyche* lygdamus; Doubleday, 1841; family Lycaenidae), is distributed throughout the western US (Pelham, 2021); we worked with the subspecies *G.l. columbia* (Skinner 1917). Their host species are members of the Fabaceae family, specifically lupines and vetches. The adults fly from the end of April to early June in Western Washington prairies (James and Nunnallee, 2011, pg. 206). The second species, ochre ringlet (*Coenonympha california*, Westwood 1851; family Nymphalidae) is also distributed throughout the western US (Pelham, 2021). Note that *C. california's* taxonomy was recently changed; it was formerly *C. tullia* (Zhang et al., 2020). We worked specifically with the subspecies *C.c. eunomia* (Dornfeld, 1967) which is concentrated mainly in the South Salish Sea region (also known as South Puget Sound). Ochre ringlets are hosted by various grass species and are bivoltine. The adults fly from early May to mid-July and from late July to September (James and Nunnallee, 2011, pg. 326).

We chose six sites in western Washington (Table 1; Figure 2). Four are part of the South Salish Sea prairie ecosystem in Thurston County (Colvin Ranch, Riverbend Ranch, Johnson Prairie, and West Rocky Prairie) and two are part of the Boistfort prairie ecosystem in Lewis County (Maynard and Mary Mallonee's farms). Four sites are grazed as part of active cattle and dairy farm operations. Of the grazed sites, two (Colvin Ranch and Mary Mallonee's farm) are managed according to conservation grazing strategies, which involve rotational grazing with a spring deferment period (Farruggia et al., 2012; Ravetto Enri et al., 2017). The other two (Riverbend Ranch and Maynard Mallonee's farm), are grazed according to conventional grazing strategies, i.e. continuous grazing with no spring deferment period. Rotational grazing is a grazing

FIGURE 1
Silvery blue butterfly male (left; photo by Samantha Bussan) and ochre ringlet male (right; photo by Christopher Jason). Female silvery blue butterflies are brown. Ochre ringlets do not exhibit sexual dimorphism.

TABLE 1 Sites and their abbreviations belonging to the three management categories.

Management Category	Sites	Management
Conventional Grazing	Maynard Mallonee's farm (MY)	Continuous grazing
	Riverbend Ranch (RB)	Continuous grazing
Conservation Grazing	Mary Mallonee's farm (MA)	Rotational grazing with spring rest
	Colvin Ranch (CO)	Rotational grazing with spring rest
Native Upland Prairie	Johnson Prairie (JP)	Invasive plant spot and broadcast treatment Prescribed fire (approx. 3–5 yr FRI) Native seeding
	West Rocky Prairie (WR)	Invasive plant spot and broadcast treatment Prescribed burning (approx. 3–5 yr FRI) Native seeding

system that moves livestock regularly between paddocks to allow the plant community to recover between grazing periods. Continuous grazing is a grazing system that allows the livestock to have access to the entire pasture throughout the season (Blanchet et al., 2000). Both Maynard and Mary Mallonee's farms fall under the umbrella of Mallonee Family Farms, which is a certified organic dairy farm, but they are located on different sites with different management regimes. The two native upland prairies (Johnson Prairie and West Rocky Prairie) are both part of the South Salish Sea prairie ecosystem and are managed with prescribed fire chemical and manual treatment of invasive plants, and native seeding. Johnson Prairie is located on Joint Base Lewis McChord and is owned by the US Department of Defense. West Rocky Prairie is owned by the Washington Department of Fish and Wildlife.

Butterfly behavior and movement observations

From April–September 2019, we quantified movement behavior at sites in different management categories (Table 1). As some of the grazed sites did not have native populations of the focal species, we collected individual butterflies from source sites (Supplementary Information) and transported them to the study site. Each individual was novel to the study site. To quantify movement behavior, following methods described by Schultz et al. (2012), we conducted observations by releasing an individual butterfly and following it for up to 15 distinct location points, for a maximum of 60 minutes. We chose the release point by haphazardly selecting a location within the study site as far from the site borders as possible, but within a resource patch if available. We recorded the individual's behavior and marked its location with a pin flag every 15 seconds. If the butterfly remained in the same location for more than one 15 second interval, we recorded the number of intervals at that location, and waited to place the pin flag until the butterfly had moved from the location. An individual was considered to have changed locations once it moved more than 30 centimeters from the previous point, which was based on the accuracy of our GPS units (15–25 cm). All observers remained at least three meters away from the butterfly for silvery blues and four meters away for the more skittish ochre ringlets. After observation, each point on the individual's flight path was recorded with a decimeter accuracy GPS unit (Thales ProMark^{TM} 3 April through August 1st, Trimble© Geo 7X August 1st through the end of the season; decimeter accuracy was achieved in post processing of the GPS data).

Behavior types recorded included flying, sitting, basking, nectaring, ovipositing, plant walking, mud puddling, walking (on the ground), and mating (Schultz, 1998; Sei, 2009). Some behaviors are sex-specific; only males exhibit mud puddling behavior, and only females oviposit and plant walk. We recorded which plant species individuals chose for nectaring, ovipositing, or plant walking. We randomly selected four location points along each path using the app *Random UX* to measure habitat characteristics (host plants, nectar plants, and vegetation height). Within a meter's radius of each point, we measured host plant volume (approximate width × length × height of each host plant "patch" within the plot) and counted flowering plant inflorescences. Following standard protocol for obtaining reliable measures of forage production, we used a Robel pole (Robel et al., 1970) placed on the center point to obtain an index of vegetation height. A Robel pole has alternating numbered bands for estimating the height of visual obstruction by the vegetation. A higher band number indicated taller vegetation.

Data analysis

We modeled butterfly movement as correlated random walks following the methods in Kareiva and Shigesada (1983) and Turchin (2015). We included only successful flight paths in our calculations; a flight path was considered successful if we recorded at least four distinct consecutive location points and we did not detect effects of the observer's presence on butterfly behavior (e.g. angling their bodies to "hide" from the observer or evasive flight patterns). To analyze our flight path data, we first calculated the step lengths (distance between points within a path) and turning angles (deviation from the straight line) from our GPS data. Butterfly behavior can differ within habitat as compared to habitat boundaries; previous studies have shown that this change occurs within approximately 10–15 meters of habitat boundaries (Haddad, 1999; Schultz and Crone, 2001; Ross et al., 2005; Crone and Schultz, 2008; Schultz et al., 2012). As our goal was to assess within-habitat (i.e. within site or management type) behavior, we used ArcMap Pro version 2.9 to create a 10 m buffer around all pasture and prairie boundaries. All points and their associated step lengths and turning angles that intersected the buffer were removed from the following calculations, while retaining the path. We calculated the within-habitat expected net squared displacement and the diffusion rate (Supplementary Information) of each observed individual (Kareiva and Shigesada, 1983; Turchin, 2015, pgs. 102, 139). Diffusion rate is a metric that is derived from step lengths, turning angles, and time in flight (Turchin, 2015, pgs. 102, 139); it is important to consider both

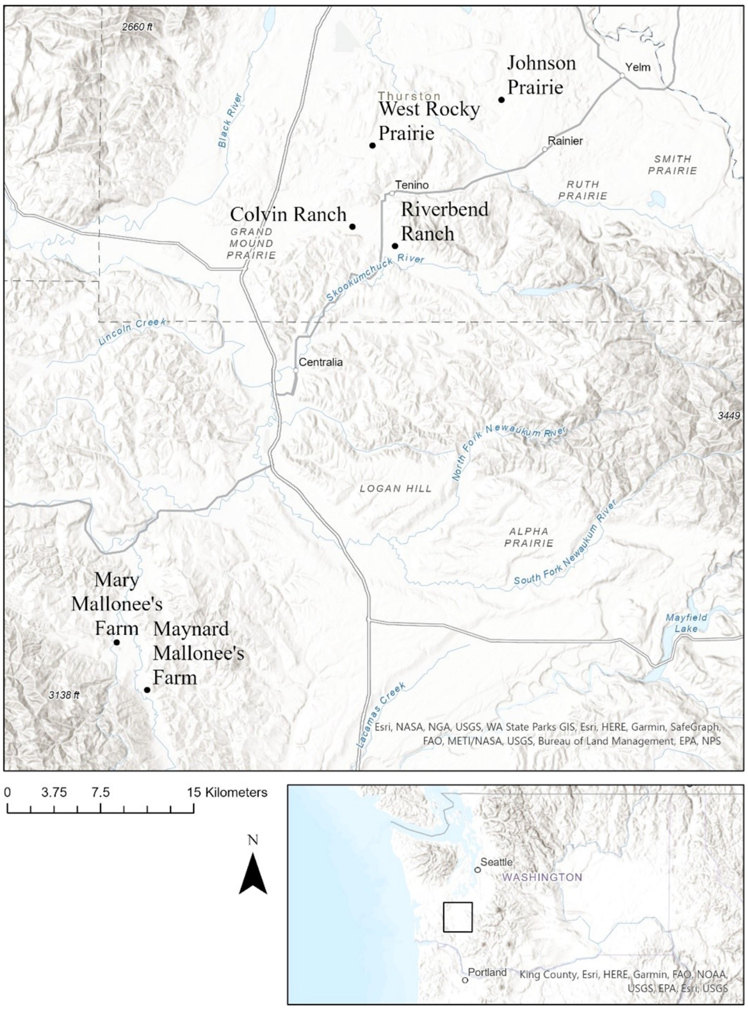


FIGURE 2
Map of study sites, located in western Washington State.

the direct measures of movement behavior and the diffusion coefficient because they may show different patterns of movement responses (e.g. Pugesek and Crone, 2022).

Effect of grazing management on butterfly movement behavior

We used similar methods for silvery blues and ochre ringlets. We fitted linear mixed models and generalized linear mixed models to examine differences in movement parameters by management type and sex. Preliminary data exploration showed potential side effects on silvery blue movement parameters. We log transformed move lengths to approximate normality and then used a linear mixed model (LMM) to evaluate them in relation to management type, sex, and an interaction between sex and management type. We also included a random effect of individual to account for pseudoreplication associated with repeated measurements of the same individual and a random effect of site. To assess turning angles as a measure of the tortuosity of the observation path (Turchin, 2015), we first calculated the cosine of the turning angles, which allowed us to assess how often individuals changed direction. More frequent direction reversals result in higher tortuosity (Turchin, 2015). Typical LMMs cannot be used with circular data (Schultz et al., 2012), so we scaled the cosine turning angles between zero (representing a 180° reversal in movement direction) and one (representing a completely straight line in movement direction) and logit transformed the resulting scaled cosines to approximate normality (Brown et al., 2017; Warchola et al., 2017). We used an LMM to assess the logit transformed scaled cosine of the turning angles in relation to management type, sex, and an interaction between sex and management type (Brown et al., 2017; Warchola et al., 2017). As above, we included random effects of individual and site. The step length and turning angle LMMs were fitted using restricted maximum likelihood (REML).

To evaluate diffusion rates, we used a generalized linear mixed model (GLMM), with diffusion rates as the response variable; sex, management type, and an interaction between sex and management type as fixed effects; and site as a random effect. We did not need to include a random effect of individual as diffusion rate is a path-level measurement and there was only one measurement of diffusion rate per individual. We used a gamma distribution with a log link for the response variable because the diffusion rates are bound by zero and infinity but have a greater mass towards zero. To assess whether there were differences in step lengths, turning angles, and diffusion rates across management types and between sexes, we compared estimated marginal means (Searle et al., 1980).

We followed similar procedures for ochre ringlet analysis. However, we were unable to include ochre ringlet females in the analysis due to low sample size per management type and site (Table 2). Therefore, the models used a similar framework to the silvery blues but used only male data and excluded female data. In addition, though ochre ringlets are bivoltine, we did not account for the two flights in the analysis due to low sample sizes in the second flight (Table 2).

Results are reported according to the framework in Muff et al. (2022), which describes relationships as having strong evidence, moderate evidence, or weak evidence according to the range of p-values as a "gradual language of evidence" rather than using an arbitrary cutoff value for statistical significance. In accordance with Muff et al. (2022), we report p-values between approximately 1 and 0.1 as having little or no evidence; p-values between 0.1 and 0.05 as weak evidence, between 0.05 and 0.01 as moderate evidence, between 0.01 and 0.001 as strong evidence, and <0.001 as very strong evidence.

Resource impacts on butterfly movement behavior

To assess the effects of habitat characteristics on butterfly movement behavior, we used partial least squares regression (PLSR; Wold, 1975). PLSR extracts latent factors that best explain, or maximize, the covariance between the explanatory and response variables (Chong and Jun, 2005; Carrascal et al., 2009). PLSR is more appropriate than traditional methods such as multiple regression or principal components analysis to assess multiple correlated explanatory variables in relation to the response variable (Chong and Jun, 2005; Carrascal et al., 2009; Scott and Crone, 2021). Several of our explanatory variables were correlated; *Vicia sativa* and *Lupinus* spp. can be both host and nectar plants for silvery blues, so host volume measurements and nectar inflorescence counts are correlated for those species.

For silvery blue butterflies, we fitted a PLSR model with diffusion rates as the response variable and potential nectar species, potential host species, site, and sex as explanatory variables. We selected potential nectar and host species for inclusion in the model based on whether we observed individual butterflies attempt nectaring or oviposition on that species at least once during the 2019 flight period. Of the three sites on which large perennial lupines were present, *Lupinus oreganus* was found only on one site (Mary Mallonee's farm), while a related species, *L. albicaulis*, was found on both other sites. To account for this, we combined *L. oreganus* and *L. albicaulis* nectar counts and host volume measurements to model them as *Lupinus* spp. nectar and *Lupinus* spp. host volume. PLSR centers the data as part of the algorithm (Mevik and Wehrens, 2007). The predictor variables were scaled by dividing each variable by its standard deviation. We validated the model using leave-one-out (LOO) cross validation. We assessed statistical significance of the components based on the minimum value of the root mean square error in the projection (RMSEP), and on the percentage of variation in the diffusion rates explained by each component (Supplementary Information) (Chong and Jun, 2005; Carrascal et al., 2009).

We followed the same approach for ochre ringlets, with the following differences. First, instead of using individual grass species for host availability, we used the median Robel index number per path as a proxy for vegetation height and therefore amount of grass available. We were able to include female ringlet paths for this analysis, but we were unable to account for site due to the limited number of female paths on some sites (Table 2).

We assessed the effects of our predictor variables through two complementary methods: variable importance in the projection (VIP) and the regression coefficients (Chong and Jun, 2005). VIP qualitatively assesses the importance of each variable using the vector of loading weights on the components (Mehmood et al., 2012). Generally, a variable is considered important if the VIP value is above 1, though values above 0.8 may be marginally important (Mehmood et al., 2012).

TABLE 2 The total number of successful observations and steps by species and sex per site.

		Ochre ringlets				Silvery blue butterflies	
		Female		Male			
Management Type	Site	Early	Late	Early	Late	Female	Male
Conventional Grazing	Riverbend Ranch	2 (23)	1 (7)	4 (25)	1 (13)	6 (30)	3 (21)
	Maynard Mallonee's Farm	1 (13)	2 (19)	5 (63)	0 (0)	5 (52)	4 (45)
Conservation Grazing	Mary Mallonee's Farm	1 (13)	1 (4)	1 (9)	3 (22)	5 (51)	5 (34)
	Colvin Ranch	1 (6)	1 (5)	5 (55)	3 (18)	6 (31)	5 (43)
Native Upland Prairie	Johnson Prairie	2 (10)	1 (11)	5 (67)	4 (29)	6 (31)	5 (61)
	West Rocky Prairie	3 (29)	2 (13)	4 (43)	3 (23)	5 (56)	5 (48)
	Total per sex per species	10 (94)	7 (59)	24 (262)	14 (105)	33 (251)	27 (252)

Number of individuals are listed in regular text and total steps across individuals in parentheses. Steps are summed across individuals within the grouping and do not include any steps within the buffer zone. Ochre ringlets are further separated by the flight period in which the observation was collected. The early flight ran May–July and the late flight ran July–September. Silvery blue butterflies have only one flight period (May), so the data are not separated by flight period.

We used the regression coefficients to interpret the direction and magnitude of the effect of the predictor variables on butterfly diffusion rates.

Package list

All analyses were completed in *R* version 4.2.1 (R Core Team 2022). We used the following packages: *moveHMM* version 1.9 (Michelot et al., 2016) for calculating move lengths and turning angles; *lme4* version 1.1-30 (Bates et al., 2015) for LMMs and GLMMs; *emmeans* version 1.8.1-1 (Lenth, 2022) for estimated marginal means; *DHARMa* version 0.4.6 (Hartig, 2022) for model diagnostics; *pls* version 2.8-1 (Liland et al., 2022) and *plsVarSel* version 0.9.8 (Mehmood et al., 2012) for partial least squares regression; and *Tidyverse* 1.3.2 (Wickham et al., 2019) for data processing and figure generation.

Results

We obtained 116 successful butterfly flight paths throughout the season, with 61 silvery blue and 55 ochre ringlet observations (Table 2). Our initial tally included 124 flight paths, but we excluded five male ochre ringlet paths where the GPS failed to record data; two male silvery blue paths that contained too many points in the border buffer zone to allow for diffusion rate calculations; and one silvery blue female whose field notes indicated that we had affected her behavior. Our observations lasted an average of 16.0 minutes for silvery blue females (ranged 1 min to 60 mins) and 14.2 minutes for silvery blue males (range: 1–58 min). We observed ochre ringlet females for an average of 36.8 minutes (range: 2.5–60 mins) and males for 12.4 minutes (range: 1.3–60 mins). Across all sites and management types, we calculated a median diffusion rate of 3.7 m^2/s (range: 0.4–17.7 m^2/s) for silvery blue males and 2.3 m^2/s (range: 0.1–25.6 m^2/s) for silvery blue females. We calculated a median diffusion rate of 3.9 m^2/s (range: 0.2–32.8 m^2/s) for ochre ringlet males and 2.4 m^2/s (range: 0.2–14.4 m^2/s) for ochre ringlet females.

Effect of grazing management on butterfly movement behavior

Silvery blue butterflies

Males and females differed in their responses to management types in terms of step lengths and diffusion rates (Table 3; Figures 3A, C; Supplementary Information Table 1). There was

TABLE 3 Results from silvery blue estimated marginal means comparison of step lengths, turning angles, and diffusion rates.

Movement Parameter	Contrast	Females					Males				
		Est.	SE	d.f.	t ratio	*p*	Est.	SE	d.f.	t ratio	*p*
Step lengths	Native Prairie/ Conventional gr.	**−1.269**	**0.446**	**4.12**	**−2.844**	**0.045 (Mod.)**	−0.219	0.460	4.62	−0.476	0.656
	Conservation gr./ Conventional gr.	**−0.972**	**0.446**	**4.12**	**−2.179**	**0.091 (Weak)**	0.097	0.466	4.86	0.208	0.843
	Conservation gr./ Native Prairie	0.297	0.446	4.12	0.666	0.541	0.316	0.448	4.17	0.706	0.518
		Est.	SE	d.f.	t ratio	*p*	Est.	SE	d.f.	t ratio	*p*
Cos turning angles	Native Prairie/ Conventional gr.	−0.393	0.727	7.16	−0.540	0.605	−1.359	0.767	8.27	−1.773	0.113
	Conservation gr./ Conventional gr.	−0.18	0.726	7.16	−0.248	0.811	−1.228	0.801	9.75	−1.534	0.157
	Conservation gr./ Native Prairie	0.213	0.732	7.33	0.290	0.780	0.131	0.726	7.16	0.181	0.862
		Ratio	SE	d.f.	Z ratio	*p*	Ratio	SE	d.f.	Z ratio	*p*
Diffusion rates	Native Prairie/ Conventional gr.	**0.224**	**0.104**	-	**−3.223**	**0.001 (Strong)**	0.936	0.478	–	−0.130	0.897
	Conservation gr./ Conventional gr.	0.588	0.276	–	−1.131	0.258	1.088	0.574	–	0.160	0.873
	Conservation gr./ Native Prairie	**2.622**	**1.26**	-	**2.005**	**0.045 (Mod.)**	1.163	0.560	–	0.313	0.754

P values are labeled with their corresponding evidence level according to Muff et al. (2022). If no level is labeled, there was no evidence of a difference between the categories. Est. refers to the estimate of the difference in means between the management types. The estimate is on the scale of the transformed data (log step lengths and logit of the scaled cosine turning angles). For GLMMs, the model computes the ratio of the comparison, instead of the difference between the categories. SE refers to standard error of the mean, and d.f. refers to degrees of freedom. Step length and turning angle degrees of freedom were calculated with the Kenward-Roger method. We do not provide d.f. for diffusion rates due to the difficulty of estimating d.f. in GLMMs with small sample sizes in R (Bolker et al., 2009). Bold text indicates at least weak levels of evidence.

strong evidence that female diffusion rates were lower in native prairie than conventional grazing, moderate evidence that diffusion rates were lower in native prairie than in conservation grazing, and no evidence that diffusion rates differed between conservation and conventional grazing (Table 3; Figure 3C). There was moderate evidence that females took shorter steps in native upland prairie than conventional grazing, while there was weak evidence that their step lengths were shorter in conservation grazing than conventional grazing and no evidence that their step lengths differed between conservation grazing and native upland prairie (Table 3; Figure 3A). Males did not differ in their move lengths or diffusion rates between management types (Figures 3A, C). We did not observe differences between sex or management type in terms of the logit transformed cosine turning angles (Table 3; Figure 3B).

Ochre ringlets

There was no evidence of an effect of management type on ochre ringlet male step lengths, cosine turning angles, or diffusion rates (Table 4; Figures 4A–C; Supplementary Information Table 2). Their behavior was similar across all management types.

Resource impacts on butterfly movement behavior

Silvery blue butterflies

We observed 40 flower species in bloom across the six sites throughout the silvery blue butterfly flight season (Supplementary Information Table 3). We observed silvery blues nectaring or attempting to nectar on thirteen species (Table 5; Figure 5A). We observed oviposition and plant walking behavior from a total of 11 individuals. Three females showed oviposition and plant walking behavior at Johnson Prairie; three females oviposited and plant walked and an additional two individuals plant walked at Mary Mallonee's farm; and one individual oviposited, one oviposited and plant walked, and one individual plant walked at West Rocky Prairie. Host plant availability differed greatly across the six sites (Figure 5B). Though *Vicia sativa* is a potential host plant, all oviposition and plant walking behavior during our observations occurred on large perennial *Lupinus* spp. (*L. oreganus* and *L. albicaulis*). We saw only one instance of an individual exhibiting plant walking behavior on *V. sativa*, at Riverbend Ranch, which

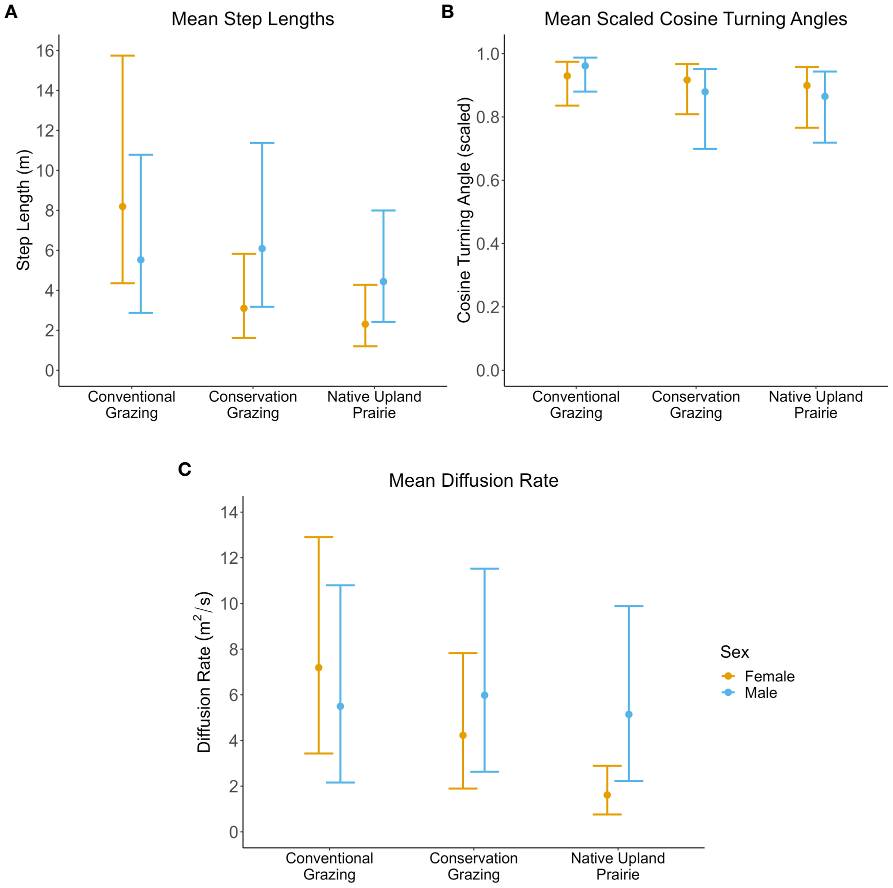


FIGURE 3
Silvery blue movement parameters by management type and sex. All error bars represent bootstrapped 95% prediction intervals. Female movement parameters are in yellow and male movement parameters are in blue. **(A)** Mean step length in meters. **(B)** Mean scaled cosine of the turning angles. Cosine turning angles were scaled between 0 (180° reversal) and 1 (straight line). **(C)** Mean diffusion rate in meters squared/second.

TABLE 4 Results from ochre ringlet male estimated marginal means comparison of step lengths, turning angles, and diffusion rates.

Movement Parameter	Contrast					
		Est.	SE	d.f.	t ratio	*p*
Step lengths	Native Prairie/Conventional gr.	0.135	0.416	2.91	0.184	0.865
	Conservation gr./Conventional gr.	0.079	0.431	3.24	0.184	0.865
	Conservation gr./Native Prairie	−0.056	0.414	2.76	−0.136	0.902
		Est.	SE	d.f.	t ratio	*p*
Cos turning angles	Native Prairie/Conventional gr.	0.304	0.577	2.78	0.526	0.638
	Conservation gr./Conventional gr.	−0.630	0.643	3.43	−0.980	0.391
	Conservation gr./Native Prairie	−0.933	0.566	2.09	−1.650	0.235
		Ratio	SE	d.f.	Z ratio	*p*
Diffusion rates	Native Prairie/Conventional gr.	1.269	0.599	–	0.505	0.614
	Conservation gr./Conventional gr.	1.141	0.618	–	0.244	0.807
	Conservation gr./Native Prairie	0.899	0.497	–	−0.192	0.848

Est. refers to the estimate of the difference in means between the management types. The estimate is on the scale of the transformed data (log step lengths and logit of the scaled cosine turning angles). SE refers to standard error of the mean, and d.f. refers to degrees of freedom. Step length and turning angle degrees of freedom were calculated with the Kenward-Roger method. We do not provide d.f. for diffusion rates due to the difficulty of estimating d.f. in GLMMs with small sample sizes in R (Bolker et al., 2009).

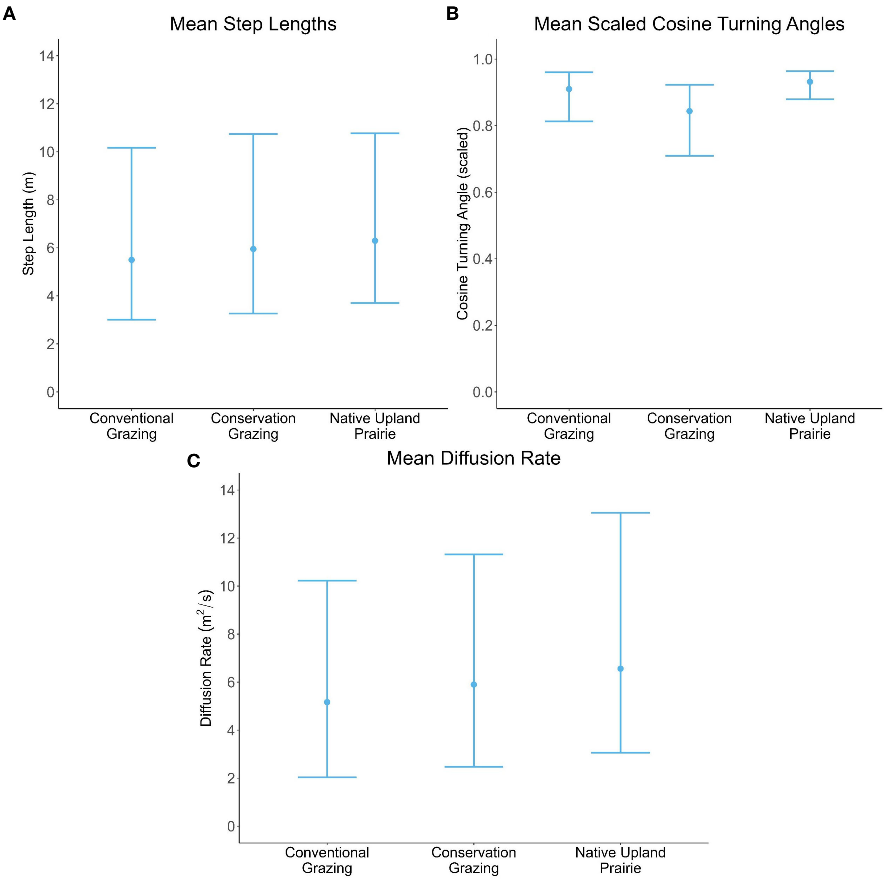


FIGURE 4
Male ochre ringlet movement parameters by management type. All error bars represent bootstrapped 95% prediction intervals. **(A)** Mean step length in meters. **(B)** Mean cosine of the turning angles. Cosine turning angles were scaled between 0 (180° reversal) and 1 (straight line). **(C)** Mean diffusion rate in meters squared/second.

TABLE 5 Silvery blue nectaring observations, time spent, and percent time nectaring on each plant species.

Nectar species	Number of nectaring observations	Time spent nectaring (minutes)	Percent time (%)
Camassia quamash	176	44.0	50.87
Cerastium arvense	2	0.5	0.58
Collinsia parviflora	3	0.75	0.87
Geramium molle	4	1.0	0.58
Geranium dissectum	1	0.25	0.87
Leucanthemum vulgare	43	10.75	12.43
Lupinus albicaulis	9	2.25	2.60
Myosotis discolor	5	1.25	1.45
Microsteris gracilis	1	0.25	0.29
Trifolium repens	39	9.75	11.27
Trifolium subterraneum	2	0.5	0.58
Vicia sativa	40	10.0	11.56
Viola adunca	18	4.5	5.20
Total	343	85.75	100

A nectaring observation was defined as an attempt at probing the flower with the proboscis at the 15 second interval. The entire interval was assigned to that plant species.

occurred after the observation had already ended. The individual left the plant without laying an egg.

Our silvery blue PLSR results and model validation (Supplementary Information) indicated that the first two components were significant and cumulatively accounted for 54.25% of the variation in silvery blue diffusion rates (R^2; 46.65% and 7.60% respectively). Our VIP results and regression coefficients indicated that the sites Riverbend Ranch (conventional grazing) and West Rocky Prairie (native upland prairie) had strong but opposite effects on silvery blue diffusion rates; diffusion rates were higher at Riverbend than West Rocky Prairie (Figures 6A–C). Riverbend had the highest VIP value and largest regression coefficients in the model (Figures 6A–C). *Lupinus* spp. volume and nectar count as well as *V. sativa* nectar count also had high VIP values and were associated with lower diffusion rates (Figures 6A–C). *V. sativa* host volume and most nectar species had little influence on silvery blue diffusion rates, with a few exceptions. *C. parviflora* and *L. vulgare* nectar counts both had high VIP values and were associated with higher diffusion rates (Figures 6A–C). Despite 50% of silvery blue nectaring behavior taking place on *Camassia quamash* (Table 5), *C. quamash* had little effect on diffusion rates (Figure 6C). *V. sativa* nectar and host measurements were less closely associated with each other than *Lupinus* spp. nectar and host measurements were (Figure 6D). Both components were loosely associated with a gradient of diffusion rates. *Lupinus* spp. host and *Lupinus* spp. nectar were associated with lower diffusion rates on both components. Variables such as site (Riverbend), which is a conventional grazing site, and *L. vulgare* were associated with higher diffusion rates on Component 1. On Component 2, site (Maynard Mallonee's farm), which is the other conventional grazing site, was associated with higher diffusion rates (Figure 6D).

Ochre ringlets

We observed 37 flower species in bloom across both ochre ringlet flight seasons (Supplementary Information Table 4). We observed ochre ringlets nectaring or attempting to nectar on 11 different species throughout both seasons (Table 6; Figure 7). *L. vulgare* and *Daucus carota* were the two most used nectar species (Table 6). We observed three females exhibiting oviposition behavior, one of which was on *Carex inops*, and the other two on thatch. We also observed plant walking behavior from four other individuals, all of which was spent crawling around in thatch near the base of plants. Ringlet female oviposition and plant walking behavior occurred in all three management types.

The ochre ringlet PLSR model was unstable because there was too little variation in the model; we were unable to use LOO to validate our model. Therefore we are unable to report the results of the ochre ringlet PLSR.

Discussion

Animal movement behavior has been used in many contexts to evaluate aspects of habitat or habitat quality (e.g. Dumont et al., 2007; Korösi et al., 2008; Dias et al., 2009; Dodge et al., 2014; Crone et al., 2019; Murphy and Boone, 2022; Pugesek and Crone, 2022); however, studies of butterfly movement have rarely been applied to evaluate habitat under grazing management (Bussan, 2022; but see Schtickzelle et al., 2007; Ehl et al., 2019). We quantified within-habitat diffusion rates to understand butterfly perceptions of habitat quality in conventional grazing, conservation grazing, and native upland prairie. We conclude that there is potential for conservation grazing to contribute to habitat in the landscape for native butterflies as long as pastures are managed to encourage diverse plant communities and important resources for focal species.

Effect of grazing management on butterfly movement behavior

Animals take shorter steps, larger turning angles, and have smaller diffusion rates in habitats that they perceive as high quality (Crone et al., 2019). This pattern has frequently been observed in butterflies; many studies have found evidence that butterflies have higher diffusion rates (Schultz, 1998; Fownes and Roland, 2002; Ovaskainen, 2004; Ross et al., 2005; Brown et al., 2017; Schultz et al., 2017; Warchola et al.,

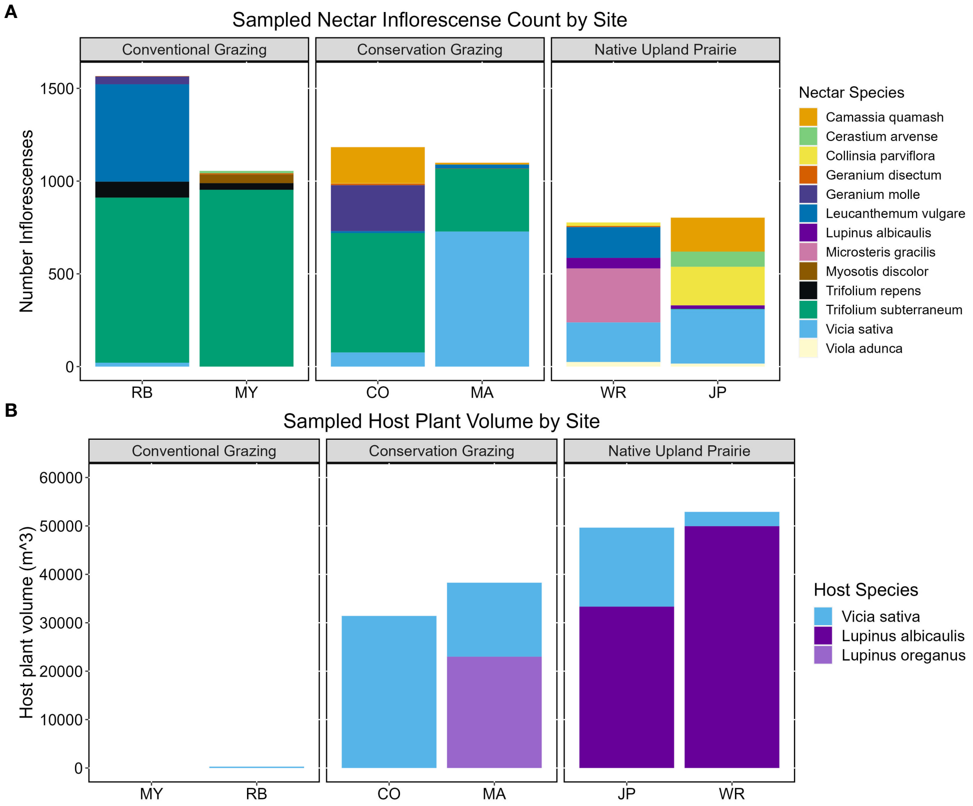


FIGURE 5
(A) Sampled silvery blue nectar species inflorescence count summed by site over the flight season. Nectar inflorescences were sampled on a path scale. **(B)** Sampled silvery blue host volume measurements summed by site in m^3. Host volume was sampled on a path scale.

2017), longer steps (Stanton, 1982; Fownes and Roland, 2002; Ross et al., 2005; Brown et al., 2017; Schultz et al., 2017) and smaller turning angles in matrix or lower quality habitats than in high quality habitats (Brown et al., 2017; Schultz et al., 2017; Warchola et al., 2017). Our observed median diffusion rates for both species within habitat types were comparable to other similar studies. For example, Schultz et al. (2017) observed lycaenids of similar size to silvery blues (25–30 mm wingspan) to have diffusion rates approximately 0.1–6.9 m^2/s in field margins with good nectar resources, 0.1–2.6 m^2/s in seminatural habitats, and 1.4–15.1 m^2/s in agricultural fields. The authors observed slightly higher diffusion rates for nymphalids than we observed for ochre ringlets, but the nymphalids in their study were slightly larger (> 40 mm wingspan compared to 35 mm ochre ringlets) and wing span is strongly correlated with diffusion rate (Schultz et al., 2017). They observed nymphalid species to have median diffusion rates approximately 6.7–48.3 m^2/s in field margins, 1.5–14.3 m^2/s in seminatural grasslands, and 2.77–12.95 m^2/s in agricultural fields.

In our study, male and female silvery blues differed in their responses to management type; female diffusion rates and step lengths differed among management types, while males did not differ in any movement parameter between management types. Female silvery blue turning angles did not differ among habitat types, though they still exhibited area restricted search behavior through reduced step lengths and lower diffusion rates in native upland prairie (Dorfman et al., 2022). Female diffusion rates and step lengths were highest in conventional grazing and lowest in native upland prairie, suggesting that females exhibited area restricted search more often in native upland prairie (Dorfman et al., 2022; Pugesek and Crone, 2022) and perceived prairie as higher quality habitat than conventionally grazed pastures (Crone et al., 2019). This is likely because of the higher host plant availability in native prairie than conventional grazing (see *Discussion: Resource impacts on butterfly movement behavior* below). Many other studies have found that female butterflies have lower diffusion rates and step lengths in high quality habitat, which is often characterized by high host plant availability (Root and Kareiva, 1984; Schultz, 1998; Schultz et al., 2012; Brown et al., 2017; Warchola et al., 2017).

Female diffusion rates were higher in conservation grazing than native upland prairie, while female step lengths were lower in conservation grazing than conventional grazing. These results suggest that female silvery blues perceived the conservation grazing sites as intermediate quality between conventional grazing and native upland prairie (Crone et al., 2019; Schultz et al., 2019). In highly fragmented, human dominated landscapes, it may not be possible to provide enough high quality habitat on reserves to maintain species populations. Other studies have shown that intermediate quality habitat can still contribute to resource availability and increase animal populations in the landscape. For example, Kahara et al. (2022) found that each 100 ha increase in intermediate quality habitat over poor quality in California's Central Valley resulted in 86 more mallards (*Anas platyrhynchos*)

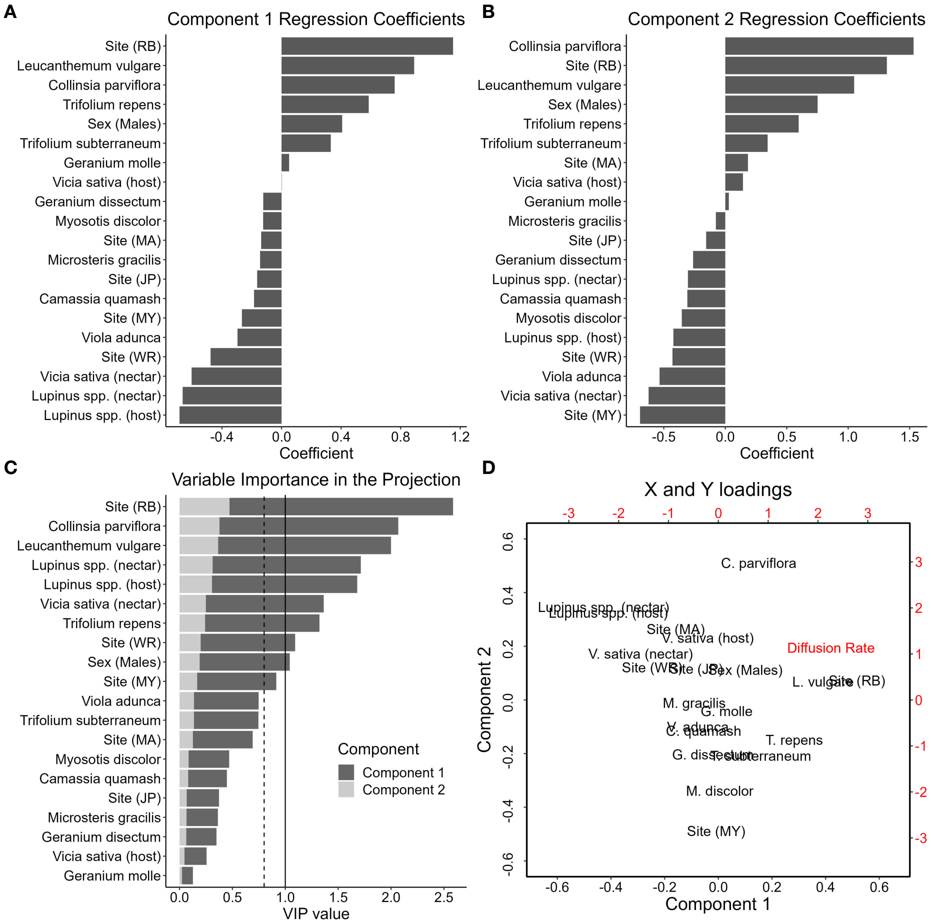


FIGURE 6
Results from the silvery blue PLSR model. **(A)** Regression coefficients from Component 1. **(B)** Regression coefficients from Component 2. **(C)** VIP loadings on Component 1 and Component 2. The solid line represents a VIP value of 1 (an important variable in the projection). The dotted line represents a VIP value of 0.8 (marginally important variable in the projection). **(D)** Biplot of predictor variable (X) and response variable (Y) loadings. X loadings are in black text and Y loadings are in red text.

using the habitat. In addition, models accounting for the presence of intermediate habitat in addition to low and high quality performed better than models accounting for only low and high quality (Kahara et al., 2022). Our results provide support for the importance of intermediate quality habitat and the importance of providing this outside of reserves; female silvery blues still oviposited and took shorter step lengths in conservation grazing than conventional grazing, though this was heavily dependent on the resources present (see *Resource impacts on butterfly movement behavior*).

Both silvery blue males and ochre ringlet males showed little difference in any movement parameter between management types, which is unsurprising. Male butterflies are often less sensitive to habitat than females because of differing behavioral traits (Fischer et al., 1999). Males tend to focus on finding mates, while female butterflies must oviposit and are therefore usually more closely associated with their host plants (Rusterholz and Erhardt, 2000; Fischer and Fiedler, 2001; Schultz et al., 2012). We were unable to obtain enough female ochre ringlet observations to include them in the grazing management analysis, but their median diffusion rates across all sites were also lower than the median male diffusion rates. It is likely that if ochre ringlets do respond in terms of their movement behavior to differences in habitat quality between management types, the response would be driven by the females (Rusterholz and Erhardt, 2000; Fischer and Fiedler, 2001; Sei, 2009).

We tested butterfly diffusion rates under different types of grazing management. Rotational grazing is frequently recommended as a way to improve habitat heterogeneity and therefore butterfly community diversity in the landscape (e.g. Balmer and Erhardt, 2000; Pöyry et al., 2004; WallisDeVries et al., 2016), yet little attention has been paid in the literature to quantitatively testing the effects of continuous and rotational grazing on butterfly populations or communities (Bussan, 2022). Ravetto Enri et al. (2017) found "biodiversity-friendly" cattle grazing systems, or rotational grazing with a rest period for one paddock during the main flowering period, to have positive effects on butterfly abundance, richness, and flower cover. However, an important confounding factor is stocking rate. Farruggia et al. (2012) compared rotational grazing with a rest period for one paddock to continuous grazing at low and high stocking rates. At low stocking rates, there was little difference in butterfly species richness or abundance between management types, but at high

TABLE 6 Ochre ringlet nectaring observations, time spent, and percent time nectaring by plant species.

Nectar species	Number of nectaring observations	Time spent nectaring (minutes)	Percent time (%)
Achillea millefolium	8	2.0	0.76
Capsella bursa-pastoris	1	0.25	0.10
Crepis capillaris	1	0.25	0.10
Cytisus scoparius	5	1.25	0.48
Daucus carota	364	91.00	35.88
Hypochaeris radicata	13	3.25	1.28
Leucanthemum vulgare	507	126.75	50.00
Lomatium triternatum	47	11.75	4.49
Ranunculus occidentalis	27	6.75	2.58
Solidago spp.	23	5.75	2.20
Trifolium repens	16	4.0	1.53
Total time	1013	253.25	100

A nectaring observation was defined as an attempt at probing the flower with the proboscis at the 15 second interval. The entire interval was assigned to that plant species.

stocking rates, richness and abundance was higher in rotational grazing than continuous. In our study, there was some evidence that silvery blue females did perceive rotational grazing with a spring rest period (conservation grazing) as higher quality than conventional grazing.

Both silvery blue butterflies and ochre ringlets are common, relatively generalist species. As a result, they may not react as strongly to differences between sites and management types as a more sensitive species (Murphy et al., 2011; Henry et al., 2019). Even our "conventional" grazing pastures did not cover the full range of grazing practices in the region, as producers willing to allow access to their land for butterfly experiments were already interested in conservation. Two grazed sites in the experiment were certified organic farms (Mary and Maynard Mallonee's farms), and the other two, regardless of grazing strategy, use little to no pesticides, which is not the case at all farms and ranches in the region. All four grazed sites maintain a minimum stubble height of three inches (7.6 cm) in accordance with accepted sustainable grazing practices for the region (Fransen et al., 2017). It is possible that we may have seen more of a contrast in movement rates if we had access to pastures that were grazed more heavily or used pesticides.

Resource impacts on butterfly movement behavior

The impact of cattle grazing on butterflies is mediated by grazing effects on resources (host plants and nectar plants) (Kruess and Tscharntke, 2002; Schtickzelle et al., 2007; van Klink et al., 2015). Consistent with many previous studies on animal movement (e.g. Zalucki and Kitching, 1982; Kuefler and Haddad, 2006; Dumont et al., 2007; Dias et al., 2009; Avgar et al., 2013; Dodge et al., 2014; Murphy and Boone, 2022), we observed strong effects of resource availability on the path scale on butterfly diffusion rates. Here we discuss mainly silvery blue diffusion rate responses to resources, since we were unable to include the results of the ochre ringlet PLSR model due to poor model fit.

Host plant availability had strong effects on silvery blue diffusion rates. As in previous studies on lycaenid movement (e.g. Schultz, 1998; Schultz and Crone, 2001; Schultz et al., 2012; Warchola et al., 2017), silvery blue female diffusion rates were strongly associated with their host plants. Greater availability of their preferred host plants (large perennial *Lupinus* spp.) on a flight path resulted in slower silvery blue diffusion rates, indicating that when host plants are present, individuals perceive habitat as higher value (i.e. resource-rich). Despite being hosted by a variety of Fabaceae species throughout the Pacific Northwest (Pyle and LaBar, 2018, pg. 235), silvery blue females only oviposited on large *Lupinus* spp. in our observations. This clear preference for *Lupinus* spp. was reflected in the PLSR model, as *V. sativa* host measurements had a low VIP value, while *Lupinus* spp. host measurements had a high VIP value and were associated with lower diffusion rates. Both native upland prairie sites (lowest diffusion rates and step lengths) and one conservation grazing site (Mary Mallonee's farm) had large perennial *Lupinus* spp. present, while both conventional grazing sites (highest diffusion rates and step lengths) and the other conservation grazing site (Colvin Ranch) did not. We observed oviposition and plant walking behavior by silvery blue females on both the native prairies and on Mary Mallonee's farm, but not on the other sites. This suggests that management for butterflies under cattle grazing should focus on improving plant community diversity to provide host plants for a variety of species, as well as adding specific host plants for focal species.

Lupinus spp. plants are often considered to be undesirable by farmers and ranchers as they are toxic to cattle and may cause pregnant cows to abort their fetuses (Panter et al., 2002). However, cattle generally will not eat *Lupinus* spp. unless their other forage has been depleted (Lopez-Ortiz et al., 2007), and we observed cattle to remove individual grass leaves from under *Lupinus* plants without touching the plant itself (Bussan, pers. observation).

Adult butterflies are usually nectar generalists (Graves and Shapiro, 2003), though they may still exhibit preferences for some plant species over others (Thomas and Schultz, 2016). We observed silvery blues to frequently use the native plant *C. quamash* in our nectar observations, although this was not reflected in the diffusion

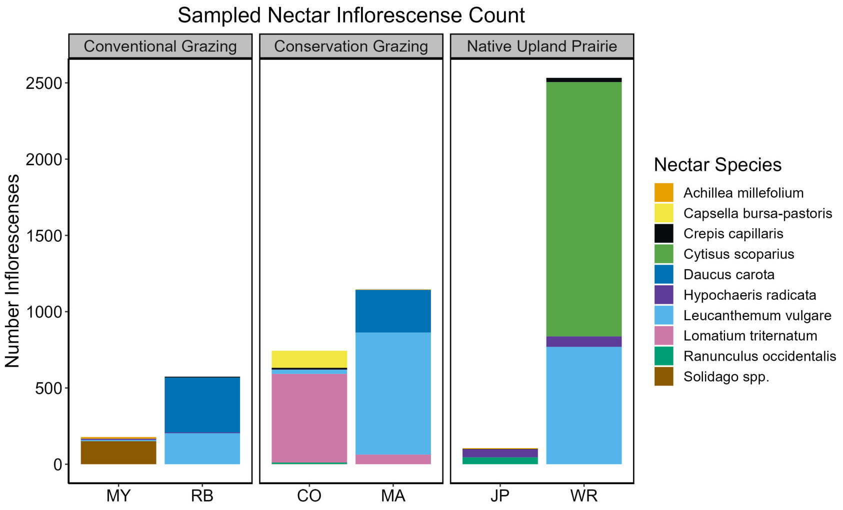


FIGURE 7
Sampled ringlet nectar species inflorescence count summed by site over both flight seasons. Nectar inflorescences were sampled on a path scale.

rates in our PLSR model. Nonnative plants can provide valuable resources for butterflies in degraded landscapes (Graves and Shapiro, 2003; Hardy and Dennis, 2008). *V. sativa*, a nonnative plant, was associated with lower diffusion rates for silvery blue butterflies and therefore higher perceived habitat quality. *V. sativa* was often one of the few nectar species available on grazed sites. We observed ochre ringlets to nectar mainly on *D. carota* and *L. vulgare*. *D. carota* was one of the few late season nectar plants available on our sites, demonstrating the importance of nectar through the full butterfly flight season. There are relatively few late season nectar species available in the landscape in western Washington (Bowcutt and Hamman, 2016). Nectar is important for other ringlet species as well: Sei (2009) observed that maritime ringlet (*Coenonympha nipisquit*) females nectared more often in areas with a high larval survival rate, though it was unclear if the nectar was a causal factor in the larval survival rate. Silvery blues and ochre ringlets nectared on mostly different species, even in parts of their flight period that overlapped, highlighting both the importance of understanding the biological needs of individual species, and of providing a variety of resources through heterogeneous management in the landscape (Dennis, 2004; Dennis et al., 2006; Jerrentrup et al., 2014; Joubert-van der Merwe et al., 2019).

Grazed pastures may have potential to act as corridors between native prairie reserves. Butterflies are known to reach higher densities in habitat patches connected by corridors than patches that are unconnected (Haddad and Baum, 1999; Haddad and Tewksbury, 2005). Butterflies move faster in matrix habitat (Haddad and Tewksbury, 2005; Brown et al., 2017; Schultz et al., 2017; Crone et al., 2019) so providing a series of “stepping stones” with important resources between reserves could increase connectivity in fragmented landscapes (Schultz, 1998). We speculate that conservation grazing pastures, as potential providers of intermediate habitat quality, may act as both habitat and valuable stepping stones for migration between high quality native prairies in the highly fragmented landscape of western Washington.

Diffusion rates are an integrated method used in the current literature for comparing incidence of area restricted search across habitats or management types (Pugesek and Crone, 2022); lower diffusion rates indicate a higher incidence of area restricted search behavior, which in turn indicates perception of high habitat quality (Crone et al., 2019). The analysis of movement behavior can provide more reliable estimates of habitat quality than occupancy or abundance data (Winker et al., 1995). Movement parameters such as step lengths, turning angles, and diffusion rates are often used to discriminate between “habitat” and “non-habitat” (i.e. matrix habitat, with border habitat as a third option) (e.g. Schultz, 1998; Ross et al., 2005; Murphy and Boone, 2022). We applied diffusion rates as a more sensitive indicator of habitat quality on a fine scale and show that conservation grazing may be an important source of intermediate quality habitat in the landscape. However, it is important to note that our study measured only butterfly behavioral response to potential habitat quality, and makes the assumption that differences in movement parameters indicate differences in quality (Crone et al., 2019). In addition, our analysis assessed mainly external effects on butterfly movement, and did not take into account internal factors (see Nathan et al., 2008 for further discussion), such as butterfly age or body mass. We did classify wing wear as a proxy for age, but were unable to include it in the analysis due to limited sample size and the fact that it is confounded by changes in the plant community as the season progresses. Future studies should measure vital rates of butterflies under different grazing management strategies to directly measure habitat quality (Schultz et al., 2019). Demographic studies would indicate whether conservation grazing has the potential to become an ecological trap (Schlaepfer et al., 2002) by attracting females to lay eggs, only to face reduced larval survival due to trampling or other effects of cattle grazing.

Implications for conservation of native butterflies

We conclude that there is potential for grazed land to contribute to butterfly habitat in the landscape, though prairie refugia should be maintained for sensitive species. Grazed habitats may be able to contribute to butterfly habitat if important resources, especially host and nectar plants, are available. Therefore, to support a wide range of butterfly species, grazing practices that support the greatest diversity of host plants should be encouraged, such as rotational grazing with a spring rest period (Ravetto Enri et al., 2017). While the IUCN recommends that 30% of the world's ecosystems be conserved by 2030 (IUCN, 2022), protected conservation areas alone are not enough to achieve this goal (Watson et al., 2014; Butchart et al., 2015). Incorporating conservation grazing into the agricultural landscape will be an important tool to conserve native species in light of accelerating habitat and biodiversity loss.

Data availability statement

Data will be made available in a data repository. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Code will be published on GitHub.

Ethics statement

This study was reviewed and permitted for scientific research by the Washington Department of Fish and Wildlife. No further ethical review was required for common invertebrates.

Author contributions

CS and SB developed the initial idea; CS obtained and provided grants and other resources; SB collected and analyzed data; SB wrote the manuscript; CS and SB edited the manuscript. Both authors contributed to the article and approved the submitted version.

Funding

This work was generously supported by Conservation Research Education and Outreach International (CREOi), Western Sustainable Agriculture Research and Education (WSARE; grant number 201207548), WSU CEREO, and Washington State University Vancouver.

Acknowledgments

We thank Sarah Hamman, Leslie New, Jonah Piovia-Scott, and the two reviewers, Jason Harmon and Adam Kőrösi, for helpful comments on previous versions of this manuscript. We also thank our collaborators and partnered organizations who facilitated our work: Stephen Bramwell, Sarah Hamman, Marty Chaney, Thurston County WSU Extension, Ecostudies Institute, and NRCS. We are deeply grateful to the farmers and ranchers who allowed us to conduct butterfly experiments on their land: Fred Colvin, Kevin Jensen, and Mary and Maynard Mallonee. We also thank the Washington Department of Fish and Wildlife and the Department of Defense (Joint Base Lewis-McChord) for access to the native prairies. Collin Edwards, Kelsey King, and Erica Henry provided invaluable statistics advice and coding assistance. We thank Christopher Jason for his instrumental help in the field. Rachael Bonoan, Kelsey King, Cassandra Doll, Chelsea Thomas, and Center for Natural Lands Management and Evergreen State College volunteers also provided field assistance. A previous version of this manuscript appeared in SB's dissertation.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1162060/full#supplementary-material

References

Avgar, T., Mosser, A., Brown, G. S., and Fryxell, J. M. (2013). Environmental and individual drivers of animal movement patterns across a wide geographical gradient. *J. Anim. Ecol.* 82, 96–106. doi: 10.1111/j.1365-2656.2012.02035.x

Balmer, O., and Erhardt, A. (2000). Consequences of succession on extensively grazed grasslands for central European butterfly communities: rethinking conservation practices. *Conserv. Biol.* 14, 746–757. doi: 10.1046/j.1523-1739.2000.98612.x

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-Effects models using lme4. *J. Stat. Software* 67 (1), 1–48. doi: 10.18637/jss.v067.i01

Beck, J. J., Hernández, D. L., Pasari, J. R., and Zavaleta, E. S. (2015). Grazing maintains native plant diversity and promotes community stability in an annual grassland. *Ecol. Appl.* 25, 1259–1270. doi: 10.1890/14-1093.1

Bigelow, D. P., and Borchers, A. (2017). *Major Uses of Land in the United States 2012. no. 178* (St. Paul, MN.: USDA Economic Research Service).

Blanchet, K., Moechnig, H., and DeJong-Hughes, J. (2000). *Grazing Systems Planning Guide* (University of Minnesota Extension Service).

Bock, C. E., and Jones, Z. F. (2004). Avian habitat evaluation: should counting birds count? *Front. Ecol. Environ.* 2, 403–410. doi: 10.1890/1540-9295(2004)002[0403:AHESCB]2.0.CO;2

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., et al. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135. doi: 10.1016/j.tree.2008.10.008

Bowcutt, F., and Hamman, S. (2016). *Vascular Plants of the South Sound Prairies. 1st edition* (Olympia, Washington: The Evergreen State College Press).

Brook, A., Zint, M., and De Young, R. (2003). Landowners' responses to an endangered species act listing and implications for encouraging conservation. *Conserv. Biol.* 17, 1638–1649. doi: 10.1111/j.1523-1739.2003.00258.x

Brown, L. M., Fuda, R. K., Schtickzelle, N., Coffman, H., Jost, A., Kazberouk, A., et al. (2017). Using animal movement behavior to categorize land cover and predict consequences for connectivity and patch residence times. *Landscape Ecol.* 32, 1657–1670. doi: 10.1007/s10980-017-0533-8

Bussan, S. K. (2022). Can cattle grazing benefit grassland butterflies? *J. Insect Conserv.* 26, 359–374. doi: 10.1007/s10841-022-00373-8

Butchart, S. H. M., Clarke, M., Smith, R. J., Sykes, R. E., Scharlemann, J. P. W., Harfoot, M., et al. (2015). Shortfalls and solutions for meeting national and global conservation area targets. *Conserv. Lett.* 8, 329–337. doi: 10.1111/conl.12158

Carrascal, L. M., Galván, I., and Gordo, O. (2009). Partial least squares regression as an alternative to current regression methods used in ecology. *Oikos* 118, 681–690. doi: 10.1111/j.1600-0706.2008.16881.x

Chong, I.-G., and Jun, C.-H. (2005). Performance of some variable selection methods when multicollinearity is present. *Chemometrics Intelligent Lab. Syst.* 78, 103–112. doi: 10.1016/j.chemolab.2004.12.011

Cortés Capano, G., Toivonen, T., Soutullo, A., and Di Minin, E. (2019). The emergence of private land conservation in scientific literature: A review. *Biol. Conserv.* 237, 191–199. doi: 10.1016/j.biocon.2019.07.010

Crone, E. E., Brown, L. M., Hodgson, J. A., Lutscher, F., and Schultz, C. B. (2019). Faster movement in nonhabitat matrix promotes range shifts in heterogeneous landscapes. *Ecology* 100, e02701. doi: 10.1002/ecy.2701

Crone, E. E., and Schultz, C. B. (2008). Old models explain new observations of butterfly movement at patch edges. *Ecology* 89, 2061–2067. doi: 10.1890/07-1173.1

Davis, K. P., Augustine, D. J., Monroe, A. P., Derner, J. D., and Aldridge, C. L. (2020). Adaptive rangeland management benefits grassland birds utilizing opposing vegetation structure in the shortgrass steppe. *Ecol. Appl.* 30, e02020. doi: 10.1002/eap.2020

Debinski, D. M., Moranz, R. A., Delaney, J. T., Miller, J. R., Engle, D. M., Winkler, L. B., et al. (2011). A cross-taxonomic comparison of insect responses to grassland management and land-use legacies. *Ecosphere* 2, art131. doi: 10.1890/ES11-00226.1

de Knegt, H. J., Hengeveld, G. M., van Langevelde, F., de Boer, W. F., and Kirkman, K. P. (2007). Patch density determines movement patterns and foraging efficiency of large herbivores. *Behav. Ecol.* 18, 1065–1072. doi: 10.1093/beheco/arm080

Delaney, J. T., Moranz, R. A., Debinski, D. M., Engle, D. M., and Miller, J. R. (2016). Exotic-dominated grasslands show signs of recovery with cattle grazing and fire. *PloS One* 11, e0165758. doi: 10.1371/journal.pone.0165758

Dennis, R. L. H. (2004). Just how important are structural elements as habitat components? Indications from a declining lycaenid butterfly with priority conservation status. *J. Insect Conserv.* 8, 37–45. doi: 10.1023/B:JICO.0000027496.82631.4b

Dennis, R. L. H., Shreeve, T. G., and Van Dyck, H. (2006). Habitats and resources: the need for a resource-based definition to conserve butterflies. *Biodiversity Conserv.* 15, 1943–1966. doi: 10.1007/s10531-005-4314-3

Dias, M. P., Granadeiro, J. P., and Palmeirim, J. M. (2009). Searching behaviour of foraging waders: does feeding success influence their walking? *Anim. Behav.* 77, 1203–1209. doi: 10.1016/j.anbehav.2009.02.002

Dodge, K. L., Galuardi, B., Miller, T. J., and Lutcavage, M. E. (2014). Leatherback turtle movements, dive behavior, and habitat characteristics in ecoregions of the Northwest Atlantic Ocean. *PloS One* 9, e91726. doi: 10.1371/journal.pone.0091726

Dorfman, A., Hills, T. T., and Scharf, I. (2022). A guide to area-restricted search: a foundational foraging behaviour. *Biol. Rev. Camb. Philos. Soc* 97, 2076–2089. doi: 10.1111/brv.12883

Dumont, C. P., Himmelman, J. H., and Robinson, S. M. C. (2007). Random movement pattern of the sea urchin *Strongylocentrotus droebachiensis*. *J. Exp. Mar. Biol. Ecol.* 340, 80–89. doi: 10.1016/j.jembe.2006.08.013

Ehl, S., Böhm, N., Wörner, M., Rákosy, L., and Schmitt, T. (2019). Dispersal and adaptation strategies of the high mountain butterfly *Boloria pales* in the Romanian Carpathians. *Front. zoology* 16, 1. doi: 10.1186/s12983-018-0298-1

Farruggia, A., Dumont, B., Scohier, A., Leroy, T., Pradel, P., and Garel, J. P. (2012). An alternative rotational stocking management designed to favour butterflies in permanent grasslands. *Grass Forage Sci.* 67, 136–149. doi: 10.1111/j.1365-2494.2011.00829.x

Fischer, K., Beinlich, B., and Plachter, H. (1999). Population structure, mobility and habitat preferences of the violet copper Lycaena helle (Lepidoptera: Lycaenidae) in Western Germany: implications for conservation. *J. Insect Conserv.* 3, 43–52. doi: 10.1023/A:1009630506216

Fischer, K., and Fiedler, K. (2001). Resource-based territoriality in the butterfly *Lycaena hippothoe* and environmentally induced behavioural shifts. *Anim. Behav.* 61, 723–732. doi: 10.1006/anbe.2000.1662

Fleischner, T. L. (1994). Ecological costs of livestock grazing in western North America. *Conserv. Biol.* 8, 629–644. doi: 10.1046/j.1523-1739.1994.08030629.x

Fownes, S., and Roland, J. (2002). Effects of meadow suitability on female behaviour in the alpine butterfly *Parnassius smintheus*. *Ecol. Entomology* 27, 457–466. doi: 10.1046/j.1365-2311.2002.00426.x

Fransen, S., Pirelli, G., Chaney, M., Brewer, L., and Robbins, S. (2017). *The Western Oregon and Washington Pasture Calendar* (Oregon State University, University of Idaho, Washington State University).

Graves, S. D., and Shapiro, A. M. (2003). Exotics as host plants of the California butterfly fauna. *Biol. Conserv.* 110, 413–433. doi: 10.1016/S0006-3207(02)00233-1

Haddad, N. M. (1999). Corridor use predicted from behaviors at habitat boundaries. *Am. Nat.* 153, 215–227. doi: 10.1086/303163

Haddad, N., and Baum, K. A. (1999). An experimental test of corridor effects on butterfly densities. *Ecol. Appl.* 9, 623–633. doi: 10.1890/1051-0761(1999)009[0623:AETOCE]2.0.CO;2

Haddad, N. M., and Tewksbury, J. J. (2005). Low-quality habitat corridors as movement conduits for two butterfly for two butterfly species. *Ecol. Appl.* 15, 250–257. doi: 10.1890/03-5327

Hamman, S. T., Dunwiddie, P. W., Nuckols, J. L., and McKinley, M. (2011). Fire as a restoration tool in pacific northwest prairies and oak woodlands: challenges, successes, and future directions. *Northwest Sci.* 85, 317–328. doi: 10.3955/046.085.0218

Hardy, P. B., and Dennis, R. L. H. (2008). Resources for British butterflies (Lepidoptera: Hesperioidea, Papilionoidea). The alien consumer component and its significance for butterfly habitats. *Eur. J. entomology* 105, 649–657. doi: 10.14411/eje.2008.089

Hartig, F. (2022). "DHARMa: residual diagnostics for hierarchical (Multi-level/mixed) regression models," in *R package version 0.4.6*. Available at: https://CRAN.R-project.org/package=DHARMa.

Henry, E., Brammer-Robbins, E., Aschehoug, E., and Haddad, N. (2019). Do substitute species help or hinder endangered species management? *Biol. Conserv.* 232, 127–130.

IUCN (2022). "IUCN position paper on the post-2020 global biodiversity framework OEWG-5 and CBD COP15," in *Fifteenth meeting of the conference of the parties (COP15)*. Eds. S.Peña Moreno and V. Romero (Montreal, Canada: International Union for the Conservation of Nature).

James, D., and Nunnallee, D. (2011). *Life Histories of Cascadia Butterflies* (Corvallis, OR: Oregon State University Press).

Jerrentrup, J. S., Wrage-Mönnig, N., Röver, K.-U., and Isselstein, J. (2014). Grazing intensity affects insect diversity via sward structure and heterogeneity in a long-term experiment. *J. Appl. Ecol.* 51, 968–977. doi: 10.1111/1365-2664.12244

Joubert-van der Merwe, L., Pryke, J. S., and Samways, M. J. (2019). Well-managed grassland heterogeneity promotes butterfly conservation in a corridor network. *J. Environ. Manage.* 238, 382–395. doi: 10.1016/j.jenvman.2019.03.021

Kahara, S. N., Skalos, D., Madurapperuma, B., and Hernandez, K. (2022). Habitat quality and drought effects on breeding mallard and other waterfowl populations in California, USA. *J. Wildl. Manage.* 86, e22133. doi: 10.1002/jwmg.22133

Kamal, S., Grodzińska-Jurczak, M., and Brown, G. (2015). Conservation on private land: a review of global strategies with a proposed classification system. *J. Environ. Plann. Manage.* 58, 576–597. doi: 10.1080/09640568.2013.875463

Kareiva, P., and Odell, G. (1987). Swarms of predators exhibit "Preytaxis" if individual predators use area-restricted search. *Am. Nat.* 130, 233–270. doi: 10.1086/284707

Kareiva, P. M., and Shigesada, N. (1983). Analyzing insect movement as a correlated random walk. *Oecologia* 56, 234–238. doi: 10.1007/BF00379695

Kerr, J. T., Sugar, A., and Packer, L. (2000). Indicator taxa, rapid biodiversity assessment, and nestedness in an endangered ecosystem. *Conserv. Biol.* 14, 1726–1734. doi: 10.1111/j.1523-1739.2000.99275.x

Konvička, M., Ričl, D., Vodičková, V., Beneš, J., and Jirků, M. (2021). Restoring a butterfly hot spot by large ungulates refaunation: the case of the Milovice military training range, Czech Republic. *BMC Ecol. Evol.* 21, 73. doi: 10.1186/s12862-021-01804-x

Körösi, A., Orvössy, N., Batáry, P., Kövér, S., and Peregovits, L. (2008). Restricted within-habitat movement and time-constrained egg laying of female *Maculinea rebeli* butterflies. *Oecologia* 156, 455–464. doi: 10.1007/s00442-008-0986-1

Kruess, A., and Tscharntke, T. (2002). Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. *Conserv. Biol.* 16, 1570–1580. doi: 10.1046/j.1523-1739.2002.01334.x

Kuefler, D., Avgar, T., and Fryxell, J. M. (2012). Rotifer population spread in relation to food, density and predation risk in an experimental system. *J. Anim. Ecol.* 81, 323–329. doi: 10.1111/j.1365-2656.2011.01917.x

Kuefler, D., and Haddad, N. M. (2006). Local versus landscape determinants of butterfly movement behaviors. *Ecography* 29, 549–560. doi: 10.1111/j.0906-7590.2006.04574.x

Kuefler, D., Hudgens, B., Haddad, N. M., Morris, W. F., and Thurgate, N. (2010). The conflicting role of matrix habitats as conduits and barriers for dispersal. *Ecology* 91, 944–950. doi: 10.1890/09-0614.1

Lebeau, J., Wesselingh, R. A., and Van Dyck, H. (2015). Butterfly density and behaviour in uncut hay meadow strips: behavioural ecological consequences of an agri-environmental scheme. *PloS One* 10, e0134945. doi: 10.1371/journal.pone.0134945

Lenth, R. (2022). "emmeans: Estimated Marginal Means, aka Least-Squares Means," in *R package version 1.8.1-1*. Available at: https://CRAN.R-project.org/package=emmeans.

Leopold, E., and Boyd, R. (1999). "An ecological history of old prairie areas in southwestern washington," in *Indians, Fire, and the Land in the Pacific Northwest* (Corvallis, OR: Oregon State University Press), 139–163.

Liland, K., Mevik, B., and Wehrens, R. (2022). "pls: partial least squares and principal component regression_," in *R package version 2.8-1*. Available at: https://CRAN.R-project.org/package=pls.

Lopez-Ortiz, S., Pfister, J. A., Launchbaugh, K. L., and Gay, C. C. (2007). Forage availability and body condition affect intake of lupine (*Lupinus leucophyllus*) by grazing cattle. *Prof. Anim. Scientist* 23, 459–466. doi: 10.1532/S1080-7446(15)31006-8

Manley, W. A., Hart, R. H., Samuel, M. J., Smith, M. A., Waggoner, J. W., and Manley, J. T. (1997). Vegetation, cattle, and economic responses to grazing strategies and pressures. *J. Range Management* 50, 638.

Maxwell, S. L., Cazalis, V., Dudley, N., Hoffmann, M., Rodrigues, A. S. L., Stolton, S., et al. (2020). Area-based conservation in the twenty-first century. *Nature* 586, 217–227. doi: 10.1038/s41586-020-2773-z

Mehmood, T., Liland, K. H., Snipen, L., and Sæbø, S. (2012). A review of variable selection methods in Partial Least Squares Regression. *Chemometrics Intelligent Lab. Syst.* 118, 62–69. doi: 10.1016/j.chemolab.2012.07.010

Mevik, B.-H., and Wehrens, R. (2007). The pls package: principal component and partial least squares regression in R. *J. Stat. Software* 18. doi: 10.18637/jss.v018.i02

Michelot, T., Langrock, R., and Patterson, T. A. (2016). moveHMM: an R package for the statistical modelling of animal movement data using hidden Markov models. *Methods Ecol. Evol.* 7 (11), 1308–1315. doi: 10.1111/2041-210X.12578

Muff, S., Nilsen, E. B., O'Hara, R. B., and Nater, C. R. (2022). Rewriting results sections in the language of evidence. *Trends Ecol. Evol.* 37, 203–210. doi: 10.1016/j.tree.2021.10.009

Murphy, M., and Boone, M. (2022). Evaluating the role of body size and habitat type in movement behavior in human-dominated systems: A frog's eye view. *Ecol. Evol.* 12, e9022. doi: 10.1002/ece3.9022

Murphy, D. D., Weiland, P. S., and Cummins, K. W. (2011). A critical assessment of the use of surrogate species in conservation planning in the Sacramento-San Joaquin Delta, California (U.S.A.). *Conserv. Biol.* 25, 873–878.

Nathan, R., Getz, W. M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., et al. (2008). A movement ecology paradigm for unifying organismal movement research. *Proc. Natl. Acad. Sci. United States America* 105, 19052–19059. doi: 10.1073/pnas.0800375105

Neilly, H., O'Reagain, P., Vanderwal, J., and Schwarzkopf, L. (2018). Profitable and sustainable cattle grazing strategies support reptiles in tropical savanna rangeland. *Rangeland Ecol. Manage.* 71, 205–212. doi: 10.1016/j.rama.2017.09.005

Nilsson, S. G., Franzén, M., and Jönsson, E. (2008). Long-term land-use changes and extinction of specialised butterflies. *Insect Conserv. Diversity.* 1, 197–207. doi: 10.1111/j.1752-4598.2008.00027.x

Noland, S., and Carver, L. (2011). *Prairie Landowner Guide for Western Washington* (The Nature Conservancy), 2.

Noss, R. F. (1994). Cows and conservation biology. *Conserv. Biol.* 8, 613–616. doi: 10.1046/j.1523-1739.1994.08030613.x

Ovaskainen, O. (2004). Habitat-specific movement parameters estimated using mark–recapture data and a diffusion model. *Ecology* 85, 242–257. doi: 10.1890/02-0706

Panter, K. E., James, L. F., Gardner, D. R.Society for Range Management (2002). Reproductive losses to poisonous plants: Influence of management strategies. *Rev. Endocrinol. Metab.* 55, 301–308. doi: 10.2458/azu_jrm_v55i3_panter

Pelham, J. P. (2021). A catalogue of the butterflies of the United States and Canada. *J. Res. Lepidoptera*. Entomological news.

Pöyry, J., Lindgren, S., Salminen, J., and Kuussaari, M. (2004). Restoration of butterfly and moth communities in semi-natural grasslands by cattle grazing. *Ecol. Appl.* 14, 1656–1670. doi: 10.1890/03-5151

Pugesek, G., and Crone, E. E. (2022). Movement of nest-searching bumblebee queens reflects nesting habitat quality. *Ecol. Entomology* 47, 719–727. doi: 10.1111/een.13156

Pyle, R. M., and LaBar, C. C. (2018). *Butterflies of the Pacific Northwest* (Portland, OR: Timber Press, inc.).

R Core Team. (2022). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/. Version 4.4.2 (2022-10-31).

Ravetto Enri, S., Probo, M., Farruggia, A., Lanore, L., Blanchetete, A., and Dumont, B. (2017). A biodiversity-friendly rotational grazing system enhancing flower-visiting insect assemblages while maintaining animal and grassland productivity. *Agriculture Ecosyst. Environ.* 241, 1–10. doi: 10.1016/j.agee.2017.02.030

Revilla, E., Wiegand, T., Palomares, F., Ferreras, P., and Delibes, M. (2004). Effects of matrix heterogeneity on animal dispersal: from individual behavior to metapopulation-level parameters. *Am. Nat.* 164, E130–E153. doi: 10.1086/424767

Robel, R. J., Briggs, J. N., Dayton, A. D., and Hulbert, L. C. (1970). Relationships between visual obstruction measurements and weight of grassland vegetation. *J. Range Manage.* 23, 295. doi: 10.2307/3896225

Root, R. B., and Kareiva, P. M. (1984). The search for resources by cabbage butterflies (*Pieris rapae*): ecological consequences and adaptive significance of markovian movements in a patchy environment. *Ecology* 65, 147–165. doi: 10.2307/1939467

Ross, J. A., Matter, S. F., and Roland, J. (2005). Edge avoidance and movement of the butterfly *Parnassius smintheus* in matrix and non-matrix habitat. *Landscape Ecol.* 20, 127–135. doi: 10.1007/s10980-004-1010-8

Rusterholz, H.-P., and Erhardt, A. (2000). Can nectar properties explain sex-specific flower preferences in the Adonis Blue butterfly *Lysandra bellargus*? *Ecol. Entomology* 25, 81–90. doi: 10.1046/j.1365-2311.2000.00233.x

Samson, F. B., Knopf, F. L., and Ostlie, W. R. (2004). Great Plains ecosystems: past, present, and future. *Wildlife Soc. Bull.* 32, 6–15. doi: 10.2193/0091-7648(2004)32[6:GPEPPA]2.0.CO;2

Schlaepfer, M. A., Runge, M. C., and Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends Ecol. Evol.* 17, 474–480. doi: 10.1016/S0169-5347(02)02580-6

Schtickzelle, N., Turlure, C., and Baguette, M. (2007). Grazing management impacts on the viability of the threatened bog fritillary butterfly *Proclossiana eunomia*. *Biol. Conserv.* 136, 651–660. doi: 10.1016/j.biocon.2007.01.012

Schultz, C. B. (1998). Dispersal behavior and its implications for reserve design in a rare Oregon butterfly. *Conserv. Biol.* 12, 284–292. doi: 10.1111/j.1523-1739.1998.96266.x

Schultz, C. B., and Crone, E. E. (2001). Edge-mediated dispersal behavior in a prairie butterfly. *Ecology* 82, 1879–1892. doi: 10.1890/0012-9658(2001)082[1879:EMDBIA]2.0.CO;2

Schultz, C. B., Franco, A. M. A., and Crone, E. E. (2012). Response of butterflies to structural and resource boundaries. *J. Anim. Ecol.* 81, 724–734. doi: 10.1111/j.1365-2656.2011.01947.x

Schultz, C. B., Haddad, N. M., Henry, E. H., and Crone, E. E. (2019). Movement and demography of at-risk butterflies: building blocks for conservation. *Annu. Rev. Entomology* 64, 167–184. doi: 10.1146/annurev-ento-011118-112204

Schultz, C. B., Pe'er, B. G., Damiani, C., Brown, L., and Crone, E. E. (2017). Does movement behaviour predict population densities? A test with 25 butterfly species. *J. Anim. Ecol.* 86, 384–393. doi: 10.1111/1365-2656.12609

Scott, E. R., and Crone, E. E. (2021). Using the right tool for the job: the difference between unsupervised and supervised analyses of multivariate ecological data. *Oecologia* 196, 13–25. doi: 10.1007/s00442-020-04848-w

Searle, S. R., Speed, F. M., and Milliken, G. A. (1980). Population marginal means in the linear model: An alternative to least squares means. *Am. Statistician* 34, 216–221. doi: 10.2307/2684063

Sei, M. (2009). Flight and oviposition behavior of the adult maritime ringlet (*Coenonympha nipisiquit* McDunnough) females in response to microhabitat. *J. Insect Behav.* 22, 87–100. doi: 10.1007/s10905-008-9156-x

Smith, L. M., and Cherry, R. (2014). Effects of management techniques on grassland butterfly species composition and community structure. *Am. midland Nat.* 172, 227–235. doi: 10.1674/0003-0031-172.2.227

Stanton, M. L. (1982). Searching in a patchy environment: foodplant selection by colis P. eriphyle butterflies. *Ecology* 63, 839–853. doi: 10.2307/1936803

Stevens, V. M., Polus, E., Wesselingh, R. A., Schtickzelle, N., and Baguette, M. (2004). Quantifying functional connectivity: experimental evidence for patch-specific resistance in the Natterjack toad (Bufo calamita). *Landscape Ecol.* 19, 829–842. doi: 10.1007/s10980-004-0166-6

Thomas, R. C., and Schultz, C. B. (2016). Resource selection in an endangered butterfly: Females select native nectar species. *J. Wildlife Manage.* 80, 171–180. doi: 10.1002/jwmg.987

Thomas, J. A., Simcox, D. J., and Clarke, R. T. (2009). Successful conservation of a threatened Maculinea butterfly. *Science* 325, 80–83. doi: 10.1126/science.1175726

Turchin, P. (2015). *Quantitative analysis of movement: measuring and modeling population resdistribution in animals and plants* (Beresta Books).

Van Horne, B. (1983). Density as a misleading indicator of habitat quality. *J. Wildlife Manage.* 47, 893–901. doi: 10.2307/3808148

van Klink, R., van der Plas, F., van Noordwijk, C. G. E. T., WallisDeVries, M. F., and Olff, H. (2015). Effects of large herbivores on grassland arthropod diversity. *Biol. Rev. Cambridge Philos. Soc.* 90, 347–366. doi: 10.1111/brv.12113

Velasco, J. (2021). *Unsettling prairies: A critical reimagining of fire management in cities* (Seattle, WA: University of Washington). Doctoral dissertation.

Vogel, J. A., Debinski, D. M., Koford, R. R., and Miller, J. R. (2007). Butterfly responses to prairie restoration through fire and grazing. *Biol. Conserv.* 140, 78–90. doi: 10.1016/j.biocon.2007.07.027

WallisDeVries, M. F., Noordijk, J., Colijn, E. O., Smit, J. T., and Veling, K. (2016). Contrasting responses of insect communities to grazing intensity in lowland heathlands. *Agriculture Ecosyst. Environ.* 234, 72–80. doi: 10.1016/j.agee.2016.04.012

Warchola, N., Crone, E. E., and Schultz, C. B. (2017). Balancing ecological costs and benefits of fire for population viability of disturbance-dependent butterflies. *J. Appl. Ecol.* 55, 800–809. doi: 10.1111/1365-2664.12983

Washington Department of Fish and Wildlife. (2022). *Westside prairie* (Olympia, WA: Washington Department of Fish & Wildlife). Available at: https://wdfw.wa.gov/species-habitats/ecosystems/westside-prairie.

Watson, J. E. M., Dudley, N., Segan, D. B., and Hockings, M. (2014). The performance and potential of protected areas. *Nature* 515, 67–73. doi: 10.1038/nature13947

Weiss, S. B. (1999). Cars, cows, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grasslands for a threatened species. *Conserv. Biol.* 13, 1476–1486. doi: 10.1046/j.1523-1739.1999.98468.x

Western Regional Climate Center. (2022) *WRCC: washington climate*. Available at: https://wrcc.dri.edu/Climate/narrative_wa.php.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., et al. (2019). Welcome to the tidyverse. *J. Open Source Software* 4 (43), 1686. doi: 10.21105/joss.01686

Winker, K., Rappole, J. H., and Ramos, M. A. (1995). The use of movement data as an assay of habitat quality. *Oecologia* 101, 211–216.

Wold, H. (1975). Soft modelling by latent variables: the non-linear iterative partial least squares (NIPALS) approach. *J. Appl. probability* 12, 117–142. doi: 10.1017/S0021900200047604

Zakkak, S., Chatzaki, M., Karamalis, N., and Kati, V. (2014). Spiders in the context of agricultural land abandonment in Greek Mountains: species responses, community structure and the need to preserve traditional agricultural landscapes. *J. Insect Conserv.* 18, 599–611. doi: 10.1007/s10841-014-9663-3

Zalucki, M. P., and Kitching, R. L. (1982). The analysis and description of movement in adult *Danaus plexippus* L. (lepidoptera: danainae). *Behaviour* 80, 174–197. doi: 10.1163/156853982X00346

Zhang, J., Cong, Q., Shen, J., Opler, P. A., and Grishin, N. V. (2020). Genomic evidence suggests further changes of butterfly names. *Taxonomic Rep. Int. Lepidoptera Survey* 8 (7), 1–41.

Zontek, K. (2007). *Buffalo Nation: American Indian Efforts to Restore the Bison* (Lincoln and London, NE: University of Nebraska Press), 29.

frontiers | Frontiers in Ecology and Evolution

TYPE Review
PUBLISHED 04 April 2024
DOI 10.3389/fevo.2024.1332985

OPEN ACCESS

EDITED BY
Darren Norris,
Universidade Federal do Amapá, Brazil

REVIEWED BY
Lalasia Bialic-Murphy,
ETH Zürich, Switzerland
Dan Xing,
Guizhou Academy of Agricultural Sciences (CAAS), China

*CORRESPONDENCE
Zhenhong Wang
✉ w_zhenhong@126.com

RECEIVED 04 November 2023
ACCEPTED 15 March 2024
PUBLISHED 04 April 2024

CITATION
Wang Z, Arratia J, Yan T, Zhang C and Chiarucci A (2024) Integrative framework of multiple processes to explain plant productivity–richness relationships. *Front. Ecol. Evol.* 12:1332985. doi: 10.3389/fevo.2024.1332985

Integrative framework of multiple processes to explain plant productivity–richness relationships

Zhenhong Wang[1]*, Juan Arratia[2], Ting Yan[1], Cong Zhang[1] and Alessandro Chiarucci[3]

[1]Key Laboratory of Subsurface Hydrology and Ecological Effects in Arid Regions, Ministry of Education, Chang`an University, Xi`an, China, [2]AGMUS Institute of Mathematics, Caribbean Computing Center for Excellence, San Juan, PR, United States, [3]Department of Biological, Geological and Environmental Science, University of Bologna, Bologna, Italy

Plant diversity and productivity, two crucial properties that sustain ecosystem structures, functions, and services, are intrinsically linked to numerous ecological fields, making productivity–richness relationships (PRR) a central ecological concern. Despite extensive research from the Darwinian era to the 21st century, the various shapes of PRR and their underlying theories have sparked ongoing debates. While several processes, theories, and integrative models have been proposed to explain PRR, a comprehensive understanding of the types of PRR, the effects of these processes on plant productivity and richness, and the relationships between PRR shapes remains elusive. This paper proposes a new integrative framework that focuses on these aspects, aiming to elucidate the diverse shapes of PRR and their interconnections. We review recent integrative methods that explain the roles of processes and the varying shapes in PRR to support this new framework. The paper traces the distinct phases in PRR research, including the discovery of PRR shapes, tests of influencing processes, and integrative research. We discuss the application of the Structural Equation Model (SEM), Statistical Dynamical Model (SDM), and Differential Dynamical Model (DDM) in integrative research. This integrative framework can guide theoretical and applied ecologists in identifying, deriving, explaining, and predicting the interconnected but distinct shapes of PRR. The humped, asymptotic, positive, negative, and irregular shapes of PRR are interconnected, with one shape potentially transforming into another. The balance between the positive and negative effects of different processes determines the different shapes of PRR, ultimately leading to a globally positive effect of plant diversity on plant productivity and other ecosystem functions.

KEYWORDS

plant diversity, productivity, dynamical models, structural equation model, ecological processes, ecosystem functions, integrative research

Introduction

Plant diversity and productivity are fundamental for the structure and functioning of ecosystems, including the composition, proportion, interrelation of organisms in the food chain and a variety of ecosystem functions (Humborg et al., 1997; Grace et al., 2016; Laforest-Lapointe et al., 2017). Ecosystems with diverse plant species are essential for achieving sustainable primary productivity and stability, although there are a few counter-examples (Bezemer & van der Putten, 2007). Additionally, diverse ecosystems can provide valuable ecosystem services, such as carbon sequestration, oxygen release, wood production, water resource regeneration, and soil erosion control (Sugden, 2018; Leclère et al., 2020). Consequently, plant productivity and richness relationships (PRR) have become a core issue for ecologists worldwide (Tilman et al., 2001; Chen et al., 2018).

Ecologists have observed various shapes of PRR across different continents and ecosystems, including forests, grasslands, lakes, and seas (Mittelbach et al., 2003; Whittaker and Heegaard, 2003; Adler et al., 2011; Pierce, 2014). However, information on the occurrence of these PRR shapes is scattered and irregular, leading to confusion among ecologists (Gillman and Wright, 2006; Whittaker and Heegaard, 2003; Pierce, 2014). Numerous processes and theories have been proposed to explain the shapes of PRR (Abrams, 1995; Willig, 2011). For instance, intra- and inter-specific competition effects have been proposed to explain PRR, clarifying specific sections or shapes of PRR (Stevens and Carson, 1999; Michalet et al., 2006). The dynamic equilibrium hypothesis has been applied to explain the growth and decline of populations in humped-shaped PRR (Huston, 1979; Chiarucci et al., 2006). Species-pool effect, environmental heterogeneity, and negative density dependence are often considered to regulate species richness, while selection effects, complementary effects, and inter-specific facilitation influence plant productivity in PRR (Zobel et al., 1998; Hector et al., 1999; Loreau et al., 2001; Grossman et al., 2017). Due to the diversity of PRR shapes and corresponding explanations, the general pattern of PRR and its underlying mechanisms have been the subject of debate since the 1950s (Abrams, 1995; Schmid, 2002; Adler et al., 2011; Duffy et al., 2017). However, ecologists have not clearly classified the types of PRR, despite identifying many different shapes and proposing various explanations. Furthermore, the positive and negative effects of each process on plant productivity and richness, as well as the relationships between different shapes of PRR, have rarely been analyzed.

Ecologists have also employed mathematical models to integrate the effects of different processes, aiming for a comprehensive explanation of PRR (Tilman et al., 1997; Loreau, 1998; Grace et al., 2014, Grace et al., 2016; Liang et al., 2016b; Wang et al., 2019). For example, competition models quantify the impact of inter-specific competition influenced by abiotic factors on plant productivity and species richness in PRR (Huston, 1979; Tilman et al., 1997). Mechanistic models, which consider selection effects, complementary effects, resource availability, and species' functional traits, have been established to reveal the effects of species richness on plant productivity in competition for limiting soil nutrients (Loreau, 1998). Structural equation models, as a form of stochastic process analysis, have been widely used to quantify the roles of different processes in regulating plant diversity, productivity, biomass, and soil organic carbon in PRR (Grace et al., 2016; Chen et al., 2018). However, these integrative methods have been applied independently and have not incorporated actual values of each process contributing to plant species richness and productivity based on sampling analysis, which would enhance the understanding of PRR shapes and their relationships.

In this review, we propose a new integrative framework to explain PRR based on multiple processes or theories and previous integrative studies. The framework incorporates processes or theories proposed by ecologists after extensive research, as well as integrative models and results of PRR. Additionally, we conduct a comprehensive review of the positive and negative effects of processes on PRR, as well as relevant theories. We also examine recent integration analyses that utilize structural equation models to quantify the roles of different processes in shaping PRR, and integration analyses that employ dynamical models to provide insights into the mechanisms underlying PRR shapes. These reviews serve as valuable support for the proposed new integrative framework. Our aim is to promote further research on PRR in the field of biodiversity and ecosystem functions.

Integration framework of multiple processes or theories

The integrative framework comprises three sections:

(1) Definition of the two types of PRR, and multiple processes influencing plant richness and productivity in PRR at the top layer (Figure 1). The two types of PRR encompass: (i) the plant productivity-species richness relationship (PSRR), where plant productivity serves as an independent variable and species richness as a dependent variable, describing the patterns of diversity influenced by productivity and other changing processes; (ii) the species richness-plant productivity relationship (SRPR), which represents the converse relationship to PSRR. In SRPR, species richness acts as the independent variable and plant productivity as the dependent variable, elucidating the effects of plant diversity on productivity and its role in regulating ecosystem functioning, stability, and services (Wang, 2017; Wang et al., 2019; Figure 1). PSRR and SRPR are closely linked to key processes in ecology. However, the classification and definition of these two types of PRR have been vague in previous studies, contributing to the ongoing debate on the shapes of PRR and the underlying mechanisms (Mittelbach et al., 2003; Cardinale et al., 2007; Whittaker, 2010; Grace et al., 2014). In the framework, processes or theories are also classified into two types, affecting or explaining PSRR and SRPR, respectively. However, some processes, such as disturbance, can influence both PSRR and SRPR (Grace et al., 2016). Generally, processes affecting PSRR or SRPR can have either positive or negative effects on species richness, plant productivity, and subsequently on PRR. Some processes may even have both positive and negative effects (Wang, 2017; Wang et al., 2019).

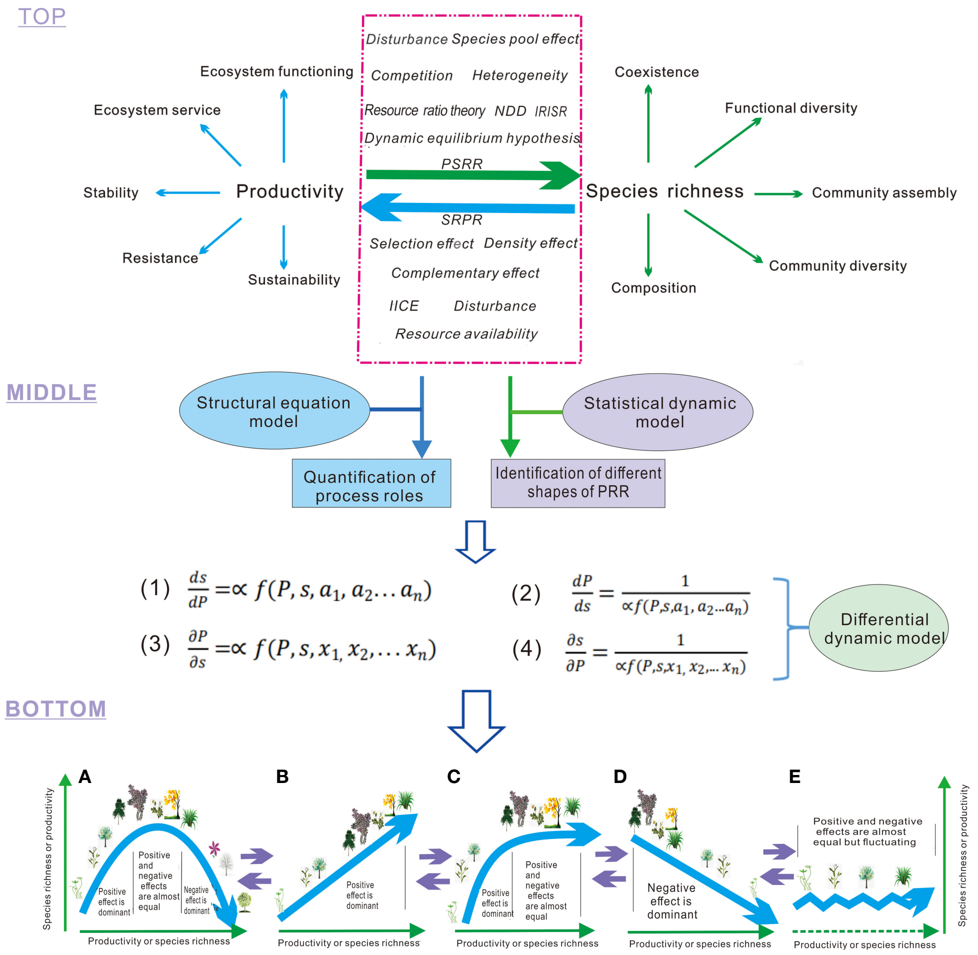


FIGURE 1
Processes acting on productivity-richness relationship (PRR). Top layer: The green arrow represents productivity as an independent variable that influences species richness and related patterns in conjunction with other processes. The blue arrow indicates species richness as an independent variable that affects productivity and related ecosystem properties in conjunction with other processes. The red dashed box encompasses various processes that directly or indirectly impact productivity or species richness, consequently altering PRR. NDD, Negative Density Dependence; PSRR, Productivity-Richness Relationship with productivity as the independent variable and species richness as the dependent variable; SRPR, Species Richness-Productivity Relationship with species richness as the independent variable and productivity as the dependent variable; IICE, Intra- and Inter-specific Competition Effects. Middle layer: The first and fourth equations represent the rates of change in species richness (S, a dependent variable) with plant productivity (P, an independent variable), respectively. These equations integrate different processes (i.e., variables, a1-an, x1-xn) to derive the shapes of PSRR. The second and third equations reflect the rates of change in plant productivity (a dependent variable) with species richness (an independent variable) and integrate diverse processes to derive the shapes of SRPR. Bottom layer: The results depict the diverse shapes of PRR derived from integrative analysis and dynamic models: **(A)** Humped; **(B)** Positive; **(C)** Asymptotic; **(D)** Negative; **(E)** Irregular. These shapes are interconnected, and one shape can transition into another shape with changes in the overall positive and negative effects of processes. **A** and **C** represent the dominant shapes of PSRR and SRPR, respectively, in the absence of exclusion of other shapes (Mittelbach et al., 2003; Fraser et al., 2015; Liang et al., 2016b). The purple arrows represent that the different forms **(A,B,C,D** and **E)** of PRR can be transformed each other.

However, the explicit definition of the positive or negative effects, or the dual effects of these processes, has been rare. Some processes have not received sufficient attention, and we provide a generalization of them in Box 1.

(2) Integrative models in the middle layer. The integrative models encompass the structural equation model, statistical dynamical model, and differential dynamical model within the framework. The structural equation model is a statistical method used to analyze the relationship between variables based on their covariance matrix. It enables the estimation, testing, and quantification of causality (Grace et al., 2016; Chen et al., 2018). The structural equation model has been applied to various practical scenarios, including multi-dependent variable analysis, latent variable analysis, and intermediate variable analysis. It can be viewed as a combination of path analysis and confirmatory factor analysis. The positive or negative effects (i.e., contributions) of multiple processes on plant species richness and productivity in PRR are quantified integrally using the structural equation model. The quantified effects (standardized) are then assigned as coefficients of the integrated processes in the dynamical model to derive the shapes of PRR. This application of the structural equation model to the framework avoids the subjective assignment of coefficients for the process variables and enhances the practicality of the differential equation in the framework.

The statistical dynamical model is a type of dynamic model that describes the occurrence of random processes. It is often employed in

BOX 1 Integrated ecological processes and theories in the framework.

(1) ***Intrinsic rate of increase in species richness with productivity (IRISR)***. IRISR is a positive process to directly increase species richness with increasing plant productivity because high productivity can increase metabolic rate, mutation rate of genes and rapid speciation, resulting in higher species richness in communities (Allen et al., 2002; Stegen et al., 2009). The process has not been explicitly defined before but it exists with a high possibility at a scale of evolutionary time. (+species richness/+/-productivity)*.

(2) ***Intra- and inter-specific competition effects (IICE)***. IICE is an effect of competition among individuals of same and different species on species richness and productivity, which include competition stress, competitive exclusion and assemblage-level thinning to decrease species richness and productivity or increase productivity (Goldberg & Miller, 1990; Huston & DeAngelis, 1994). (-species richness/+/-productivity)

(3) ***Dynamic equilibrium hypothesis***. The hypothesis proposes that poor competitors are excluded rapidly in highly productive habitats with rare disturbance, leading to low diversity; a strong disturbance also results in the disappearance of inadaptable species, leading to low species richness; with moderate disturbances, diversity remains relatively high in the habitats of any productivity to form the peak of the humped shape of PSRR (Huston, 1979; Michalet et al., 2006). (+/-species richness/+/-productivity).

(4) ***Resource ratio theory.*** Resource ratio theory argues that as the availability of any one resource R_1 increases, another resource R_2 is likely to become limiting; because different species are superior competitors for different resources, a balanced resource supply between R_1 and R_2 can help maintain species coexistence (Tilman, 1982; Cardinale et al., 2009). (+species richness).

(5) ***Species-pool effect***. Species pools are a set of plant species with each species of a community, local, or regional flora being a member of any community, local, or regional species pool, with different degrees of probability; species-pool effect is a contribution of species from a species pool to species richness in the community on a certain scale (Zobel et al., 1998; Foster et al., 2004). (+species richness).

(6) ***Disturbances***. Disturbances are some processes such as grazing, fire, severe windstorms, wave damage, land cover alterations, habitat fragmentation, and forest destruction, which often alters plant productivity and species richness, primarily via a negative or positive effect (Hughes et al., 2007; Wu et al., 2019). (-/+species richness and productivity)

(7) ***Environmental heterogeneity***. Environmental heterogeneity is locally diverse configurations in resource types with different availability levels along with more complex configurations in abiotic and biotic resources and more heterogeneities but environmental heterogeneity is the configurations of diverse habitats, i.e., habitat heterogeneity, on a landscape scale (Amarasekare, 2003; Lasky et al., 2014). (+species richness).

(8) ***Density effects***. Density effects are an ecological process resulting in species richness with increasing number of plant individuals in a plant community; plant density increases with increasing species richness also leads to high and low biomass production at low and high inter-specific and intra-specific competition levels, respectively (Marquard et al., 2009). (+species richness/+/-productivity).

(9) ***Negative density dependence*** (***NDD***). NDD is a process by which population growth rates decline at high densities as a result of natural enemies (e.g., predators, pathogens, or herbivores) and/or competition for space and resources to lead to the coexistence of species (Yenni et al., 2012; LaManna et al., 2017a, LaManna et al., 2017b). (+ species richness).

(10) ***Selective and complementary effects***. Selection effect is the standard positive covariance effect, as a diverse community stochastically contains highly productive species (Balvanera et al., 2006; Loreau et al., 2001); complementary effect refers to an effect caused by species`differentiation in resource use and/or inter-specific facilitation at higher levels of species richness (Balvanera et al., 2006; Cardinale et al., 2007). (+productivity).

(11) ***Resource availability***. Resource availability is relatively higher quantities of limited resources which ensures that weaker competitors are able to capture the limited resources for the maintenance of a population leading to the diversity and productivity of coexisting species (Tilman, 1982; Cardinale et al., 2009). (+ productivity/+species richness).

* "+" or "-" represents positive or negative effect on species richness or productivity.

meta-analysis and sampling analysis to identify the shapes of PRR (Mittelbach et al., 2003; Liang et al., 2016b). In the framework, statistical dynamical models can determine the occurrence ratios of different-shaped PRR. The differential dynamical model is a type of dynamic model used to describe the continuous change of dependent and independent variables regulated by multiple processes. Ecologists commonly establish such models to derive the shapes of PRR based on assumed parameter values of processes. These models further reveal how the shapes of PRR occur under the regulation of these processes and how they are linked with each other, i.e., underlying mechanisms (Loreau, 1998; Wang, 2017; Wang et al., 2019). In the framework, the actual parameter values of processes from the analysis of structural equation models may be introduced to differential dynamical models for deriving the shapes of PRR which have been identified by statistical dynamic models. Therefore, the three types of models are related to each other.

(3) Integrative results in the bottom layer. As shown in Figure 1, the integrative framework allows for the derivation of five typical shapes of the PRR by applying the three types of models discussed earlier. This approach differs from previous methods that relied on assumed coefficients to determine the shapes of PRR (Loreau, 1998; Liang et al., 2016b; Wang, 2017; Wang et al., 2019). When the positive effects of integrated processes dominate, the PRR shapes exhibit an upward trend. Conversely, when the negative effects of integrated processes dominate, the PRR shapes show a downward trend. When the positive and negative effects of integrated processes are approximately equal, the PRR shapes display a horizontal or fluctuating pattern. Finally, when the positive and negative effects of integrated processes successively dominate, the PRR shapes exhibit a humped pattern. This integrative framework effectively resolves the long-standing debate surrounding the shapes of PRR and their underlying mechanisms (Schmid, 2002; Adler et al., 2011; Duffy et al., 2017).

The integrative framework provides an explanation for the occurrence of different shapes in the productivity-richness relationship observed in the real world, considering the effects of multiple variables. It can specifically demonstrate which processes are strong or weak, and whether they have a positive or negative effect, thereby determining the shapes of the PSRR and SRPR. In contrast, a meta-analysis or statistical dynamical approaches such as $P=\alpha(X)S^B$ cannot achieve this level of understanding. While statistical dynamical models can be used to simply identify the shapes of SRPR (Liang et al., 2016b), the integrative framework allows for tracking the dynamics of the interactions among different processes that influence the shapes of PSRR and SRPR. For example, it can capture the dynamics of species-pool effects and inter-specific competition by utilizing differential equations, which offer greater flexibility in dealing with variable dynamics compared to statistical dynamical methods. Ecologists can identify the inflection points at which the shapes of PSRR and SRPR change from one pattern to another, and determine the corresponding processes or integrative processes responsible for these changes (Wang et al., 2019). Consequently, the integrative framework provides a clearer understanding of the underlying mechanisms driving PSRR and SRPR, resolving key debates regarding the drivers of hump-shaped patterns and other patterns. By combining the strengths of structural equation models, statistical dynamical models, and differential dynamical models while avoiding their shortcomings, this framework presents a novel technology roadmap for deriving the shapes of PSRR and SRPR.

The integrative framework has broad applications in the study of diversity patterns, ecosystem functions and services, underlying mechanisms, and ecosystem management. Ecologists can start by conducting field vegetation investigations to collect data on productivity, species richness, and the processes influencing productivity and species richness in a particular research region, either through new data collection or using existing datasets. The interaction relationships among productivity, species richness, and influencing processes can then be analyzed using structural equation modeling, providing factor loadings and determinant coefficients through analysis. Subsequently, the field data can be used to identify the shapes of PSRR and SRPR using statistical dynamical models under specific conditions, thereby determining the shapes of PRR. The differential equation set for PSRR and SRPR can be established by utilizing the factor loadings as coefficients for the variables of productivity, species richness, and processes. Mathematical methods such as Fortran or Python can be employed to solve the equations and obtain solutions for each variable, including productivity, species richness, and processes. The dynamics of these variables can be modeled with changes in other variables such as disturbance and resource availability, and compared with the shapes identified by statistical dynamical models. The differential equations can be further refined to predict PSRR and SRPR for management purposes in similar regions. These methods are also applicable to purely theoretical research.

The following review includes two sections that utilize structural equation models and dynamical models (both statistical and differential) to analyze the integration of processes in PRR and explain the formation of PRR shapes. These sections serve to recapitulate the contributions of previous integration research on PRR while highlighting certain research limitations. These limitations align with the issues that the integration framework proposed in this review aims to address. As a result, these two sections provide valuable support for the proposed new integrative framework.

Integration analysis with structural equation models to quantify the roles of processes in PRR

Previous studies have recognized that individual processes or theories can only explain specific sections or dominant shapes of PRR, although they have contributed to the understanding of PRR (Axmanová et al., 2012; Pierce, 2014). As the dominant shapes of PRR have been challenged by diverse patterns, some researchers have argued that PRR is variable, complex, and scale-dependent, influenced by numerous abiotic and biotic processes (Grace et al., 2007; Willig, 2011). Consequently, ecologists have shifted their focus towards incorporating more processes to explain the shapes of PRR, utilizing structural equation models to integrate different processes within the bivariate relationship of plant richness and productivity (Grace et al., 2014, Grace et al., 2016). The structural equation model approach allows for the calculation of the role values of each process affecting species richness and productivity based on field investigations and meta-analyses of previous studies.

In one specific integration, Grace et al. (2014) established a causal network for the humped shape of PSRR, assuming the hump as the basic shape. Using a structural equation model, the corresponding processes influencing plant richness and productivity in the humped shapes were quantified. Surprisingly, this analysis did not support the assumed humped shape of PSRR but instead revealed alternative shapes and influencing processes. This study demonstrates how causal networks can be established through hypotheses and explicit tests to explain PSRR as an abstracting system, providing powerful predictions beyond bivariate analysis. Building upon this concept, further structural equation modeling was employed to integrate competing theories into a multi-process hypothesis and evaluate it using global data from 1,126 plots in grass-dominated sites (Grace et al., 2016). The variables measured included plant species richness, productivity, total biomass, and various drivers such as soil fertility, climate, heterogeneity, soil suitability, and shading. In contrast to a bivariate species richness-productivity model, this modeling approach explained 61% of the variation in richness at the site and plot levels, quantifying the roles of different processes in regulating PSRR and SRPR (Figure 2).

In another integration, field observations from 6,098 forest, shrubland, and grassland sites across China were collected to integrally quantify the first-type effects of climate, soils, and human impacts on soil organic carbon (SOC) storage, as well as the second-type effects mediated by species richness, above-ground net primary productivity (ANPP), and below-ground biomass (BB), using a structural equation model (Chen et al., 2018). The analysis revealed a positive SRPR and a positive biomass-SOC relationship. Favorable climates (high temperature and precipitation) consistently had a negative effect on SOC storage but a positive effect on species richness, ANPP, and BB. The positive relationships between species richness and ANPP/BB offset the negative effect of

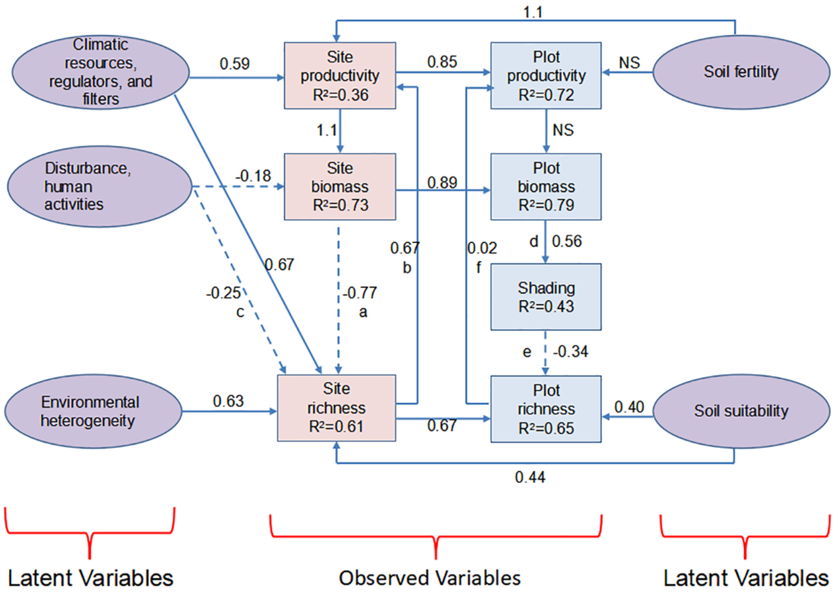


FIGURE 2
Roles of multiple processes in PRR quantified by a structural equation model. This figure illustrates the roles of multiple processes in PRR as quantified by a structural equation model. Solid arrows indicate positive effects, while dashed arrows represent negative effects. The digits alongside the lines indicate the magnitude of these effects. The lowercase letters represent the different plots for the data of collection. NS, no significance. Adapted from Grace et al. (2016).

favorable climate on SOC storage. Maintaining high levels of diversity can enhance soil carbon sequestration (Chen et al., 2018). These results are supported by other local studies conducted in China and Canada (Chen et al., 2018; Huang et al., 2018; Chen et al., 2020).

The aforementioned studies by Grace et al. (2014, 2016) primarily focused on PRR influenced by abiotic processes, while the study by Chen et al. (2018) attempted to reveal the underlying mechanisms linking SOC storage with PRR. The results indicated that species richness had positive effects on productivity, biomass, and subsequently SOC storage, highlighting the regulation of PRR by diverse processes. Structural equation modeling represents a significant advancement in the analysis of PRR beyond two-dimensional variables of plant productivity and diversity. However, the data on species richness, productivity, and abiotic and biotic processes used in structural equation models are often collected simultaneously. Abiotic and biotic processes continuously vary and exhibit hysteresis in the regulation of PRR. In other words, the sampled abiotic and biotic processes, such as soil fertility, when plant richness and productivity are measured, will primarily affect plant richness and productivity in the future. Additionally, a single application of a structural equation model cannot identify the shapes of PRR. Therefore, it is necessary to consider dynamic processes when establishing a model network to assess the effects of processes on PRR. Nevertheless, the role values of different processes in regulating plant richness and productivity, quantified by structural equation models, can be used as coefficients for independent and dependent variables in dynamic models. The application of a structural equation model alone cannot derive or model the shapes of PRR or reveal underlying mechanisms. Instead, it encourages us to leverage its advantages in combination with other methods within the integrative framework.

Integration analysis with dynamical models to explain the shapes of PRR

In order to predict the variation of species richness in PRR and elucidate the underlying mechanisms, ecologists have previously developed integrative models such as the CSR strategy, non-equilibrium interaction model, multispecies patch-occupancy model, resource-ratio model, and modified neutral model (Grime, 1974; Huston, 1979; Hastings, 1980; Tilman, 1982; Kadmon and Benjamini, 2001). These models, with their respective differences, aimed to understand the mechanisms of plant diversity and could be integrated to explain the humped shape of PRR, which was widely accepted by many ecologists at that time (Figure 3A). To explain the shapes of SRPR, integrative models were developed to characterize inter-specific competitive interactions among randomly chosen species and a spatially structured ecosystem competing for a limiting soil nutrient. These models were based on complementary effects, inter-specific facilitation, and selection effects, which provided an explanation for why species richness had positive effects on productivity (Tilman et al., 1997; Loreau, 1998; Loreau et al., 2001; Figure 3B). These theoretical approaches represented early integration analyses with dynamical models and significantly contributed to the understanding of the underlying mechanisms of SRPR.

However, these early integrative models were primarily designed to integrate the important processes suggested (or

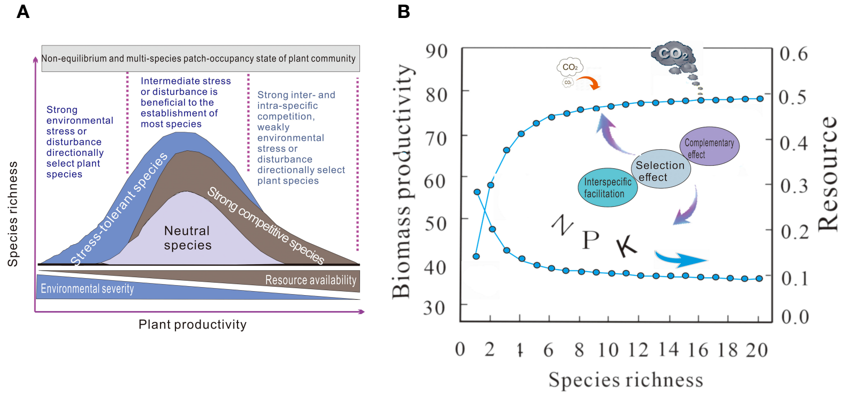


FIGURE 3
Two dominant shapes of PSRR and SRPR in early integrative studies. **(A)** Humped shape of PSRR: The figure illustrates the humped shape of PSRR. In this shape, the plant community exists in a non-equilibrium state with multi-species patch occupancy along a gradient of resource availability and environmental severity. On the high environmental severity side, strong environmental stress or disturbance selects for stress-tolerant species adapted to such conditions, resulting in low species richness. Conversely, on the high resource availability side, strong competitive species dominate the competition for limiting resources, such as light, excluding other species that freely immigrate but are not adapted to such competitive habitats, leading to low species richness. Intermediate levels of stress or disturbance between the two sides favor both neutral and stress-tolerant species, and strong competitive species can also thrive with neutral species, allowing for the coexistence of multiple species and maintaining high richness. **(B)** Relationship among species richness, productivity, and resource-use intensity in SRPR: The figure depicts the relationship among species richness, productivity, and resource-use intensity in SRPR. An ecosystem with high species richness exhibits complementarity in resource use, leading to increased resource absorption by plants and higher productivity. At the same time, inter-specific competition is intense in the ecosystem. Additionally, as species richness increases, more productive and reciprocal species occur in the ecosystem, resulting in high productivity. This phenomenon is attributed to the selection effect and inter-specific facilitation, where more productive species are favored and occur in greater numbers as species richness increases.

excluded) by researchers to explain (or support) the widely accepted shapes of PRR. While these studies made efforts to reveal the mechanisms of PRR, the focused integrative methods weakened the universality of the results regarding the diverse shapes of PRR. Recent integrative analyses using dynamical models have taken a different approach. On one hand, they have moved away from focused studies that only consider a few processes related to the dominant shapes of PRR, such as the effects of environmental heterogeneity, resource availability, plant density, trait variability, etc., to clarify the underlying mechanisms (Hodapp et al., 2016; Wang, 2017; Hodapp et al., 2018; Wang et al., 2019). On the other hand, unlike early integration, these analyses have attempted to incorporate as many processes as possible that have been identified by ecologists as factors influencing plant richness and productivity (Box 1). These integrative analyses focus on two types of methods: using statistical dynamic models to test the shapes of PRR observed in literature and field studies, and using differential dynamic models to integrate multiple processes in order to derive the shapes of PRR and analyze the underlying mechanisms.

Statistical dynamic model

To address the limitations of early integrative studies that focused only on dominant shapes of PRR, ecologists have employed statistical dynamic models. These models combine statistical and dynamic methods, originating from weather forecasting models, to test the occurrence ratios of different shapes of PRR in previous species-assembly experiments and field investigations (Cardinale et al., 2007; Adler et al., 2011). One commonly used statistical dynamic model is meta-analysis, which analyzes study cases to determine the shapes of PRR as a function of various dynamic factors such as scales, investigation methods, plant taxa, grains, and regions (Mittelbach et al., 2003; Gillman and Wright, 2006; Cardinale et al., 2007; Whittaker, 2010). Meta-analyses have indicated that, while there is still debate regarding the shapes of PRR, the humped shape is dominant for PSRR in all collected cases, with a relatively lower probability of occurrence for other shapes such as negative, U-shaped, and unrelated forms (Mittelbach et al., 2003; Adler et al., 2011; Fraser et al., 2015; Figure 4A). For SRPR, a positive or asymptotic shape is dominant compared to other shapes (Cardinale et al., 2007; Duffy et al., 2017). It is evident that scales, investigation methods, and plant taxa influence these statistical results. However, meta-analysis fails to capture the changes in PRR and the relationships between different shapes of PPR, as it provides static results without considering the impact of plant productivity, diversity, or other processes affecting PRR. Nevertheless, statistical models are valuable tools for identifying and validating the shapes of PRR in previous study cases within the framework (Figure 1).

Another statistical dynamic model is the use of simple regression with empirical equations or direct regression analysis to demonstrate the different shapes of PRR based on field sampling results (Axmanová et al., 2012; Steudel et al., 2012; Huang et al., 2018). In such models, the coefficient of species richness (independent variable) is utilized to determine the shapes of SRPR corresponding to the sampling results

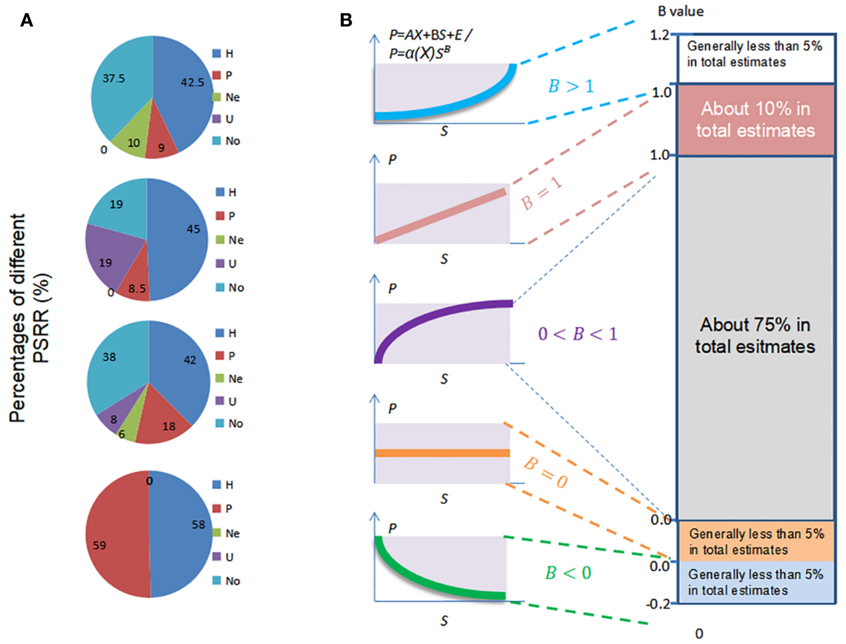


FIGURE 4
Shapes of PSRR and SRPR based on multiple references cited in the text. **(A)** Statistical results of the shapes of PSRR observed in study cases at various scales, including local, landscape, regional, and continental to global scales. The shapes are represented by the following abbreviations: H (humped), P (positive), Ne (negative), U (U-shaped), and No (unrelated). **(B)** Sampling results of the shapes of SRPR based on the coefficient *B* representing the effect of tree diversity on forest productivity. Left: *B* values ranging from 0 to 1 correspond to positive and asymptotic shapes, while *B* $\leq$ 0 corresponds to level and negative shapes. Right: Dominance of different shapes based on the distribution of the sampling data. Tree diversity is represented by *S*, and productivity is represented by *P*. Adapted from Liang et al. (2016b).

(Figure 4B). For instance, an empirical dynamical model $P=\alpha{\cdot}f(X){\cdot}S^B$(P, productivity; X, environmental factors such as soil and climate; S, species richness; α, coefficient; B, the effects of species richness on productivity) was employed to quantify the dependence of productivity on species richness and measure the marginal productivity, which represents the change in productivity resulting from a one-unit decline in species richness, while accounting for climatic, soil, and plot-specific covariates (Liang et al., 2016b). When $B > 1$, the shape of SRPR is concave-down; when $B = 1$, the shape is positive; when $1 > B > 0$, the shape is asymptotic; when $B = 0$, the shape is parallel (no effect); when $B < 0$, the shape is negative. Direct sampling data from various sources indicated that the average θ was 0.26, suggesting a predominantly positively asymptotic shape. Other forms occupied only a small percentage. A sampling study across the Amazon Basin, involving 90 one-hectare plots, also demonstrated the dominant positively asymptotic effect of taxonomic and evolutionary diversity on productivity, which was separated from environmental factors using generalized least-squares modeling (De Souza et al., 2019). These field sampling results were consistent with meta-analyses of other ecologists' studies, although meta-analysis represents a secondary form of sampling (Hooper et al., 2005; Grace et al., 2007; Forrester & Bauhus, 2016; Duffy et al., 2017).

The statistical dynamical models based on field sampling are effective and straightforward approaches for identifying the shapes of PRR. Additionally, by utilizing a coefficient known as marginal productivity—the change in productivity resulting from a one-unit decline in species richness—the relationship between different shapes of PRR can be defined in a simple manner. However, these models have limited flexibility in considering variables other than productivity and species richness (represented by variable X). This limitation hinders the ability to reveal the interactions among these processes since X is often quantified using linear methods rather than non-linear ones (Liang et al., 2016b). In reality, the non-linear interactions of other processes significantly impact PRR, as demonstrated by earlier studies examining interactions among disturbance, competition, stress, resource availability, and more (Grime, 1974; Huston, 1979; Hastings, 1980; Tilman, 1982). Unfortunately, the statistical dynamical models fail to adequately quantify these non-linear interactions of other processes, leading to increased errors in explaining the shapes of PRR.

Differential dynamical model

Some ecologists argue that PRR is governed by diverse and complex processes, and to clarify the shapes of PRR, it is necessary to assess the different effects of these processes on plant richness and productivity and simulate their interactions (Willig, 2011; Grace et al., 2014; Wang, 2017). In line with this perspective, a set of differential equations, known as the PSRR model, was established based on the positive and/or negative effects of 21 widely accepted processes on plant productivity and species richness, as identified in the relevant literature (Wang et al., 2019). These equations integrate the effects of these processes into a comprehensive measure of plant productivity, allowing for the derivation of the shapes of PSRR.

Each process is assigned a different parameter value to represent its strength, and these parameter values can be adjusted to regulate the strengths of the processes. Plant richness is explicitly defined as a dependent variable, while plant productivity serves as an independent variable in the equations, quantifying the effects of plant productivity on species richness. Subsequently, the PSRR model is transformed into the SRPR model, which represents the feedback relationships to PSRR. In the SRPR model, plant productivity is determined as a dependent variable, and species richness as an independent variable. Using the PSRR model, the five typical shapes of PSRR, the dynamics of IICE (Box 1), and the effects of the species pool on these shapes with increasing productivity were derived and verified using field data (Wang et al., 2019; Figure 5). It was observed that the shapes of PSRR can change from one shape to another by altering the parameter values representing the strengths of the processes. Since the same set of parameters is used in the SRPR model, the diverse shapes of SRPR can also be derived. These derivations indicate that different strengths of processes acting on species richness and productivity give rise to different shapes of PSRR and SRPR. Specifically, when the integrated processes show a dominant positive effect, the shape of PSRR or SRPR is linear or asymptotic; when the integrated processes show a dominant negative effect, the shape of PSRR or SRPR is negative; and when the integrated processes successively show a dominant positive and negative effect, the shape of PSRR or SRPR is humped. These integrative methods can explain the documented PSRR and SRPR patterns observed in empirical studies conducted over several decades on various terrestrial, freshwater, and marine taxa from different regions of the world (Mittelbach et al., 2003; Gillman & Wright, 2006; Whittaker, 2010; Grace et al., 2014; Liang et al., 2016b; Fichtner et al., 2017). Furthermore, these results reveal the connections between the different shapes of PSRR and SRPR and the underlying processes (Wang, 2017; Wang et al., 2019).

While the differential dynamical model offers a flexible solution for revealing the dynamical interactions of different processes affecting PRR and can elucidate the mechanisms underlying PRR, it is challenging to determine the coefficients of the numerous variables in the model. Moreover, the shapes of PRR derived or modeled using this non-linear differential model are generally diverse and require validation using field sampling data. Therefore, the structural equation model and statistical dynamical model can complement the limitations of the differential dynamical model within an integrative framework.

The recent integrative studies using statistical and differential dynamic models (Cardinale et al., 2007; Liang et al., 2016a, b; Wang et al., 2019) have improved the limited universality of results obtained by earlier studies that primarily integrated only a few processes to explain the accepted dominant shapes of PRR. The differential dynamical model provides insights into why and how the diverse PRR patterns discovered by statistical dynamical models based on meta-analysis and field sampling occur in the real world. Based on the differential dynamic model, it has been found that: (i) ecological processes that have a positive or negative effect on plant richness and productivity in PSRR and SRPR can vary temporally or spatially; (ii) processes that have a strongly positive effect at one productivity or richness level may have a weakly positive or negative effect at another level; and (iii) the integration of all positive and/or negative effects of processes, species richness, and plant productivity into a total effect (which continually changes but may be positive or negative) fundamentally determines the shapes of PSRR and SRPR (Wang et al., 2019; Leclère et al., 2020). However, these integrative methods still require further improvement. Theoretically, integrative methods are based on the analysis of processes affecting plant richness and productivity to establish dynamical models of PRR (Tilman et al., 1997; Loreau, 1998; Wang et al., 2019). The parameter values representing the effects of processes on PRR in dynamical models are often assumed and subjectively determined, although many derived PRR shapes have been validated by field data. Such an approach can influence the reliability of the derived PRR shapes. Therefore, within the framework of explaining PRR, we propose that the parameter values representing the effects of processes on PRR in the PRR dynamical models should be determined by quantifying the roles of different processes in the regulation of PPR in the field using a structural equation model (Figure 1).

Conclusions

PRR has been a subject of extensive debate and research in ecology. Over time, research on PRR has progressed through distinct stages, including the identification of different PRR shapes, investigations of influencing processes, and integrative studies involving vegetation analysis, manipulation experiments, and theoretical analysis. The central focus of the debate has been on determining the dominant shapes of PRR and understanding the underlying mechanisms.

Recent integrative research, which involves analyzing and integrating the effects of respective processes influencing PRR, has revealed that the humped, asymptotic, positive, negative, and irregular shapes of PRR are interconnected. These shapes are not fixed, and one shape of PRR can transition into another. The balance between the positive and negative effects of different processes plays a crucial role in determining the various shapes of PRR. Furthermore, this balance leads to plant diversity having a globally positive effect on plant productivity and other ecosystem functions.

Respective and integrative research represent two types of methods employed to study the ecological processes influencing PRR. Respective research focuses on testing the effects of individual processes on PRR and uncovering the underlying mechanisms. Integrative research, on the other hand, examines the relative roles and interactions of processes in regulating PRR in real-world settings, as well as the relationships between different PRR shapes. PRR is considered a fundamental ecological issue that spans populations, communities, ecosystems, and landscapes. Ecologists have long been

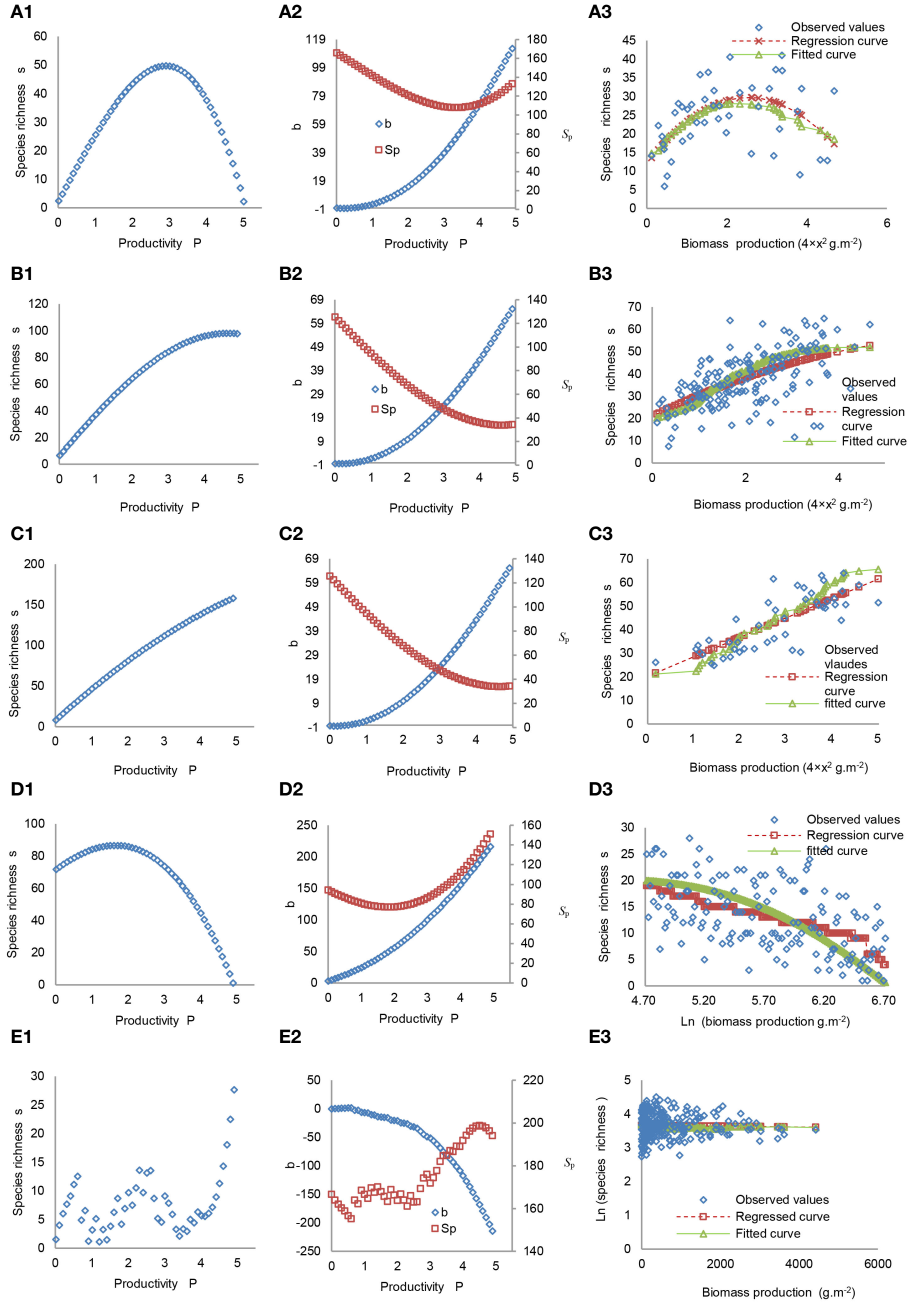


FIGURE 5
Typical Shapes of PSRR. **(A1–E1)** These curves represent the humped, asymptotic, positive, negative, and irregular shapes, respectively, derived by the PSRR model, which incorporates almost all processes affecting species richness. Adapted from Wang et al. (2019). **(A2–E2)** These curves illustrate the dynamics of intra- and inter-specific competition effects (b) and the potential species-pool effect (Sp), which directly influence the shapes of PSRR. **(A3–E3)** These curves depict the observed species richness along a productivity gradient at a local plot across Germany, Czech Republic, Russia, USA, and Australia, respectively. The regression curves represent the results fitted based on these observed species richness and productivity. The fitted curves correspond to the outcomes obtained by fitting the observed data with the PSRR model. Notably, there was no significant difference between the fitted and observed species richness.

interested in PRR and the ecological processes that affect it, which has led to the development of various ecological theories.

Future studies on PRR should emphasize the relationships between metabolic rates related to resource availability and productivity, gene mutation rates, and increasing plant diversity, as these factors are evolutionarily significant. It is essential to identify the relative importance of each process and understand their interactions for the advancement of integrative studies. While significant progress has been made in understanding PRR, it is crucial for ecologists to carefully differentiate between the two types of PRR influenced by respective and integrative processes. Confusion between these types of PRR and different research methods can contribute to additional debates and challenges in the field.

Author contributions

ZW: Conceptualization, Funding acquisition, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. JA: Software, Validation, Writing – review & editing. TY: Data curation, Methodology, Software, Writing – review & editing. CZ: Data curation, Formal Analysis, Writing – original draft. AC: Conceptualization, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by the Fundamental Research Funds for the central Universities (No.300111230018; No. 300102292902).

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

References

Abrams, P. A. (1995). Monotonic or unimodal diversity-productivity gradients: what does competition theory predict? *Ecology* 76, 2019–2027. doi: 10.2307/1941677

Adler, P. B., Seabloom, E. W., Borer, E. T., Hillebrand, H., Hautier, Y., Hector, A., et al. (2011). Productivity is a poor predictor of plant species richness. *Science* 333, 1750–1752. doi: 10.1126/science.1204498

Allen, A. P., Brown, J. H., and Gillooly, J. F. (2002). Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science* 297, 1545–1548. doi: 10.1126/science.1072380

Amarasekare, P. (2003). Competitive coexistence in spatially structured environments: a synthesis. *Ecol. Lett.* 6, 1109–1122. doi: 10.1046/j.1461-0248.2003.00530.x

Axmanová, I., Chytrý, M., Zelený, D., Li, C. F., Vymazalová, M., Danihelka, J., et al. (2012). The species richness-productivity relationship in the herb layer of European deciduous forests. *Global Ecol. Biogeography* 21, 657–667. doi: 10.1111/j.1466-8238.2011.00707.x

Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Raffaelli, D., and Schmid, B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecol. Lett.* 9, 1146–1156. doi: 10.1111/j.1461-0248.2006.00963.x

Bezemer, T. M., and van der Putten, W. H. (2007). Diversity and stability in plant communities. *Nature* 446, E6–E7. doi: 10.1038/nature05749

Cardinale, B. J., Hillebrand, H., Harpole, W. S., Gross, K., and Ptacnik, R. (2009). Separating the influence of resource "availability" from resource "imbalance" on productivity-diversity relationships. *Ecol. Lett.* 12, 475–487. doi: 10.1111/j.1461-0248.2009.01317.x

Cardinale, B. J., Wright, J. P., Cadotte, M. W., Carroll, I. T., Hector, A., Srivastava, D. S., et al. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proc. Natl. Acad. Sci.* 104, 18123–18128. doi: 10.1073/pnas.0709069104

Chen, Y., Huang, Y. Y., Niklaus, P. A., Castro-Izaguirre, N., Clark, A. T., Bruelheide, H., et al. (2020). Directed species loss reduces community productivity in a subtropical forest biodiversity experiment. *Nat. Ecol. Evol.* 4, 550–559. doi: 10.1038/s41559-020-1127-4

Chen, S. P., Wang, W. T., Xu, W. T., Wang, Y., Wan, H. W., Chen, D. M., et al. (2018). Plant diversity enhances productivity and soil carbon storage. *Proc. Natl. Acad. Sci.* 115, 4027–4032. doi: 10.1073/pnas.1700298114

Chiarucci, A., Viciani, D., Winter, C., and Diekmann, M. (2006). Effects of productivity on species area curves in herbaceous vegetation: evidence from experimental and observational data. *Oikos* 115, 475–483. doi: 10.1111/j.2006.0030-1299.15116.x

De Souza, F. C., Dexter, K. G., Phillips, O. L., Pennington, R. T., Neves, D., Sullivan, M. J. P., et al. (2019). Evolutionary diversity is associated with wood productivity in Amazonian forests. *Nat. Ecol. Evol.* 3, 1754–1761. doi: 10.1038/s41559-019-1007-y

Duffy, J., Godwin, C., and Cardinale, B. (2017). Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature* 549, 261–264. doi: 10.1038/nature23886

Fichtner, A., Härdtle, W., Li, Y., Bruelheide, H., Kunz, M., and von Oheimb, G. (2017). From competition to facilitation: how tree species respond to neighborhood diversity. *Ecol. Lett.* 20, 892–900. doi: 10.1111/ele.12786

Forrester, D. I., and Bauhus, B. J. (2016). A review of processes behind diversity-productivity relationships in Forests. *Curr. Forestry Rep.* 2, 45–61. doi: 10.1007/s40725-016-0031-2

Foster, B. L., Dickson, T. L., Murphy, C. A., Karel, I. S., and Smith, V. H. (2004). Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. *J. Ecol.* 92, 435–449. doi: 10.1111/j.0022-0477.2004.00882.x

Fraser, L. H., Pither, J., Jentsch, A., Sternberg, M., Zobel, M., Skarizadeh, D., et al. (2015). Worldwide evidence of a unimodal relationship between productivity and plant species richness. *Science* 349, 302–305. doi: 10.1126/science.aab3916

Gillman, L. N., and Wright, S. D. (2006). The influence of productivity on the species richness of plants: a critical assessment. *Ecology* 87, 1234–1243. doi: 10.1890/0012-9658(2006)87[1234:TIOPOT]2.0.CO;2

Goldberg, D. E., and Miller, T. E. (1990). Effects of different resource additions on species diversity in an annual plant community. *Ecology* 71, 213–225. doi: 10.2307/1940261

Grace, J. B., Adler, P. B., Harpole, W. S., Borer, E. T., and Seabloom, E. W. (2014). Causal networks clarify productivity-richness interrelations, bivariate plots do not. *Funct. Ecol.* 28, 787–798. doi: 10.1111/1365-2435.12269

Grace, J. B., Anderson, T. M., Seabloom, E. W., Borer, E. T., Adler, P. B., Harpole, W. S., et al. (2016). Integrative modeling reveals mechanisms linking productivity and plant species richness. *Nature* 529, 390–393. doi: 10.1038/nature16524

Grace, J. B., Anderson, T. M., Smith, M. D., Seabloom, E., Andelman, S. J., Meche, G., et al. (2007). Does species diversity limit productivity in natural grassland communities? *Ecol. Lett.* 10, 680–689. doi: 10.1111/j.1461-0248.2007.01058x

Grime, J. P. (1974). Vegetation classification by reference to strategies. *Nature* 250, 26–31. doi: 10.1038/250026a0

Grossman, J. J., Cavender-Bares, J., Hobbie, S. E., Reich, P. B., and Montgomery, R. A. (2017). Species richness and traits predict overyielding in stem growth in an early-successional tree diversity experiment. *Ecology* 98, 2601–2614. doi: 10.1002/ecy.1958

Hastings, A. (1980). Disturbance, coexistence, history, and competition for space. *Theor. Population Biol.* 18, 363–373. doi: 10.1016/0040-5809(80)90059-3

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G., et al. (1999). Plant diversity and productivity experiments in European grasslands. *Science* 286, 1123–1127. doi: 10.1126/science.286.5442.1123

Hodapp, D., Borer, E. T., Harpole, W. S., Lind, E. M., Seabloom, E. W., Adler, P. B., et al. (2018). Spatial heterogeneity in species composition constrains plant community responses to herbivory and fertilization. *Ecol. Lett.* 21, 1364–1371. doi: 10.1111/ele.13102

Hodapp, D., Hillebrand, H., Blasius, B., and Ryabov, A. B. (2016). Environmental and trait variability constrain community structure and the biodiversity-productivity relationship. *Ecology* 97, 1463–1474. doi: 10.1890/15-0730.1

Hooper, D. U., Chapin, F. S.III, Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., et al. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75, 3–35. doi: 10.1890/04-0922

Huang, Y. Y., Chen, Y. X., Castro-Izaguirre, N., Baruffol, M., Brezzi, M., Lang, A., et al. (2018). Impacts of species richness on productivity in a large-scale subtropical forest experiment. *Science* 362, 80–83. doi: 10.1126/science.aat6405

Hughes, A. R., Byrnes, J. E., Kimbro, D. L., and Stachowicz, J. J. (2007). Reciprocal relationships and potential feedbacks between biodiversity and disturbance. *Ecol. Lett.* 10, 849–864. doi: 10.1111/j.1461-0248.2007.01075.x

Humborg, C., Ittekkot, V., Cociasu, A., and Bodungen, B. V. (1997). Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. *Nature* 386, 385–388. doi: 10.1038/386385a0

Huston, M. A. (1979). A general hypothesis of species diversity. *Am. Nat.* 113, 81–101. doi: 10.1086/283366

Huston, M. A., and DeAngelis, D. L. (1994). Competition and coexistence: the effects of resource transport and supply rates. *Am. Nat.* 144, 954–977. doi: 10.1086/285720

Kadmon, R., and Benjamini, Y. (2001). Effects of productivity and disturbance on species richness: a neutral model. *Am. Nat.* 167, 939–946. doi: 10.1086/504602

Laforest-Lapointe, I., Paquette, A., Messier, C., and Kembel, S. W. (2017). Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* 546, 145–147. doi: 10.1038/nature22399

LaManna, J. A., Belote, R. T., Burkle, L. A., Catano, C. P., and Myers, J. A. (2017a). Negative density dependence mediates biodiversity-productivity relationships across scales. *Nat. Ecol. Evol.* 1, 1107–1115. doi: 10.1038/s41559-017-0225-4

LaManna, J. A., Mangan, S. A., Alonso, A., Bourg, N. A., Brockelman, W. Y., Bunyavejchewin, S., et al. (2017b). Plant diversity increases with the strength of negative density dependence at the global scale. *Science* 356, 1389–1392. doi: 10.1126/science.aar3824

Lasky, J. R., Uriarte, M., Boukili, V. K., Erickson, D. L., Kress, W. J., and Chazdon, R. L. (2014). The relationship between tree biodiversity and biomass dynamics changes with tropical forest succession. *Ecol. Lett.* 17, 1158–1167. doi: 10.1111/ele.12322

Leclère, D., Obersteiner, M., Barrett, M., Butchart, S. H. M., Chaudhary, A., Palma, A. D., et al. (2020). Bending the curve of terrestrial biodiversity needs an integrated strategy. *Nature* 585, 551–556. doi: 10.1038/s41586-020-2705-y

Liang, J. J., Crowther, T. W., Picard, N., Wiser, S., Zhou, M., Alberti, G., et al. (2016b). Positive biodiversity-productivity relationship predominant in global forests. *Science* 354, 1–12. doi: 10.1126/science.aaf8957

Liang, J. J., Watson, J. V., Zhou, M., and Lei, X. (2016a). Effects of productivity on biodiversity in forest ecosystems across the United States and China. *Conserv. Biol.* 30, 308–317. doi: 10.1111/cobi.12636

Loreau, M. (1998). Biodiversity and ecosystem functioning: A mechanistic model. *Proc. Natl. Acad. Sci.* 95, 5632–5636. doi: 10.1073/pnas.95.10.5632

Loreau, M., Naeem, S., Inchasusti, P., Bengtsson, J., Grime, J. P., Hector, A., et al. (2001). Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294, 804–808. doi: 10.1126/science.1064088

Marquard, E., Weigelt, A., Roscher, C., Gubsch, M., Lipowsky, A., and Schmid, B. (2009). Positive biodiversity–productivity relationship due to increased plant density. *J. Ecol.* 97, 696–704. doi: 10.1111/j.1365-2745.2009.01521.x

Michalet, R., Brooker, R. W., Cavieres, L. A., Kikvidze, Z., Lortie, C. J., Pugnaire, F. I., et al. (2006). Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecol. Lett.* 9, 767–773. doi: 10.1111/j.1461-0248.2006.00935.x

Mittelbach, G. G., Steiner, C. F., Scheiner, S. M., and Steiner, C. H. (2003). What is the observed relationship between species richness and productivity? *Reply. Ecol.* 84, 3390–3395. doi: 10.1890/03-3077

Pierce, S. (2014). Implications for biodiversity conservation of the lack of consensus regarding the humped-back model of species richness and biomass production. *Funct. Ecol.* 28, 253–257. doi: 10.1111/1365-2435.12147

Schmid, B. (2002). The species richness–productivity controversy. *Trends Ecol. Evol.* 17, 113–114. doi: 10.1016/S0169-5347(01)02422-3

Stegen, J. C., Enquist, B. J., and Ferriere, R. (2009). Advancing the metabolic theory of biodiversity. *Ecol. Lett.* 12, 1001–1015. doi: 10.1111/j.1461-0248.2009.01358.x

Steudel, B., Hector, A., Friedl, T., Löffler, C., Lorenz, M., Wesche, M., et al. (2012). Biodiversity effects on ecosystem functioning change along environmental stress gradients. *Ecol. Lett.* 15, 1397–1405. doi: 10.1111/j.1461-0248.2012.01863.x

Stevens, M. H. H., and Carson, W. P. (1999). Plant density determines species richness along experimental gradients. *Ecology* 80, 455–465. doi: 10.1890/0012-9658(1999)080[0455:PDDSRA]2.0.CO;2

Sugden, A. M. (2018). Tree diversity improves forest productivity. *Science* 362, 41–43. doi: 10.1126/science.2018.362.6410.twis

Tilman, D. (1982). *Resource competition and community structure* (New Jersey, USA: Princeton University Press). doi: 10.1515/9780691209654

Tilman, D., Lehman, C. L., and Thomson, K. T. (1997). Plant diversity and ecosystem productivity: Theoretical considerations. *Proc. Natl. Acad. Sci.* 94, 1857–1861. doi: 10.1073/pnas.94.5.1857

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., and Lehman, C. (2001). Diversity and productivity in a long-term grassland experiment. *Science* 294, 843–845. doi: 10.1126/science.1060391

Wang, Z. H. (2017). Process strengths determine the forms of the relationship between plant species richness and primary productivity. *PloS One* 12, e0185884. doi: 10.1371/journal.pone.0185884

Wang, Z. H., Chiarucci, A., and Arratia, F. J. (2019). Integrative models explain the relationships between species richness and productivity in plant communities. *Sci. Rep.* 9, 13730. doi: 10.1038/s41598-019-50016-3

Whittaker, R. J. (2010). Meta-analyses and mega-mistakes: calling time on meta-analysis of the species richness-productivity relationship. *Ecology* 91, 2522–2533. doi: 10.1890/08-0968.1

Whittaker, R. J., and Heegaard, E. (2003). What is the observed relationship between species richness and productivity? *Comment. Ecol.* 84, 3384–3390. doi: 10.1890/02-3128

Willig, M. R. (2011). Biodiversity and productivity. *Science* 333, 1709–1710. doi: 10.1126/science.1212453

Wu, J. H., Li, M., Sebastian, F., Ma, W. L., Wang, X. T., Zhang, X. Z., et al. (2019). Impacts of grazing exclusion on productivity partitioning along regional plant diversity and climatic gradients in Tibetan alpine grasslands. *J. Environ. Manage.* 231, 635–645. doi: 10.1016/j.jenvman.2018.10.097

Yenni, G., Adler, P. B., and Ernest, S. K. M. (2012). Strong self-limitation promotes the persistence of rare species. *Ecology* 93, 456–461. doi: 10.1890/11-1087.1

Zobel, M., van der Maarel, E., and Dupré, C. (1998). Species pool: the concept, its determination and significance for community restoration. *Appl. Vegetation Sci.* 1, 55–66. doi: 10.2307/1479085

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 08 May 2024
DOI 10.3389/fevo.2024.1405459

Check for updates

OPEN ACCESS

EDITED BY
Helen McCreery,
Tufts University, United States

REVIEWED BY
Michał Filipiak,
Jagiellonian University, Poland
Madeleine Ostwald,
University of California, Santa Barbara,
United States

*CORRESPONDENCE
Kristin M. Robinson
Robinson.Kristin@outlook.com

RECEIVED 22 March 2024
ACCEPTED 22 April 2024
PUBLISHED 08 May 2024

CITATION
Robinson KM and Baudier KM (2024) Stingless bee foragers experience more thermally stressful microclimates and have wider thermal tolerance breadths than other worker subcastes.
Front. Ecol. Evol. 12:1405459.
doi: 10.3389/fevo.2024.1405459

Stingless bee foragers experience more thermally stressful microclimates and have wider thermal tolerance breadths than other worker subcastes

Kristin M. Robinson* and Kaitlin M. Baudier

School of Biological, Environmental and Earth Sciences, The University of Southern Mississippi, Hattiesburg, MS, United States

Introduction: The current state of anthropogenic climate change is particularly concerning for tropical insects, species predicted to be the most negatively affected. Researching climatic tolerance in social insects is challenging because adaptations exist at both individual and societal levels. Division of labor research helps to bridge the gap between our understanding of these adaptations at different scales, which is important because social insects comprise a tremendous portion of global animal biomass, biodiversity, and ecosystem services. Considering how individual physiologies construct group-level adaptations can improve climate change impact assessments for social species. *Tetragonisca angustula* is a neotropical stingless bee species that exhibits high worker subcaste specialization with a morphologically distinct soldier caste.

Methods: We used this species to investigate 1) whether age- and size-differentiated subcastes differ in thermal tolerance, 2) which worker subcaste operates closest to their thermal limits, and 3) the extent to which this species selects active foraging times to offset thermal stress. We measured the thermal tolerance (CT_{max} and CT_{min}) of small-bodied foragers and two soldier subcastes (hovering guards and standing guards) in *T. angustula*.

Results and discussion: Despite body size differences between foragers and guards, no differences in the upper or lower thermal limits were observed. However, the average thermal tolerance breadth of foragers was significantly larger than that of guards, and foraging sites were more thermally variable than nest sites, supporting the Climatic Variability Hypothesis at a microclimate scale and in the context of division of labor. Warming tolerance was significantly lower among small-bodied foragers compared to hovering and standing guards. The magnitude of warming tolerances indicated low risk of imminent climate change impacts in this environment but suggests that increasing temperatures and heatwave prevalence may cause foragers to meet their upper thermal limits before other subcastes. Foraging occurred at a narrower range of temperatures than would challenge critical temperatures, with higher morning activity. Directionally increasing temperatures will likely confine these preferred foraging temperatures to a narrower time window. Further study is needed to elucidate how foragers may shift times of activity in response to anthropogenic warming, but changing climates may impact plant pollination rates in natural and agricultural systems.

KEYWORDS

climate change, Jataí, Meliponini, Microclimates, social insects, *Tetragonisca angustula*, thermal tolerance, warming tolerance

Introduction

While there are a wide range of abiotic threats currently faced by insects, anthropogenic climate change is of particular concern, especially in the tropics where increasingly variable temperature and precipitation patterns are predicted to be most intense (Deutsch et al., 2008; Diamond et al., 2012; Gonzalez et al., 2021; IPCC, 2022). Because the tropics have less seasonal variation in temperature, species adapted to tropical environments have narrower ranges of climatic tolerance, as stated by the Climatic Variability Hypothesis (CVH) or Janzen's rule (Janzen, 1967; Addo-Bediako et al., 2000; Gaston et al., 2009; Sunday et al., 2019). The performance of ectothermic animals are highly subject to changes in climate because they either match their body temperature to the environment or they behaviorally thermoregulate to change their internal temperature (Clusella-Trullas et al., 2011; Sunday et al., 2019). Small ectothermic animals, like insects, are even more highly subject to variations in temperature and humidity due to their lower surface area to volume ratio, less lipid storage, and high metabolic rates (Gibbs et al., 2003; Bujan et al., 2016). Tropical insects with narrow ranges of thermal tolerance, as predicted by the CVH, are particularly at risk of thermal stress due to anthropogenic climate change (Deutsch et al., 2008; Diamond et al., 2012).

Eusocial insects are particularly in need of further climate change studies because of their contribution to the planet's biomass, biodiversity, and proficiency at implementing ecosystem services such as biological control, seed dispersal, and pollination (Elizalde et al., 2020). It is predicted that climate change will reduce social insect species-level and colony-level ranges, having major implications for agriculture and natural ecosystems (Friedman et al., 2019; Souza-Junior et al., 2020; Gonzalez et al., 2021; Maia-Silva et al., 2021). When studying eusocial insects, it is essential to account for both individual-level and colony-level variation to understand how species respond to climatic stressors (Baudier and O'Donnell, 2017). Overlooking intracolony variation in thermal performance may lead to improperly estimating climatic risk and conservation needs. Because eusocial insects have different castes that are all essential to the functioning of the colony, it is important to assess whether one caste may operate closer to its functional limits, acting as a limiting factor for colony-level (Baudier and O'Donnell, 2017; Menzel and Feldmeyer, 2021).

In many eusocial insects, such as in stingless bees (Tribe: Meliponini), previous studies have used estimates of thermal tolerance to predict responses to climate change (Torres et al., 2007; Macías-Macías et al., 2011; da Silva et al., 2017; Hrncir et al., 2019; Souza-Junior et al., 2020; Maia-Silva et al., 2021; Gonzalez et al., 2022c), but division of labor is rarely taken into account. The threat of increasing climate variability to stingless bees is high and these insects are among the most diverse and abundant pollinators in the tropics (Hrncir et al., 2016; Quezada-Euán et al., 2018). Due to its high degree of worker sub-caste specialization (Grüter et al., 2012, Grüter et al., 2017; Baudier et al., 2019), the stingless bee *Tetragonisca angustula* is a species of particular interest for researching how division of labor may mitigate or exacerbate the effects of climate variation on a bee colony. *Tetragonisca angustula* is one of very few bee species known to exhibit a morphologically distinct soldier subcaste (Grüter et al., 2012), a characteristic most commonly found and studied in termites and ants (Abbot, 2022). Colonies of *T. angustula* have two types of guards within the soldier subcaste: hovering guards that hover outside the nest entrance and standing guards that patrol the nest entrance on foot (Wittmann, 1985; Bowden et al., 1994; Kärcher & Ratnieks, 2009; Grüter et al., 2012). These guards exhibit discrete division of labor and do not repeatedly switch back and forth between hovering and standing guarding (Grüter et al., 2011), and they exhibit age polyethism with their task allocations shifting as they age from adult forager to hovering guard and finally to the role of standing guard (Baudier et al., 2019). In addition to this species' conservation value as a widespread native neotropical pollinator, understanding the thermal adaptations of *T. angustula* also has applied value in agriculture. This species is one of the most common stingless bees used in farming operations across Latin America from Southern Mexico to Southern Brazil (Jaffé et al., 2015; Quezada-Euán et al., 2018).

The high degree of division of labor in *T. angustula* makes them a good model species in which to compare how different worker subcastes respond to temperature stressors. Here we address the following questions: 1) Do age- and size-differentiated task groups differ in thermal tolerance? 2) which worker subcaste operates closest to its thermal limits in its respective functional microclimate? 3) Do foragers use behavioral thermoregulation, in the form of shifting their times of foraging activity, to offset the thermal stress of these functional microclimates?

Thermal performance variation across morphologically distinct worker-subcastes within colonies is poorly studied in stingless bees, likely because bee species that exhibit these polymorphic worker subcastes are less common. However, in ant species with polymorphic workers, higher heat tolerance and lower cold tolerance typically correspond to a larger body size trend (Cerdá and Retana, 1997; Baudier et al., 2018; Roeder et al., 2021) that is more often observed in the tropics than in temperate regions (Roeder et al., 2021). When looking at a microclimate level, this size-thermal tolerance relationship seems to be stronger in more stable environments where the species are adapted to less climatic variation (Baudier et al., 2018). Based on this, we would predict that the larger-bodied guards would be able to withstand higher temperatures than smaller workers. Cold tolerance appears to be more complex and less related to size (Baudier et al., 2018; Roeder et al., 2021). However, even in tropical systems, measuring cold tolerance is important as it can be more geographically limiting (Bishop et al., 2017), and it allows for the calculation of thermal tolerance breadth which indicates how much thermal variability each individual can tolerate (Sánchez-Echeverría et al., 2019).

Few studies have assessed this size-tolerance relationship within colonies of individual bee species. Studies have been conducted comparing across individuals within the same species, but not necessarily within the same colony, which report no effect of body size on upper or lower thermal tolerance within three subspecies (Maebe et al., 2021), and an effect of larger body size decreasing lower thermal tolerance and increasing upper thermal tolerance only in one of three species assessed (Oyen et al., 2016). Many studies have been conducted regarding the size-thermal tolerance relationship between species of bees, or between populations of a single bee species, with conflicting results. A study by da Silva et al. (2021) in Fiji indicated no effect of body size on upper or lower thermal tolerance across several bee species except one species that had higher thermal tolerance among larger-bodied bees, and further studies have also found no effect of body size on CT_{max} across several species in temperate climates (Hamblin et al., 2017; Burdine and McCluney, 2019), across lab-reared *Bombus* subspecies (Maebe et al., 2021), and across populations of *Apis mellifera* in Mexico (Sánchez-Echeverría et al., 2019; Barreiro et al., 2024). Other studies in temperate climates have found that increasing body size is correlated with decreasing cold tolerance across and within several species (Peters et al., 2016), and a significant effect of increasing body size on increasing heat tolerance and decreasing cold tolerance across three *Bombus* species (Oyen et al., 2016). In a few examples of tropical bee species, one study reports larger body sizes across four *Bombus* species were correlated to lowered CT_{min} (when looking within species, this trend was only maintained in one species) while there was no effect on CT_{max} across species (Gonzalez et al., 2022a), and another study reports no strong relationship between size and thermal tolerance across stingless bee species (Gonzalez et al., 2022c). It is not yet clear whether there is a reliable size-tolerance relationship trend in bee species, and it is especially unclear whether any trends exist between polymorphic castes within genetically similar colonies of a single species.

Age may also affect thermal tolerance, but its role is currently unclear. A review by Bowler and Terblanche (2008) indicated that there is a general trend across insect taxa of decreasing upper thermal tolerance as an individual advances through life stages which would lead us to predict that the age transition of hovering guards to standing guards would follow this trend. However, when just looking at adult stages, tolerance is highest at eclosion and decreases during early adulthood to a stable level with age, but the effect of age on thermal tolerance is complex and species-dependent (Bowler and Terblanche, 2008). Some studies on social insects find no effect of age on thermal tolerance (Oyen and Dillon, 2018; Baudier et al., 2022), and some results are more complex, showing freshly eclosed ants with higher CT_{min} but no effect of age on CT_{max} (Baudier and O'Donnell, 2016). Many of these studies that examine how thermal tolerance changes with age in social insects usually compare young, freshly eclosed adults to all other adults (Baudier and O'Donnell, 2016; Oyen and Dillon, 2018; Roeder et al., 2021; Baudier et al., 2022), or compare life stages such as larvae to adults (Mitchell et al., 1993; Kingsolver and Buckley, 2020). There is not as much work comparing middle-aged adults to older adults (Bowler and Terblanche, 2008), especially for social insects, as would be the case for the comparison between hovering guards to standing guards in *T. angustula*.

In addition to the possible effects of size and age, the foragers have to spend more time compared to the guards in thermally variable environments away from the nest, which is thermally regulated via worker behavior and nest site selection (Jones and Oldroyd, 2006; Torres et al., 2007; Hrncir et al., 2019; Perez and Aron, 2020; Maia-Silva et al., 2021). Under the CVH, we predict that the foragers would have a wider range of thermal tolerance. Most tests of the CVH focus on large-scale adaptations to biogeographic contrasts in climate or species-level differences in tolerance (Bishop et al., 2017; Sunday et al., 2019; Sklenář et al., 2023) rather than assessing differences in microclimates across microhabitats experienced within colonies of eusocial insects (e.g. Kaspari et al., 2015; Baudier et al., 2018; Villalta et al., 2020). In fact, not evaluating microclimate effects can lead to inaccurately assessing climate change risk (Pincebourde and Casas, 2015). Applying the CVH to a microclimate scale, improves our understanding of how individual physiologies function in the context of the colony as a superorganism (Johnson and Linksvayer, 2010). Not only are foragers likely experiencing more variable environments, but they are likely also facing more extreme high temperatures when foraging compared to the guards performing their tasks at the nest. This leads us to predict that the foragers might be operating closer to their thermal limits than the guards. If this is the case, we also predict that foragers may employ behavioral strategies to adjust the times at which the forage to reduce their exposure, such as was found by Maia-Silva et al. (2021).

Methods

Field site

All field and lab work were conducted on-site at La Selva Research Station (10.43°N, 84.00°W), a lowland tropical rainforest in Heredia province, Costa Rica from May to June of 2022 and 2023.

Testing for differences in thermal tolerance across subcaste

To assess the differences in thermal tolerance across subcastes of *T. angustula*, we estimated the critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) of the most abundant worker subcastes that spend the most time outside of the nest: foragers, hovering guards, and standing guards, using the dynamic method (Lutterschmidt and Hutchison, 1997). We subjected 5 foragers, 5 hovering guards, and 5 standing guards from each of the 10 colonies to each thermal tolerance assay (CT_{max} and CT_{min}) in 2022, for a total of 150 bees for the CT_{max} assay and 150 bees for CT_{min}. All bees only underwent one of the two assays. One additional forager, hovering guard, and standing guard were collected from each colony to act as controls (bees placed in the assay apparatus, but not exposed to thermal ramping). Similar to Baudier et al. (2019), foragers were identified as bees that immediately flew away upon exiting the nest and were not carrying detritus which would indicate a waste-removal worker. Guards were bees observed engaging in guarding tasks at the nest entrance for at least 20 seconds each. Standing guards were identified as those guards standing in place on the nest entrance tube while facing the entrance during those 20 seconds. Hovering guards were identified as those flying or hovering in place while facing towards the nest entrance.

We collected bees via a combination of aerial net, forceps, and aspirator from their nests into 33 mL (9 dram) vials each with a paper towel moistened with DI water. Each vial was transported inside of an insulated bag which contained an ice pack at the bottom wrapped with a towel to keep the bees from experiencing heat or cold stress during transportation. A Thermochron iButton data logger (Maxim Integrated, San Jose, California) was placed in the bag with the vials to measure the temperature during transport. Transport temperature averaged 22.9°C which was not much cooler than the lab environment (averaging 24.3°C) where the assays were conducted. The time from when bees were collected until the assay began was less than 2 hours. We placed each bee individually into 1.5 mL microcentrifuge tubes with a drop of approximately 1.7 mg commercially processed *Apis mellifera* honey per tube. Preliminary data collection showed that supplying honey reduced starvation-associated mortality over the course of the assay, as evidenced by improved (100%) control survival. Providing a source of food and hydration also minimized variation in starvation and hydration statuses among subject bees before the assay began. The caps of the tubes were filled with cotton and closed to prevent the bees from seeking thermal refuge in the tops of the tubes.

Tubes were haphazardly placed within the dry heating or cooling block depending on their respective assignment. The assay began with the blocks set at 30°C for both cooling and heating. We ramped the temperature up for CT_{max} or down for CT_{min} by 2°C every 10 minutes (averaging 0.2°C min^{-1}). Slower ramping rates offer finer resolution, enabling detection of small differences among individuals. This was the slowest ramping rate that was comparable to other bee thermal tolerance studies but was not so slow as to elicit signs of starvation or desiccation (as evidenced by control performance) (Gonzalez et al., 2022b).

Mobility was checked every 10 minutes after each 2°C change. Mobility loss was defined as a lack of movement aside from the onset of spasms after tapping or flipping the tube to elicit a movement response (Lutterschmidt and Hutchison, 1997; Terblanche et al., 2011), representing the loss of the ability of an animal to escape its thermal environment or perform functions necessary for survival (Mitchell et al., 1993; Kovac et al., 2014; da Silva et al., 2017). The most extreme temperature at which a bee retained mobility was considered its CT_{max} or CT_{min}, respectively. The controls were placed in the same type of microcentrifuge tube under the same conditions for the same duration as the assays, except they were held at room temperature which averaged 24.3°C. At the end of the assay, all bees were frozen at -15°C for a minimum of 24 hours before being placed in a drying oven at 50°C for 3 days. Preliminary tests showed no change in mass after 48 hours at this temperature, and previous studies on much larger bee species have found similar temperatures between 50 - 60°C to be sufficient for obtaining dry mass (Hagen and Dupont, 2013; Ostwald et al., 2022). Dry weights were then measured to the nearest 0.1 mg.

For the statistical analysis, we first checked whether the dry mass differed among subcastes as previous studies indicated using a generalized linear mixed effect analysis with colony as the random variable (Table 1). We used separate generalized linear mixed effect analyses with colony as a random variable to assess whether CT_{max} or CT_{min} differs among subcaste (Table 1). Because CT_{max} and CT_{min} are count-like integer data, the Poisson statistical family was used for these analyses. For these models, only subcaste was used as a predictor variable rather than including both subcaste and dry mass since dry mass and subcaste are highly correlated. Thermal tolerance breadth was calculated as the difference between the average CT_{max} and the average CT_{min} per subcaste per colony. We analyzed the difference in thermal tolerance breadth across subcaste with a linear mixed effect model that included colony as a random variable (Table 1).

Comparing warming tolerance across subcastes

To assess how close each worker subcaste is to their thermal limit while performing daily tasks in their respective microclimates, we calculated warming tolerance for each individual bee. An animal's warming tolerance represents how much an organism's environment can warm before it affects the organism's critical functioning (Deutsch et al., 2008; Diamond et al., 2012). Warming tolerance was calculated as the difference between each bee's CT_{max} and the average highest daily temperature they experienced in their respective microclimate outside the nest. For foragers, these microclimate sites were areas where *T. angustula* was observed foraging on flowers or resin during haphazard searches for these resources throughout the forest, and natural and human-made clearings. Three floral foraging sites were found in 2023 (a palm inflorescence, *Clibadium* sp., and *Stachytarpheta* sp.). Resin foraging sites were located with other bee species present, but none with *T. angustula*.

To record these temperatures, Hygrochron iButton data loggers (Maxim Integrated, San Jose, California) were placed for approximately three days outside each nest, recoding data every 15 minutes. We placed each iButton within a 3D-printed white plastic housing that prevented direct sunlight and rainfall from encountering the probe while allowing for free airflow around it (The University of Southern Mississippi Eagle Maker Hub; Supplementary Figure S1). Each iButton was placed within 15 cm laterally at each nest entrance. This distance was chosen to be as close as possible to the nest entrance while not disturbing the activity of the bees, indicated by foragers approaching

TABLE 1 Summary of mixed effect statistical models.

Statistical Model	Statistical Family	Response Variable	Predictor Variable(s)	Random Variable
Generalized linear mixed effect	Inverse gaussian	Dry mass (mg)	~ Subcaste	+ (1|Colony ID)
Generalized linear mixed effect	Poisson	CT_{max}	~ Subcaste	+ (1|Colony ID)
Generalized linear mixed effect	Poisson	CT_{min}	~ Subcaste	+ (1|Colony ID)
Linear mixed effect model	Gaussian	Thermal tolerance breadth	~ Subcaste	+ (1|Colony ID)
Linear mixed effect model, weighted to account for heteroscedasticity	Gaussian	Warming tolerance	~ Subcaste	+ (1|Colony ID)

Statistical families were chosen as the best fit for the model based on assumptions of residual normality, residual homoscedasticity, data type, and AIC scores.
All statistical analyses were conducted using R statistical software version 4.1.3.

but withdrawing, and guards turning away from the nest entrance to face the data logger. The iButtons at each foraging site were placed within approximately 30 cm of a resource being foraged by *T. angustula*. We estimated warming tolerance as the difference between the average warmest daily temperature recorded by these iButtons and each bee's CT_{max}. The warmest average daily temperature was calculated for the guards by averaging the highest temperature experienced outside their respective nest for each day. Because the foragers were collected directly from their colonies, they could not be associated directly with one foraging site over another. The warmest average daily temperature for foragers was averaged across foraging sites across days to be used as the environmental temperature for all foragers. Both foraging site and nest entrance temperature data were collected in 2023. We compared the difference in warming tolerance between subcastes by using a weighted linear mixed effect model with colony as a random variable (Table 1). The weights were included to account for the heteroscedasticity caused by outliers in the otherwise normally distributed data.

Comparing foraging and nest site microclimates

To understand whether the daily temperatures of foraging sites differ from nest sites during the day (0600 - 1800), the temperature data collected by the iButtons (as described in the previous section) were also used to calculate the average hourly temperatures at each foraging site and nest site. Daytime temperatures (0600 - 1800) were subset from one full day (0000 - 2359) recorded from each foraging and nest site in 2023. The temperatures were averaged for every hour at each site. Levene's Test was used to assess whether foraging sites were more variable than nesting sites. A Mann Whitney U test was used to assess whether the medians differed significantly between foraging sites and nesting sites.

Influence of temperature on forager activity

The daily activity of foragers was recorded at the nest sites and the foraging sites. In 2023, each nest and foraging site were visited once during two-hour intervals from 0530 to 1730, approximately sunrise to sunset. These visits did not always occur within the same day for each colony because data was not collected during rainy weather when the bees were not active. At the foraging sites, the times that foragers were present or absent were recorded while an iButton was recording temperature data. At the nesting sites, the forager activity data was recorded as the number of foragers leaving the nest per minute which was recorded at 8 nests. The temperature data at the nests was not recorded concurrently with the activity data, but the nest temperature was compiled from 13 nests. From these data, we compared the average hourly temperatures throughout the day to the times when the bees were active to see if there were differences between the available temperatures and the selected temperatures at each foraging site and nest site.

In 2022, we also concurrently recorded forager activity while recording nest temperatures data using iButtons. Forager activity was still measured as the number of foragers leaving per minute which was able to be directly correlated to temperature data at each nest to provide a comparison between the peak forager activity and the peak temperature throughout the day.

Results

Differences in thermal tolerance across subcaste

Despite the morphological, age, and task differences, standing guards, hovering guards, and foragers did not differ in CT_{max} ($n = 150$; $\chi^2 = 0.099$, df = 2, $p = 0.952$; Figure 1A) or CT_{min} ($n = 151$; $\chi^2 = 0.363$, df = 2, $p = 0.834$; Figure 1B). Dry mass was significantly different among the subcastes ($\chi^2 = 22.596$, df = 2, $p < 0.001$; Supplementary Figure S2). Foragers were significantly smaller than the hovering guards (Tukey HSD; $p < 0.001$) and standing guards (Tukey HSD; $p < 0.001$) by an average of 10%. Even when testing whether just body size is a predictor variable of CT_{max} or CT_{min}, no significant effect was found (generalized linear mixed effect model with Poisson distribution: CT_{max} $\chi^2 = 0.093$, df = 1, $p = 0.761$; CT_{min} $\chi^2 = 0.078$, df = 1, $p = 0.781$; Supplementary Figure S3).

When assessing thermal tolerance breadth, the difference between the upper and lower thermal tolerances, there was a significant difference among the subcastes (χ^2 = 7.412, df = 2, p = 0.025; Figure 1C). The foragers exhibited a significantly larger thermal tolerance breadth compared to the hovering guards (Tukey HSD; p = 0.041) and a marginally significantly larger thermal tolerance breadth than standing guards (Tukey HSD; p = 0.058). On average, the foragers have a thermal tolerance breadth that is 0.67 ± 0.02°C larger than the guards.

Warming tolerance across subcaste

To assess how close each worker subcaste is operating to its upper thermal tolerance, warming tolerance was compared across subcastes and a significant difference was found (χ^2 = 918.06, df = 2, $p < 0.001$; Figure 1D). Foragers had the smallest warming tolerance in comparison to the hovering guards (Tukey HSD; $p < 0.001$) and standing guards (Tukey HSD; $p < 0.001$). On average, the warming tolerance of the foragers was 3.38 ± 0.06°C lower than the guards. Body size was not included as a predictor of warming tolerance in this model because it was colinear with subcaste, and because dry mass did not have a significant effect on CT_{max} (Supplementary Figure S4).

Foraging versus nest site microclimates

Foraging sites had significantly more variable temperatures than the nesting sites (Levene's Test: $F_{1,190}$ = 10.317, p = 0.002; Figure 2). The median temperature of the foraging sites (29.22°C) was also significantly greater than the median temperature of the nesting sites (27.85°C) (Mann-Whitney U: W = 3507.5, p = 0.020; Figure 2). The median of the foraging sites was 1.37°C greater than the nesting sites.

Temperature and forager activity

The time at which all three foraging sites had foragers present was between 0900 to 1100 which ranged in hourly temperature across sites between 28.00 - 39.78°C with an overall average temperature of 32.92°C. For each foraging site, the respective range of times and temperatures at which there were foragers present was as follows 0900 to 1100: 28.00 - 29.40°C; 0700 to 1100: 26.36 - 34.39°C; 0700 to 1300: 26.52 - 40.42°C. Foraging site temperatures peaked between 1100 to 1200 with the sites ranging from 28.05 - 40.42°C with an overall average of 33.46°C (Figure 3A). The times when the foragers were most active at the nests was between 0800 and 1400 which ranged in temperature across sites from 24.26 - 36.75°C with an overall average temperature of 29.38°C, and the peak activity was between 1000 and 1200 when the temperature ranged between 26.63 - 36.75°C and averaged 30.56°C (Figure 3B). The peak temperature at the nest sites, while lower in magnitude than at the foraging sites, was still within the same time range from 1100 to 1200 with the sites ranging from 27.11 - 36.75°C and averaging 31.01°C.

When comparing forager activity at the nest site to temperature intensity, the peak of temperature intensity was between 1200 and 1400 which ranged from 29.8 - 31.6°C with an average of 30.6°C (Figure 4A), while the peak of forager activity between 1000 and 1200 ranged in temperature from 26.8 - 30.6°C and averaged 28.53°C (Figure 4B).

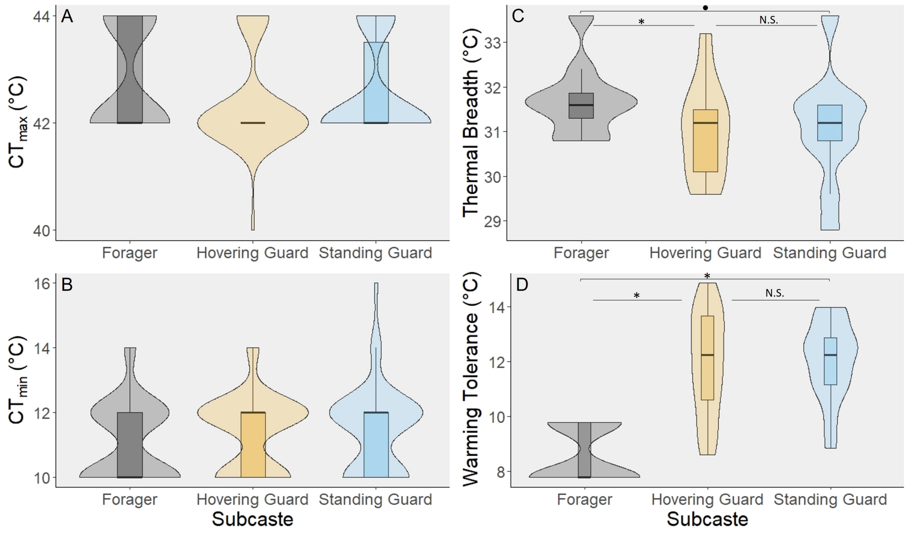


FIGURE 1

Tetragonisca angustula worker subcastes did not differ in critical thermal tolerance limits **(A, B)** but differ in thermal tolerance breadth and warming tolerance **(C, D)**. Violin plots layered over each box plot represent the distribution of data as a shape. Results of *post-hoc* Tukey HSD tests are shown graphically as follows: *indicates $p < 0.05$, • indicates $0.05 < p < 0.10$, and N.S. indicates $p > 0.1$. For both CT_{max} [**(A)**, n=150] and CT_{min} [**(B)**, n=151], the spread of the data is strongly hourglass-shaped and concentrated at every 2°C due to the ramping rate of the assay and how often the bees were checked for mobility loss. **(C)** Because thermal tolerance breadth shows the difference between CT_{max} and CT_{min} for each subcaste, the values for CT_{max} and CT_{min} were averaged for each subcaste for each colony (n = 30). **(D)** Warming tolerance (n=150) is calculated as the average daily high temperature subtracted from CT_{max}. This number represents how close an organism is regularly operating to their upper thermal limit.

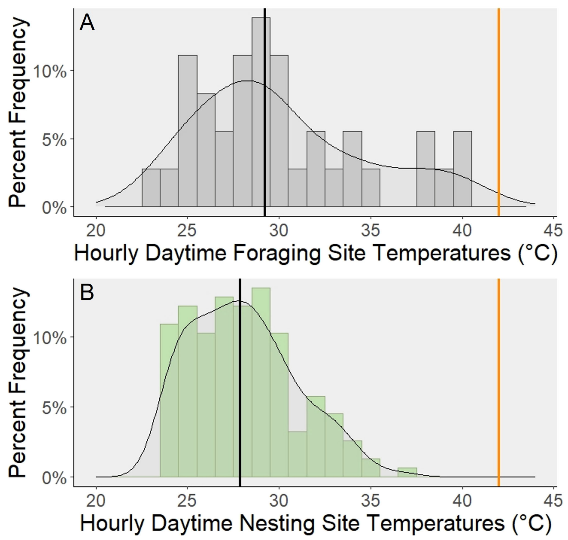


FIGURE 2
Comparison of daytime (0600 - 1800) temperatures between two microclimates experienced by different worker subcastes. These histograms show the percentage of temperatures that fall within each range of temperatures throughout the day. These daytime temperatures (0600 - 1800 were subset from one full day (0000 - 2359) recorded at each site. The curve fitted to each plot is a density curve. The bold vertical orange line on the righthand side represents the median upper thermal tolerance (CT_{max}) of all subcastes. The bold vertical black line represents the median temperature for each site type. **(A)** The daytime temperatures across 3 floral foraging sites are shown on the top panel. **(B)** The daytime temperatures across 13 nest sites are shown in the bottom panel.

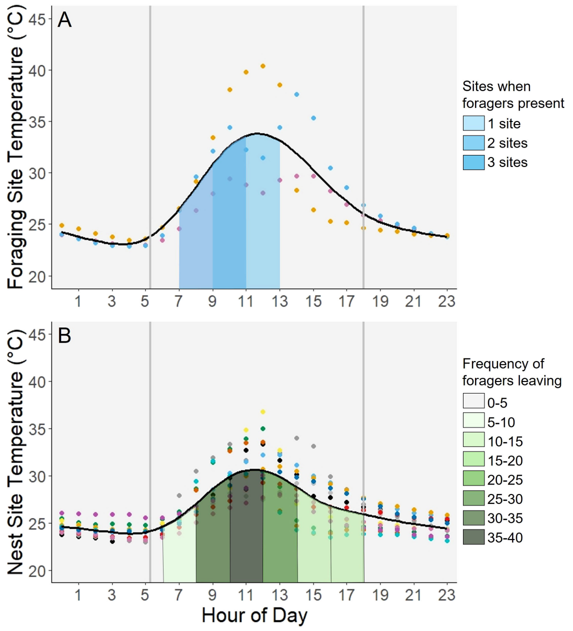


FIGURE 3
Comparison of presence at foraging sites and activity at nest sites. The above graphs show the change in temperature throughout the day at foraging sites (n=3) and nesting sites (n=13). Each color of dot corresponds to a specific foraging or nest site. The vertical gray lines represent sunrise and sunset. The curved line fit to the graphs was added using a Generalized Additive Model to produce a smoothed line representing the average. **(A)** Forager activity at the foraging sites was measured as presence or absence of *T. angustula* foragers. For each foraging site, the space under the curve was shaded when foragers were present. The overlap of these shaded portions, where the color is darkest, represents the time periods when all sites had active foragers. **(B)** The number of foragers leaving each nest per minute was averaged and binned so that darker shaded areas on the graph would indicate a higher average number of foragers leaving the nest. The temperature data was recorded from 13 nests. Activity data was recorded and averaged from 8 nests.

Discussion

Despite the differences in body size, age, and task group, no difference was found between the upper and lower tolerances among foragers, hovering guards, or standing guards. This does not follow the trend within colonies of tropical ants for larger-bodied workers to have higher heat tolerance and lower cold tolerance (Cerdá and Retana, 1997; Baudier et al., 2018; Roeder et al., 2021). Our results more closely follow the lack of relationship between intraspecific size variation and thermal tolerance in bumblebees (Maebe et al., 2021) and honeybees (Sánchez-Echeverría et al., 2019; Barreiro et al., 2024), and are in-line with cross-species comparisons of tropical stingless bees that found no relationship between body size and either upper or lower thermal tolerance (Gonzalez et al., 2022c). However, we report that foragers did have a larger thermal tolerance breadth than hovering guards or standing guards. Finding significant differences with thermal tolerance breadth but no difference when individually assessing CT_{max} and CT_{min}, suggests that the effect of body size on thermal tolerance may be small, only appearing when calculating the average breadth for each subcaste in each colony perhaps due to the resolution of our CT_{max} and CT_{min} assays being 2°C. We also found that foraging sites were more thermally variable during the day than nest sites. Although this effect of subcaste on thermal tolerance breadth was small, it supports the Climatic Variability Hypothesis at a microclimate scale within social insect colonies: workers that experience more variable microclimates while performing their tasks have a larger thermal tolerance breadth.

We found that foragers had significantly lower warming tolerances at foraging sites than the guards at their most common patrolling site just outside of nest entrances. This is consistent with previous studies reporting that stingless bee foragers experience greater thermal risk than any other workers (Perez and Aron, 2020; Maia-Silva et al., 2021). The warming tolerance values for the foragers are still well above zero which does not indicate an imminent risk of approaching upper thermal tolerance limits while performing daily tasks. However, with predicted global temperature increases (IPCC, 2022), foragers will likely be the subcaste most at risk. By the end of the 21st century, the average annual temperature in Costa Rica is predicted to increase by approximately 4°C (Hidalgo et al., 2013; Almazroui et al., 2021). However, this predicted increase is an estimated average value, meaning that the daily high temperatures experienced by the foragers during different times of the year could be well above that 4°C, especially because heatwaves are also increasing in intensity and frequency (Wu et al., 2023). Therefore, not only might the foragers reach their upper critical temperature in their

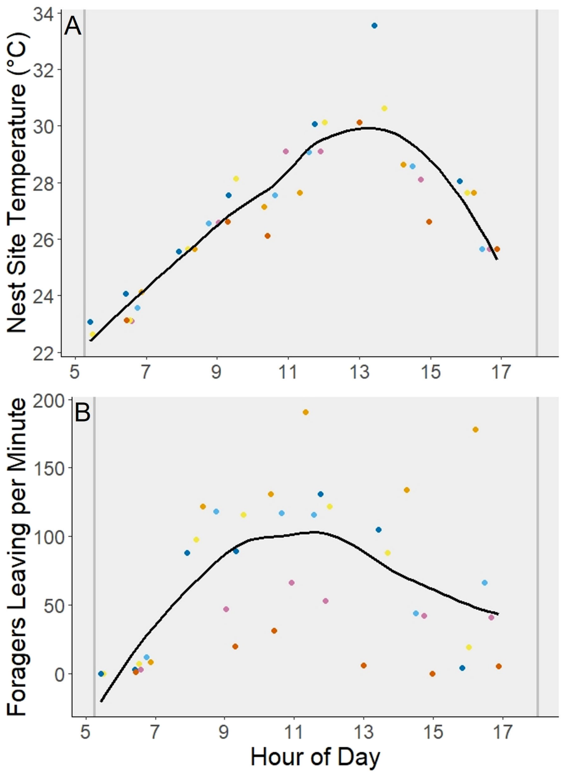


FIGURE 4
Comparison of daytime nest temperatures to forager activity data. Each color of dot corresponds to a specific nest site (n = 6). At each time point, the temperature **(A)** and the number of foragers leaving per minute **(B)** were recorded such that the data directly correspond. The vertical gray lines represent sunrise and sunset. The curved line fit to the graphs was added using the Locally Weighted Scatterplot Smoothing method to produce a smoothed line representing the average.

functional environments sooner than predicted, but foragers likely experience sub-lethal thermal stress long before reaching their upper critical temperature.

For the thermal tolerance assays, we provided honey to reduce control mortality even though some studies suggest that providing sugar sources increases the thermal tolerance of individuals in some eusocial species (Macías-Macías et al., 2011; Bujan and Kaspari, 2017; Maia-Silva et al., 2021; but see: Oyen and Dillon, 2018; Gonzalez et al., 2022b). Aside from being a necessary step to reduce mortality in our assay, we believe that this methodology may mimic a more realistic scenario in which bees are able to seek out food and hydration to reduce the effects of extreme temperatures. Providing honey in these assays may result in overestimates of thermal tolerance (and, in turn, warming tolerance), however this still allows us to compare between worker subcastes within each colony. Additionally, the choice of ramping rate for this study was selected to be slow enough to detect intracolony differences and to mimic the rate of similar studies on bees while not being so slow to cause desiccation or starvation (Gonzalez et al., 2022b). Comparing thermal tolerance studies with different ramping rates and starting temperatures should be done with caution because a faster ramping rate has the potential to estimate a lower CT_{min} by 1.1 - 2.6°C and a higher CT_{max} by 5.3 - 6.9°C (Gonzalez et al., 2022b). Again, this may limit comparisons between this study and other studies but does not hinder our ability to compare subcastes within the colony.

The thermal stress experienced by foragers during peak temperatures at foraging sites may be driving other trends in forager activity. The foraging sites sampled, while few, showed forager activity to be shifted slightly earlier in the day when temperatures were less intense compared to the activity of the foragers leaving the nest (Figure 3). More specifically, when comparing the times of forager activity at the nest and temperature intensity, it also seems to indicate that the activity does not necessarily correspond and peak at the same time as the temperature (Figure 4). Activity at foraging sites peaked earlier in the day, with bees not as likely to return to foraging sites at similar temperatures in the afternoon. Based on these coupled time and temperature preferences, warming temperatures may cause foragers to continue shifting their times of activity to narrower windows of time earlier in the morning.

Future studies should be conducted investigating whether the driving factor of peak foraging activity is due to thermal preferences, flowering time, or interspecific dominance at the sites. This is often done by setting up forager bait traps on a gradient of thermal environments, however, attempts to attract *T. angustula* to honey bait traps at this site were previously unsuccessful (Breed et al., 1999). We only sampled three foraging sites in this study because although nests were dense, *T. angustula* foragers were sparsely observed across surveyed foraging sites. The lack of *T. angustula* at many surveyed foraging sites may be explained by foraging dominance of other bee species present at the dense patches of understory floral resources which are easier for researchers to find (Johnson and Hubbell, 1974; Nagamitsu and Inoue, 1997; Lichtenberg et al., 2011). Smaller bees, like *T. angustula*, are also known to more commonly forage on smaller, dispersed flowers (Johnson and Hubbell, 1974; Nagamitsu and Inoue, 1997; Lichtenberg et al., 2011). We were also not able to survey upper canopy foraging sites which, according to some studies in the Brazilian Atlantic forests, *T. angustula* more frequently visits than the understory (Ramalho, 2004; Bastos et al., 2020). However, research would need to be conducted to be certain about whether *T. angustula* also forages more often in the upper canopy in Costa Rica because this type of behavior could be dependent on seasonal resource availability and community composition affecting foraging dominance. If *T. angustula* foragers were more prevalent in the upper canopy than the understory at the site of this study, we would be underestimating the thermal risk experienced by the foragers because the canopy is warmer on average than the understory (Bujan et al., 2016).

Lastly, we acknowledge the limitations of some of the microclimate data recorded by the iButtons as we were limited in the number of days sampled at each site. However, this tropical region does not experience major climate fluctuations beyond the transition of the dry to the rainy season. This research occurred during the rainy season, so the microclimate data is more representative of that season. During the rainy season, the activity of bees can fluctuate more based on the amount of precipitation because they avoid flying when there is heavy rain. Because of this, sampling of activity and temperature data was restricted to days of regular activity with less precipitation. Also, the nest temperatures reported in this study recorded via iButton were used for both guard subcastes, however, it is worth noting that standing guards may also experience slight differences in their thermal environment from hovering guards due to conduction with the directly contacted resinous nest tube entrance. Similarly, the foragers experience temperatures that are warmer than ambient air when they make contact with superheated boundary layers of plants in sun patches (Kaspari et al., 2015).

The results of this study suggest that the predominantly small-bodied foragers of *T. angustula* are more physiologically adapted than soldiers to life in variable environments. However, while foraging, foragers also operate closer to their physiological limits. As such, it is likely that increasing environmental temperatures will affect the foragers before affecting the other worker subcastes that also spend time outside of the nest. We also report that foragers show signs of behavioral thermoregulation to buffer extreme thermal conditions by selecting amenable times of day to search for resources (Figures 3, 4). Foragers may also reduce how far they travel to forage when experiencing hotter temperatures, as shown in other stingless bee species (Souza-Junior et al., 2020). It is likely that many ectothermic animals will need to resort to behavioral adaptations in response to climate change rather than physiological adaptations because temperatures are changing faster than an organism could evolutionarily adapt (Sunday et al., 2014; Wong and Candolin, 2015). Among social insects, nest site selection is an important first step for passive nest thermoregulation (Jones and Oldroyd, 2006; Perez and Aron, 2020), but, in the case of rapidly changing temperatures, stingless bees are not capable of easily moving their nests nor can they move them great distances (Cronin et al., 2013). Therefore, most of the behavioral mitigation of thermal stress will likely come from changes in thermoregulatory behaviors.

Behavioral adaptations to mitigate thermal stress in anthropogenically warmed climates can reduce pollination efficiency which is of both conservation and agricultural concern (Wong and Candolin, 2015). Foragers shifting the times that they forage may also affect which flowers receive the most pollination which could affect composition of plant communities in the long-term (Scaven and Rafferty, 2013; Descamps et al., 2021). Here we used physiological data to assess which essential task was the most thermally limited, then focused in-depth on thermally relevant behaviors of that task group. We estimated the average tolerable window of foragers to be from 11.06°C to 42.80°C based on thermal tolerance data, however, behavioral data at foraging sites indicated a narrower preferred foraging range of 28.00°C to 39.78°C, with a bias towards foraging at these temperatures in the morning. The upper range of preferred foraging temperatures is much closer to the average forager's CT_{max} than suggested by the warming tolerances. Were behavioral data to be excluded from this study, the result would be an underestimate of future sub-lethal effects of climate change. In this respect, when studying climate-change effects on populations, especially in social species, behavioral data should be interpreted together with physiological and microclimate data. Research lacking this information could inaccurately estimate species' responses to change.

Data availability statement

The datasets presented in this study can be found in Dryad online digital repository: https://doi.org/10.5061/dryad.0k6djhb7s. Printable 3D designs of the iButton housings used in this study are available via Zenodo: https://doi.org/10.5281/zenodo.10850673. Additional figures and analyses can be found in the Supplementary Material.

Ethics statement

No ethical approval was required to conduct this study as research on insects is not currently subject to IACUC ethical regulations. An effort was made to reduce the number of individuals by only collecting non-reproductive bees from 10 colonies. While a power analysis would be ideal to determine the minimum number of individuals needed, current power analyses available are not well suited to accounting for the replication of social insects at both the individual and the colony level simultaneously. We could not anesthetize individuals before undergoing thermal tolerance assays because common anesthetic methods would have affected the results. The fast ramping rate reduced time spent in the assay as well as minimized the potential of other stressors such as starvation, which providing honey also helped to mitigate. All individuals were euthanized in the freezer as stated in the methods because that was the most feasible method of euthanasia at our field site. While the status of freezing as a humane method to kill insects is currently being debated (Fischer et al., 2023), these bees, aside from the controls, were already dead or close to dying by the end of these assays before being placed in the freezer. The preliminary thermal tolerance assays resulted in the deaths of 71 bees evenly distributed across 6 colonies before our methods were refined to improve transport methods and prevent starvation. Subsequent testing to refine the methodology resulted in 21 deaths. All bees that survived during preliminary tests were released back to their colonies. Approximately half of the bees that died during this study, were also used for an unrelated project that required dead bees to dissect which minimized the number of deaths for that project. We recommend that future studies run an initial small-scale test to ensure the survival of the controls and verify the methods. Even if a species has previously undergone similar assays, as was the case with *T. angustula*, different populations may exhibit differences in their sensitivity.

Author contributions

KR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. KB: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Funding for this work was provided by the University of Southern Mississippi to KB, and additional travel funding was provided to KR by the International Union for the Study of Social Insects North American Section via the Charles Michener Bee Research Grant.

Acknowledgments

We thank the staff at La Selva Research Station and the Organization for Tropical Studies for access to the forests in which this study was conducted. We thank Enrique Castro, Orlando Vargas, Danilo Brenes, Benjamin Sadd, and Karen Robles López for permit assistance, onsite field support, and useful discussion that led to the improvement of this manuscript. We thank the University of Southern Mississippi Eagle Maker Hub for assisting with the design and printing of the iButton housings. Research was conducted with permission from the Costa Rican government (SINAC), permit numbers ACC-PI-re-62-2022 and ACC-PI-LC-169-2023.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1405459/full#supplementary-material

References

Abbot, P. (2022). Defense in social insects: Diversity, division of labor, and evolution. *Annu. Rev. Entomol.* 67, 407–436. doi: 10.1146/annurev-ento-082521-072638

Addo-Bediako, A., Chown, S. L., and Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proc. R. Soc Lond. B* 267, 739–745. doi: 10.1098/rspb.2000.1065

Almazroui, M., Islam, M. N., Saeed, F., Saeed, S., Ismail, M., Ehsan, M. A., et al. (2021). Projected changes in temperature and precipitation over the United States, Central America, and the Caribbean in CMIP6 GCMs. *Earth Syst. Environ.* 5, 1–24. doi: 10.1007/s41748-021-00199-5

Barreiro, J. B., Ratoni, B., Baena-Díaz, F., González-Tokman, D., and Dáttilo, W. (2024). Thermal tolerance of honeybees (*Apis mellifera* L.) changes across an elevation gradient in the Mexican transition zone. *Sociobiology* 71, e10155–e10155. doi: 10.13102/sociobiology.v71i1.10155

Bastos, B. P., Lima, L. C. L., and Da Costa Dórea, M. (2020). Pollen sources used by *Tetragonisca angustula* (Latreille 1811) (Apidae, meliponini) in the atlantic forest, Northern Coast of Bahia. *Sociobiology* 67, 535–544. doi: 10.13102/sociobiology.v67i4.5596

Baudier, K. M., D'Amelio, C. L., Malhotra, R., O'Connor, M. P., and O'Donnell, S. (2018). Extreme insolation: Climatic variation shapes the evolution of thermal tolerance at multiple scales. *Am. Nat.* 192, 347–359. doi: 10.1086/698656

Baudier, K. M., and O'Donnell, S. (2016). Structure and thermal biology of subterranean army ant bivouacs in tropical montane forests. *Insect. Soc* 63, 467–476. doi: 10.1007/s00040-016-0490-2

Baudier, K. M., and O'Donnell, S. (2017). Weak links: How colonies counter the social costs of individual variation in thermal physiology. *Curr. Opin. Insect Sci.* 22, 85–91. doi: 10.1016/j.cois.2017.06.004

Baudier, K. M., Ostwald, M. M., Grüter, C., Segers, F. H. I. D., Roubik, D. W., Pavlic, T. P., et al. (2019). Changing of the guard: mixed specialization and flexibility in nest defense (*Tetragonisca angustula*). *Behav. Ecol.* 30, 1041–1049. doi: 10.1093/beheco/arz047

Baudier, K. M., Ostwald, M. M., Haney, B. R., Calixto, J. M., Cossio, F. J., and Fewell, J. H. (2022). Social factors in heat survival: Multiqueen desert ant colonies have higher and more uniform heat tolerance. *Physiol. Biochem. Zoology* 95, 379–389. doi: 10.1086/721251

Bishop, T. R., Robertson, M. P., Van Rensburg, B. J., and Parr, C. L. (2017). Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants. *Ecol. Entomology* 42, 105–114. doi: 10.1111/een.12364

Bowden, R. M., Garry, M. F., and Breed, M. D. (1994). Discrimination of con- and heterospecific bees by *trigona* (*Tetragonisca*) *angustula* guards. *J. Kansas Entomological Soc.* 67, 137–139. Available at: http://www.jstor.org/stable/25085499.

Bowler, K., and Terblanche, J. S. (2008). Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev.* 83, 339–355. doi: 10.1111/j.1469-185X.2008.00046.x

Breed, M. D., McGlynn, T. P., Sanctuary, M. D., Stocker, E. M., and Cruz, R. (1999). Distribution and abundance of colonies of selected meliponine species in a Costa Rican tropical wet forest. *J. Trop. Ecol.* 15, 765–777. doi: 10.1017/S0266467499001169

Bujan, J., and Kaspari, M. (2017). Nutrition modifies critical thermal maximum of a dominant canopy ant. *J. Insect Physiol.* 102, 1–6. doi: 10.1016/j.jinsphys.2017.08.007

Bujan, J., Yanoviak, S. P., and Kaspari, M. (2016). Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. *Ecol. Evol.* 6, 6282–6291. doi: 10.1002/ece3.2355

Burdine, J. D., and McCluney, K. E. (2019). Differential sensitivity of bees to urbanization-driven changes in body temperature and water content. *Sci. Rep.* 9 1643. doi: 10.1038/s41598-018-38338-0

Cerdá, X., and Retana, J. (1997). Links between worker polymorphism and thermal biology in a thermophilic ant species. *Oikos* 78, 467–474. doi: 10.2307/3545608

Clusella-Trullas, S., Blackburn, T. M., and Chown, S. L. (2011). Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *Am. Nat.* 177, 738–751. doi: 10.1086/660021

Cronin, A. L., Molet, M., Doums, C., Monnin, T., and Peeters, C. (2013). Recurrent evolution of dependent colony foundation across eusocial insects. *Annu. Rev. Entomol.* 58, 37–55. doi: 10.1146/annurev-ento-120811-153643

da Silva, C. R. B., Beaman, J. E., Dorey, J. B., Barker, S. J., Congedi, N. C., Elmer, M. C., et al. (2021). Climate change and invasive species: A physiological performance comparison of invasive and endemic bees in Fiji. *J. Exp. Biol.* 224, jeb230326. doi: 10.1242/jeb.230326

da Silva, M. A., Ferreira, N., da, S., da Silva Teixeira-Souza, V. H., Maia-Silva, C., de Assis de Oliveira, F., et al. (2017). On the thermal limits for the use of stingless bees as pollinators in commercial greenhouses. *Journal of Apicultural Research* 56, 81–90. doi: 10.1080/00218839.2016.1260380

Descamps, C., Jambrek, A., Quinet, M., and Jacquemart, A.-L. (2021). Warm temperatures reduce flower attractiveness and bumblebee foraging. *Insects* 12, 493. doi: 10.3390/insects12060493

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6668–6672. doi: 10.1073/pnas.0709472105

Diamond, S. E., Sorger, D. M., Hulcr, J., Pelini, S. L., Toro, I. D., Hirsch, C., et al. (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Glob Change Biol.* 18, 448–456. doi: 10.1111/j.1365-2486.2011.02542.x

Elizalde, L., Arbetman, M., Arnan, X., Eggleton, P., Leal, I. R., Lescano, M. N., et al. (2020). The ecosystem services provided by social insects: traits, management tools and knowledge gaps. *Biol. Rev.* 95, 1418–1441. doi: 10.1111/brv.12616

Fischer, B., Barrett, M., Adcock, S., Barron, A., Browning, H., Chittka, L., et al. (2023). Guidelines for Protecting and Promoting Insect Welfare in Research. *Insect Welfare Research Society*. doi: 10.13140/RG.2.2.19325.64484

Friedman, D. A., Greene, M. J., and Gordon, D. M. (2019). The physiology of forager hydration and variation among harvester ant (*Pogonomyrmex barbatus*) colonies in collective foraging behavior. *Sci. Rep.* 9, 5126. doi: 10.1038/s41598-019-41586-3

Gaston, K. J., Chown, S. L., Calosi, P., Bernardo, J., Bilton, D. T., Clarke, A., et al. (2009). Macrophysiology: A conceptual reunification. *Am. Nat.* 174, 595–612. doi: 10.1086/605982

Gibbs, A. G., Fukuzato, F., and Matzkin, L. M. (2003). Evolution of water conservation mechanisms in *Drosophila*. *J. Exp. Biol.* 206, 1183–1192. doi: 10.1242/jeb.00233

Gonzalez, V. H., Cobos, M. E., Jaramillo, J., and Ospina, R. (2021). Climate change will reduce the potential distribution ranges of Colombia's most valuable pollinators. *Perspect. Ecol. Conserv.* 19, 195–206. doi: 10.1016/j.pecon.2021.02.010

Gonzalez, V. H., Oyen, K., Aguilar, M. L., Herrera, A., Martin, R. D., and Ospina, R. (2022a). High thermal tolerance in high-elevation species and laboratory-reared colonies of tropical bumble bees. *Ecol. Evol.* 12, e9560. doi: 10.1002/ece3.9560

Gonzalez, V. H., Oyen, K., Ávila, O., and Ospina, R. (2022b). Thermal limits of Africanized honey bees are influenced by temperature ramping rate but not by other experimental conditions. *J. Thermal Biol.* 110, 103369. doi: 10.1016/j.jtherbio.2022.103369

Gonzalez, V. H., Oyen, K., Vitale, N., and Ospina, R. (2022c). Neotropical stingless bees display a strong response in cold tolerance with changes in elevation. *Conserv. Physiol.* 10, coac073. doi: 10.1093/conphys/coac073

Grüter, C., Kärcher, M., and Ratnieks, F. L. W. (2011). The natural history of nest defence in a stingless bee, *Tetragonisca angustula* (Latreille) (Hymenoptera: Apidae), with two distinct types of entrance guards. *Neotropical Entomology* 40, 55–61. doi: 10.1590/S1519-566X2011000100008

Grüter, C., Menezes, C., Imperatriz-Fonseca, V. L., and Ratnieks, F. L. W. (2012). A morphologically specialized soldier caste improves colony defense in a neotropical eusocial bee. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1182–1186. doi: 10.1073/pnas.1113398109

Grüter, C., Segers, F. H. I. D., Menezes, C., Vollet-Neto, A., Falcón, T., von Zuben, L., et al. (2017). Repeated evolution of soldier sub-castes suggests parasitism drives social complexity in stingless bees. *Nat. Commun.* 8, 1–7. doi: 10.1038/s41467-016-0012-y

Hagen, M., and Dupont, Y. L. (2013). Inter-tegular span and head width as estimators of fresh and dry body mass in bumblebees (*Bombus* spp.). *Insect. Soc* 60, 251–257. doi: 10.1007/s00040-013-0290-x

Hamblin, A. L., Youngsteadt, E., López-Uribe, M. M., and Frank, S. D. (2017). Physiological thermal limits predict differential responses of bees to urban heat-island effects. *Biol. Lett.* 13, 20170125. doi: 10.1098/rsbl.2017.0125

Hidalgo, H. G., Amador, J. A., Alfaro, E. J., and Quesada, B. (2013). Hydrological climate change projections for Central America. *J. Hydrology* 495, 94–112. doi: 10.1016/j.jhydrol.2013.05.004

Hrncir, M., Jarau, S., and Barth, F. G. (2016). Stingless bees (Meliponini): senses and behavior. *J. Comp. Physiol. A* 202, 597–601. doi: 10.1007/s00359-016-1117-9

Hrncir, M., Maia-Silva, C., Teixeira-Souza, V. H., da, S., and Imperatriz-Fonseca, V. (2019). Stingless bees and their adaptations to extreme environments. *J. Comp. Physiol. A* 205, 415–426. doi: 10.1007/s00359-019-01327-3

IPCC. (2022). *Climate change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.* (New York, USA: Cambridge University Press). doi: 10.1017/9781009325844

Jaffé, R., Pope, N., Carvalho, A. T., Maia, U. M., Blochtein, B., Carvalho, C. A. L., et al. (2015). Bees for development: Brazilian survey reveals how to optimize stingless beekeeping. *PLoS One* 10, e0121157. doi: 10.1371/journal.pone.0121157

Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *Am. Nat.* 101, 233–249. doi: 10.1086/282487

Johnson, L. K., and Hubbell, S. P. (1974). Aggression and competition among stingless bees: Field studies. *Ecology* 55, 120–127. doi: 10.2307/1934624

Johnson, B. R., and Linksvayer, T. A. (2010). Deconstructing the superorganism: Social physiology, groundplans, and sociogenomics. *Q. Rev. Biol.* 85, 57–79. doi: 10.1086/650290

Jones, J. C., and Oldroyd, B. P. (2006). Nest thermoregulation in social insects. *Adv. Insect Physiol.* 33, 153–191. doi: 10.1016/S0065-2806(06)33003-2

Kärcher, M. H., and Ratnieks, F. L. W. (2009). Standing and hovering guards of the stingless bee *Tetragonisca angustula* complement each other in entrance guarding and intruder recognition. *J. Apicultural Res.* 48, 209–214. doi: 10.3896/IBRA.1.48.3.10

Kaspari, M., Clay, N. A., Lucas, J., Yanoviak, S. P., and Kay, A. (2015). Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biol.* 21, 1092–1102. doi: 10.1111/gcb.12750

Kingsolver, J. G., and Buckley, L. B. (2020). Ontogenetic variation in thermal sensitivity shapes insect ecological responses to climate change. *Curr. Opin. Insect Sci.* 41, 17–24. doi: 10.1016/j.cois.2020.05.005

Kovac, H., Käfer, H., Stabentheiner, A., and Costa, C. (2014). Metabolism and upper thermal limits of *Apis mellifera carnica* and *A. m. ligustica*. *Apidologie* 45, 664–677. doi: 10.1007/s13592-014-0284-3

Lichtenberg, E. M., Hrncir, M., Turatti, I. C., and Nieh, J. C. (2011). Olfactory eavesdropping between two competing stingless bee species. *Behav. Ecol. Sociobiol* 65, 763–774. doi: 10.1007/s00265-010-1080-3

Lutterschmidt, W. I., and Hutchison, V. H. (1997). The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Can. J. Zool.* 75, 1553–1560. doi: 10.1139/z97-782

Macías-Macías, J. O., Quezada-Euán, J. J. G., Contreras-Escareño, F., Tapia-Gonzalez, J. M., Moo-Valle, H., and Ayala, R. (2011). Comparative temperature tolerance in stingless bee species from tropical highlands and lowlands of Mexico and implications for their conservation (Hymenoptera: Apidae: Meliponini). *Apidologie* 42, 679–689. doi: 10.1007/s13592-011-0074-0

Maebe, K., De Baets, A., Vandamme, P., Vereecken, N. J., Michez, D., and Smagghe, G. (2021). Impact of intraspecific variation on measurements of thermal tolerance in bumble bees. *J. Thermal Biol.* 99, 103002. doi: 10.1016/j.jtherbio.2021.103002

Maia-Silva, C., Silva Pereira, J., Freitas, B. M., and Hrncir, M. (2021). Don't stay out too long! Thermal tolerance of the stingless bees *Melipona subnitida* decreases with increasing exposure time to elevated temperatures. *Apidologie* 52, 218–229. doi: 10.1007/s13592-020-00811-z

Menzel, F., and Feldmeyer, B. (2021). How does climate change affect social insects? *Curr. Opin. Insect Sci.* 46, 10–15. doi: 10.1016/j.cois.2021.01.005

Mitchell, J. D., Hewitt, P. H., and van der Linde, T. C. D. K. (1993). Critical thermal limits and temperature tolerance in the harvester termite *Hodotermes mossambicus* (Hagen). *J. Insect Physiol.* 39, 523–528. doi: 10.1016/0022-1910(93)90085-6

Nagamitsu, T., and Inoue, T. (1997). Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. *Oecologia* 110, 432–439. doi: 10.1007/s004420050178

Ostwald, M. M., Fox, T. P., Hillery, W. S., Shaffer, Z., Harrison, J. F., and Fewell, J. H. (2022). Group-living carpenter bees conserve heat and body mass better than solitary individuals in winter. *Anim. Behav.* 189, 59–67. doi: 10.1016/j.anbehav.2022.04.012

Oyen, K. J., and Dillon, M. E. (2018). Critical thermal limits of bumble bees (*Bombus impatiens*) are marked by stereotypical behaviors and are unchanged by acclimation, age, or feeding status. *J. Exp. Biol.* 221, jeb.165589. doi: 10.1242/jeb.165589

Oyen, K. J., Giri, S., and Dillon, M. E. (2016). Altitudinal variation in bumble bee (*Bombus*) critical thermal limits. *J. Thermal Biol.* 59, 52–57. doi: 10.1016/j.jtherbio.2016.04.015

Perez, R., and Aron, S. (2020). Adaptations to thermal stress in social insects: recent advances and future directions. *Biol. Rev.* 95, 1535–1553. doi: 10.1111/brv.12628

Peters, M. K., Peisker, J., Steffan-Dewenter, I., and Hoiss, B. (2016). Morphological traits are linked to the cold performance and distribution of bees along elevational gradients. *J. Biogeography* 43, 2040–2049. doi: 10.1111/jbi.12768

Pincebourde, S., and Casas, J. (2015). Warming tolerance across insect ontogeny: influence of joint shifts in microclimates and thermal limits. *Ecology* 96, 986–997. doi: 10.1890/14-0744.1

Quezada-Euán, J. J. G., Nates-Parra, G., Maués, M. M., Imperatriz-Fonseca, V. L., and Roubik, D. W. (2018). Economic and cultural values of stingless bees (Hymenoptera: Meliponini) among ethnic groups of tropical America. *Sociobiology* 65, 534–557. doi: 10.13102/sociobiology.v65i4.3447

Ramalho, M. (2004). Stingless bees and mass flowering trees in the canopy of Atlantic Forest: a tight relationship. *Acta Bot. Bras.* 18, 37–47. doi: 10.1590/S0102-33062004000100005

Roeder, K. A., Roeder, D. V., and Bujan, J. (2021). Ant thermal tolerance: A review of methods, hypotheses, and sources of variation. *Ann. Entomological Soc. America* 114, 459–469. doi: 10.1093/aesa/saab018

Sánchez-Echeverría, K., Castellanos, I., Mendoza-Cuenca, L., Zuria, I., and Sánchez-Rojas, G. (2019). Reduced thermal variability in cities and its impact on honey bee thermal tolerance. *PeerJ* 7, e7060. doi: 10.7717/peerj.7060

Scaven, V. L., and Rafferty, N. E. (2013). Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Curr. Zoology* 59, 418–426. doi: 10.1093/czoolo/59.3.418

Sklenář, P., Jaramillo, R., Wojtasiak, S. S., Meneses, R. I., Muriel, P., and Klimeš, A. (2023). Thermal tolerance of tropical and temperate alpine plants suggests that 'mountain passes are not higher in the tropics.'. *Global Ecol. Biogeogr* 32, 1073–1086. doi: 10.1111/geb.13678

Souza-Junior, J. B. F., Teixeira-Souza, V. H., da, S., Oliveira-Souza, A., de Oliveira, P. F., de Queiroz, J. P. A. F., et al. (2020). Increasing thermal stress with flight distance in stingless bees (*Melipona subnitida*) in the Brazilian tropical dry forest: Implications for constraint on foraging range. *J. Insect Physiol.* 123, 104056. doi: 10.1016/j.jinsphys.2020.104056

Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., et al. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proc. Natl. Acad. Sci.* 111, 5610–5615. doi: 10.1073/pnas.1316145111

Sunday, J., Bennett, J. M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A. L., et al. (2019). Thermal tolerance patterns across latitude and elevation. *Philos. Trans. R. Soc. B: Biol. Sci.* 374, 20190036. doi: 10.1098/rstb.2019.0036

Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., Rako, L., le Roux, P. C., and Chown, S. L. (2011). Ecologically relevant measures of tolerance to potentially lethal temperatures. *J. Exp. Biol.* 214, 3713–3725. doi: 10.1242/jeb.061283

Torres, A., Hoffmann, W., and Lamprecht, I. (2007). Thermal investigations of a nest of the stingless bee *Tetragonisca angustula* Illiger in Colombia. *Thermochimica Acta* 458, 118–123. doi: 10.1016/j.tca.2007.01.024

Villalta, I., Oms, C. S., Angulo, E., Molinas-González, C. R., Devers, S., Cerdá, X., et al. (2020). Does social thermal regulation constrain individual thermal tolerance in an ant species? *J. Anim. Ecol.* 89, 2063–2076. doi: 10.1111/1365-2656.13268

Wittmann, D. (1985). Aerial defense of the nest by workers of the stingless bee *Trigona (Tetragonisca) angustula* (Latreille) (Hymenoptera: apidae). *Behav. Ecol. Sociobiology* 16, 111–114. doi: 10.1007/BF00295143

Wong, B. B. M., and Candolin, U. (2015). Behavioral responses to changing environments. *Behav. Ecol.* 26, 665–673. doi: 10.1093/beheco/aru183

Wu, S., Luo, M., Zhao, R., Li, J., Sun, P., Liu, Z., et al. (2023). Local mechanisms for global daytime, nighttime, and compound heatwaves. *NPJ Clim Atmos Sci.* 6, 1–13. doi: 10.1038/s41612-023-00365-8

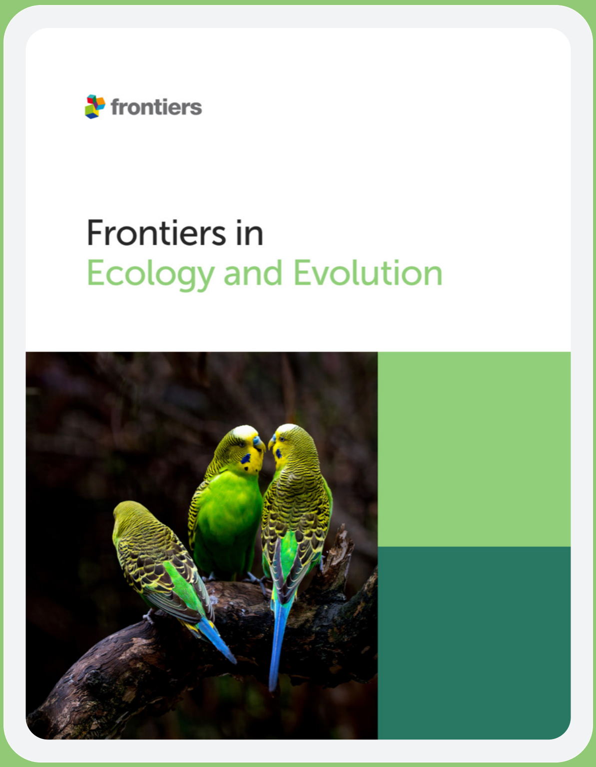
Frontiers in
Ecology and Evolution

Ecological and evolutionary research into our natural and anthropogenic world

This multidisciplinary journal covers the spectrum of ecological and evolutionary inquiry. It provides insights into our natural and anthropogenic world, and how it can best be managed.

Discover the latest Research Topics

See more →



frontiers

Frontiers in
Ecology and Evolution

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact