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Assessing species bias in amphibian research on endocrine disruptors: beyond *Xenopus laevis*

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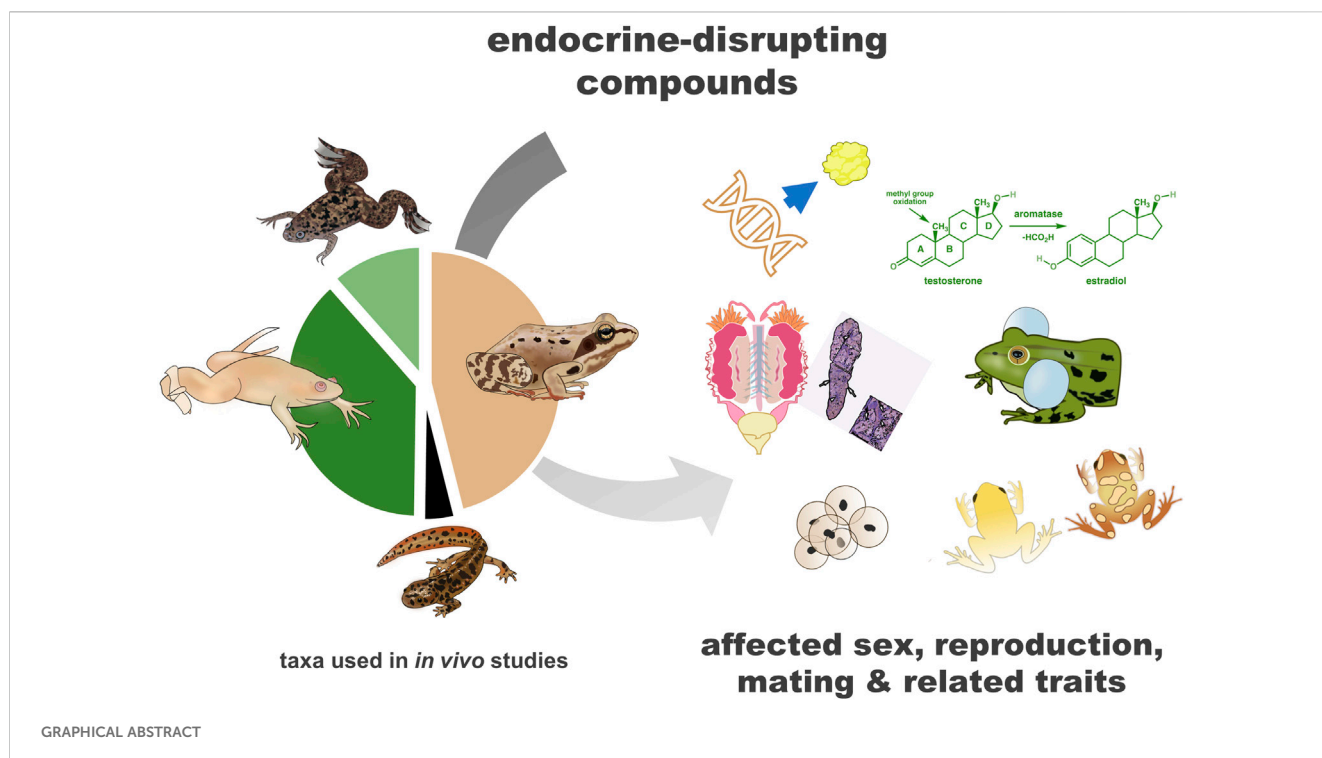
Due to their high sensitivity to hormonal agents, in recent years, amphibians have been proposed as bioindicators for the presence of endocrine-disrupting compounds (EDCs) in the environment. EDCs are a chemically diverse group of compounds, mainly of anthropogenic origin, that can interfere with hormone receptors. The escalating global environmental pollution with endocrine-disrupting compounds (EDCs) poses a significant threat to wildlife and human health. Amphibians are at high risk of exposure to EDCs in the environment, as they produce eggs without a protective shell, possess highly permeable skin, and most of them undergo an aquatic life phase, where they are chronically exposed to EDCs in the water. This exposure raises concerns about the contribution of EDCs to the dramatic decline of amphibian populations and underscores the necessity of environmental-relevant studies in this area. Despite the critical need, amphibians have attracted relatively little research focus in this regard. Although above 200 experimental studies on the topic of EDCs and sex, reproduction, and related traits in amphibians have been conducted, a significant portion of them rely on model species from the *Xenopus* genus, which do not fully represent the diverse group of amphibians. Additionally, these studies often use strict laboratory conditions that fail to mimic natural exposure scenarios. Our extensive review highlights the research gaps and emphasizes the importance of incorporating a broader range of amphibian species to understand the true impact of EDCs. We advocate for more studies in environmentally relevant settings and the use of native species to enhance the ecological validity of findings.

KEYWORDS

EDC, environmental pollution, model species, ecotoxicology, one health

1 Introduction

Amphibians have often been used in experiments concerning the impact of hormonal agents on reproductive parameters (Kloas et al., 2024). The most notable example includes the use of frogs from the *Xenopus* genus in the creation and validation of pregnancy tests (Wallingford, 2022). Due to their high sensitivity to hormonal agents, in recent years, amphibians have also been proposed as possible bioindicators of the presence of endocrine-disrupting compounds (EDCs) in the environment (Lambert et al., 2016). EDCs are concerning, mostly human-generated, exogenous substances that mimic the action of endogenous hormones (European Commission, 1996). EDCs are a chemically diverse group that includes mainly synthetic compounds present in, e.g., pesticides, plasticizers, surfactants, drugs, and personal care products. EDCs can interact with hormone receptors, or by other mechanisms, target estrogenic, androgenic, and thyroid pathways of action (for the review see



La Merrill et al., 2020). These compounds are found in significant quantities in soil, atmosphere, and surface waters worldwide (Ankley et al., 2018; Annamalai and Namasivayam, 2015; Caliman and Gavrilescu, 2009; Singh et al., 2023).

Amphibians are at high risk of exposure to EDCs in the environment, as they possess highly permeable skin and produce eggs without a protective shell (Wells, 2010). Moreover, most of them undergo an aquatic life phase during embryonic and larval development, where they are chronically exposed to all compounds, such as EDCs, in the surrounding water. In the context of the development of body systems and sex differentiation taking place at this time, they are extremely susceptible to the potential harmful effects of EDCs (Huang et al., 2005; Kloas et al., 1999; Kloas, 2002). Because of this, amphibians can act as excellent bioindicators of EDCs' presence in the environment (Lambert et al., 2016), which is important from the perspective of biomonitoring and human health, especially in a comprehensive approach, such as One Health. Studies on the impact of EDCs are crucial also because EDCs are suggested to contribute to the worldwide dramatic decline of amphibian populations, underlining the urgent need for more studies with environmental-relevant settings on this topic (Kloas et al., 2024; Luedtke et al., 2023; Salla et al., 2024a).

In comparison to other groups of vertebrates, particularly fish, amphibians are still an understudied group of animals in the context of endocrine disruptors (Johnson et al., 2017; Kloas and Lutz, 2006; Kloas et al., 2024). It is important to remember that limiting research to only selected taxa carries significant risks, with the most glaring example being the thalidomide scandal, which would not have had such disastrous effects had it been tested on a wider range of species. Thalidomide, a drug prescribed to pregnant women in the late 1950s and early 1960s, caused severe birth

defects in thousands of children because its teratogenic effects were not detected in the limited animal models initially used for testing (Swaters et al., 2022). Apart from studying the impact of EDCs on different groups of vertebrates, the diversity of species should also be taken into account.

In the case of amphibians, some authors suggested, that popular ecotoxicological protocols, such as The Frog Embryo Teratogenesis Assay-Xenopus (FETAX) used by American Society of Testing and Materials (Dumont et al., 1983; Testing and Materials, 1998) for studying potential endocrine-disrupting effects of industrial substances are limited due to the use of the single species from the *Xenopus* genus. The good example is the ongoing discussion on the safety of pesticide atrazine (Hayes et al., 2002; Hoskins et al., 2019; LaFiandra et al., 2008; Spolyarich et al., 2010; Storrs and Semlitsch, 2008), which in the studies on *Xenopus* spp. and other amphibians produced different results. Species diversity in ecotoxicological studies is crucial when thinking about how these findings might apply to different ecosystems and the environment as a whole (Luedtke et al., 2023). The current study was designed as a literature review to explore research gaps in studies on the impact of endocrine-disrupting compounds (EDCs) on the amphibian reproductive system. A primary focus was placed on examining the diversity of species involved in existing research and identifying possible, correlated issues.

2 Methods of literature search

We extensively reviewed the studies published by the end of 2024 on the topic of EDCs and their impact on sex and sex-related traits of amphibians. As sex-related traits, we understood the sex accessory structures: genital ducts and derivatives of these structures; and the secondary sexual characteristics: all differences

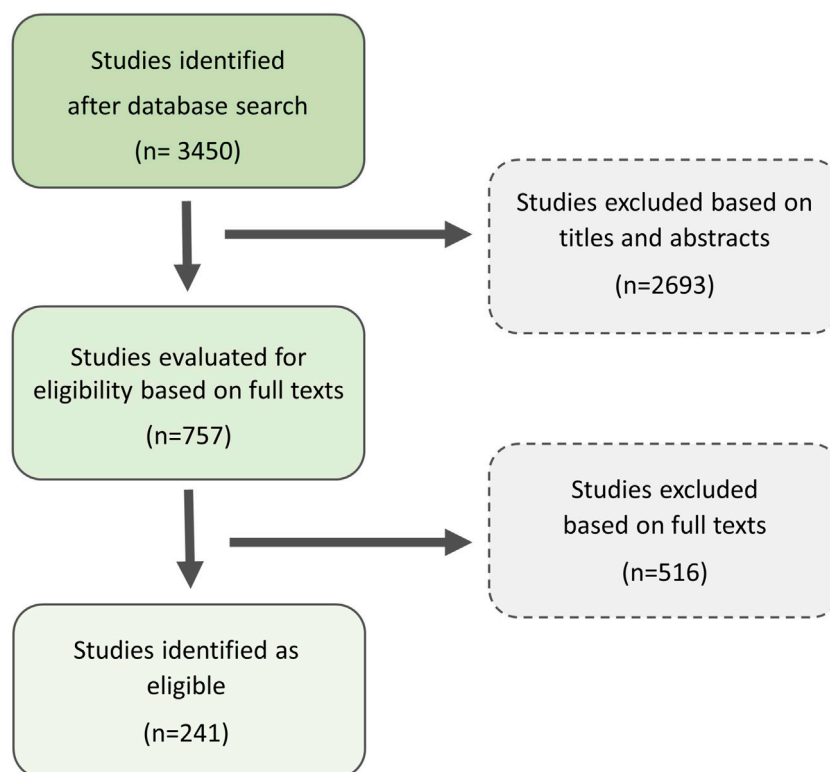


FIGURE 1
Scheme of literature search approach based on two electronic databases for the search: Web of Science Core Collection® and Google Scholar.

between the sexes resulting from sexual maturation, other than gonads and sex accessory structures (Sever and Staub, 2011). In the search, we also included the impact of EDCs on hormone signaling, expression of reproduction-related genes, sexually dimorphic behavior, mating, and fertility of amphibians. We focused on experimental studies. Analysis of research focused on EDCs and thyroid-dependent functions was beyond the scope of this review.

We used two electronic databases for the search: Web of Science Core Collection® and Google Scholar. In the database Web of Science Core Collection® the following Boolean search string was used: TS=(amphibia* OR frog* OR toad* OR newt* OR salamander* OR tadpole* OR caecilian* OR anura* OR caudata* OR urodela* OR apoda*) AND TS=(sex* OR gonad* OR test* OR ovar* OR hormone* OR vitellogenin OR reproduct* OR fertilit* OR oocyt* OR sperm* OR breeding* OR behaviour* OR nuptial pad* OR gene*) AND TI=(“endocrine disruptor*” OR endocrine* OR EDC* OR pollutant* OR pharm* OR estro* OR *estra* OR andro* OR *estradiol* OR EE2* OR *gestagen* OR progesterone* OR levonorgestrel* OR diethylstilbestrol* OR “aromatase inhibitor*” OR flutamide* OR finasteride* OR *phenol* OR *paraben* OR *cide* OR *azine* OR methoxychlor* OR chlorpyrifos* OR organochlorine* OR *dichlorodiphenyl* OR DDD* OR DDE* OR DDT* OR azocyclotin* OR glyphosate* OR Roundup* OR butachlor* OR *phosphate* OR thiophanate* OR triadimefon* OR *azole* OR vinclozolin* OR thiophanate* OR tamoxifen* OR pyrimethanil* OR tributyltin* OR triphenyltin* OR fludioxonil* OR metaxyl* OR MAXIM OR triclosan* OR fertilizer* OR nitrate* OR ammonium* OR “heavy metal*” OR

arsenic* OR chromium* OR cadmium* OR lead* OR surfactant* OR detergent* OR plasticizer* OR “flame retardant*” OR TBBPA* OR TCBPA* OR *phenyl* OR PCB* OR PBDE* OR phthalate* OR styrene* OR *chlor* OR benzene* OR “UV filter*” OR camphor* OR 4-MBC* OR 3-BC* OR trenbolone* OR microcystin* OR MCLR* OR *acid* OR salt*).

A similar strategy of search strategy was used in Google Scholar. The keywords for the search were chosen based on a preliminary literature review and updated each time a new type of EDC or potential EDC was encountered during the search. To select potentially useful studies, the titles and abstracts of 3,450 articles found were first scanned for those remotely relevant to the topic. 757 potentially relevant studies were identified. Next, after reading the full papers, we were left with 241 works, which we included in the review (Figure 1). Studies were chosen if they fulfilled the inclusion criteria: (a) the publication was written in English; (b) the study was experimental and (c) the study provided data on the impact of the exposition of amphibians or amphibian tissue on the chemical compound on sex and/or at least one, defined by us, sex-related trait. The journal or date of publication of the paper was not relevant. Unpublished results from Master’s and PhD Dissertation papers were not included.

The nomenclature of species mentioned in the article follows Frost (2024), with some modifications. A full list of English common and Latin names of all mentioned species, as well as their taxonomy, can be found in the Supporting Information (Supplementary Table S5). For describing changes within the

TABLE 1 Nomenclature, systematic position, sex determination system (GSD – genetic sex determination, TSD – temperature-dependent sex determination) and ecological traits of species of amphibians in experimental studies on the impact of endocrine-disrupting compounds on sex and sex-related traits with external exposition to compound. Please note that some studies used more than one species.

Order, Family	Latin species name	Sex determination system	Ecology of larvae	Diet of larvae	Ecology of adult individuals	IUCN Red List Status Account	Geographical distribution	Number of studies on larvae / on post-metamorphic forms
Anura, Bombinatoridae	<i>Bombina bombina</i>	unknown	lentic: benthic	primarily herbivorous	fossorial, terrestrial, aquatic	LC	Central and Eastern Europe	1/0
Anura, Bombinatoridae	<i>Bombina variegata</i>	XX/XY	lentic: benthic	primarily herbivorous	fossorial, terrestrial, aquatic	LC	terrestrial	1/0
Anura, Bufonidae	<i>Anaxyrus americanus</i>	unknown	lentic/lotic: benthic	herbivorous - detritivorous; scavenger	terrestrial	LC	Eastern North America	2/0
Anura, Bufonidae	<i>Bufo bufo</i>	ZW/ZZ	lentic: benthic	herbivorous - detritivorous	terrestrial, aquatic	LC	widespread in Europe, range extends east to Siberia and south to the North Africa	3/0
Anura, Bufonidae	<i>Bufo gargarizans</i>	ZW/ZZ	lentic/lotic: benthic	herbivorous - detritivorous	terrestrial, aquatic	LC	China, Taiwan	1/0
Anura, Bufonidae	<i>Bufotes viridis</i>	XX/XY	lentic/lotic: benthic	herbivorous - detritivorous	terrestrial, aquatic	LC	Central and Eastern Europe, Sweden, Estonia, range extends east to Siberia, south to the Middle East	5/0
Anura, Bufonidae	<i>Rhinella arenarum</i>	unknown	lentic/lotic: benthic ¹	herbivorous - detritivorous; carnivorous ¹	terrestrial, aquatic	LC	southern parts of South America	1/0
Anura, Bufonidae	<i>Sclerophrys regularis</i>	unknown	lentic: benthic	no data	terrestrial, aquatic	LC	sub-Saharan Africa, from West Africa to Central and East Africa, range extends into parts of North Africa and the Arabian Peninsula	1/0
Anura, Bufonidae	<i>Strauchbufo raddei</i>	XX/XY	lotic: benthic	herbivorous - detritivorous	terrestrial; populations in arid areas littoral	LC	China, Korea, Democratic People's Republic of Mongolia, Russian Federation	0/1
Anura, Bufonidae	<i>Hoplobatrachus rugulosus</i>	GSD unknown, observed TSD	lentic/lotic: suctorial ²	herbivorous; carnivorous ²	terrestrial, aquatic, arboreal	LC	Southeast Asia; widely farmed species	2/0
Anura, Bufonidae	<i>Rhinella icterica</i>	unknown	lentic/lotic: benthic	primarily herbivorous	terrestrial	LC	Argentina, Brazil, Paraguay	1/0
Anura, Dicroglossidae	<i>Euphlyctis cyanophlyctis</i>	ZW/ZZ, observed TSD	lotic: benthic	primarily detritivorous, scavenger, carnivorous	fossorial, terrestrial, aquatic	LC	South Asia	3/0
Anura, Dicroglossidae	<i>Fejervarya limnocharis</i>	XX/XY	lotic/lentic: pelagic, neustonic ³	planktivorous-herbivorous ³	terrestrial, aquatic, arboreal	LC	Southeast Asia; southern parts of Pakistan, Bhutan and China; Japan	0/1

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TABLE 1 (Continued) Nomenclature, systematic position, sex determination system (GSD – genetic sex determination, TSD – temperature-dependent sex determination) and ecological traits of species of amphibians in experimental studies on the impact of endocrine-disrupting compounds on sex and sex-related traits with external exposition to compound. Please note that some studies used more than one species.

Order, Family	Latin species name	Sex determination system	Ecology of larvae	Diet of larvae	Ecology of adult individuals	IUCN Red List Status Account	Geographical distribution	Number of studies on larvae / on post-metamorphic forms
Anura, Dicroglossidae	<i>Fejervarya vittigera</i>	unknown	lotic/lentic: pelagic, neustonic ⁴	planktivorous-herbivorous ⁴	terrestrial, aquatic, arboreal	LC	Philippines	0/1
Anura, Dicroglossidae	<i>Sphaerothera pashchima</i>	unknown	lotic: benthic ⁵	no data	terrestrial ⁵	unknown	India	1/0
Anura, Hylidae	<i>Acris blanchardi</i>	unknown	lotic/lentic: benthic ⁶	primarily herbivorous ⁶	terrestrial, aquatic	LC	North America	3/1
Anura, Hylidae	<i>Dryophytes versicolor</i>	unknown	lotic/lentic	herbivorous - detritivorous	terrestrial, arboreal	LC	North America	3/0
Anura, Hylidae	<i>Hyla arborea</i>	XX/XY, observed TSD	lotic/lentic: benthic, pelagic	herbivorous - detritivorous	terrestrial, arboreal	LC	widespread in Europe, range extends north to Denmark and Sweden, east to Ukraine and southern part of Russia	5/0
Anura, Hylidae	<i>Hyla intermedia</i>	XX/XY	lotic/lentic: benthic, pelagic ⁷	herbivorous - detritivorous ⁷	terrestrial, arboreal	LC	Italy	2/0
Anura, Hyperoliidae	<i>Hyperolius argus</i>	unknown	lentic: benthic	primarily herbivorous	terrestrial, arboreal	LC	Southeast Africa	2/0
Anura, Hyperoliidae	<i>Hyperolius viridiflavus</i>	XX/XY	lentic/lotic: benthic ⁸	primarily herbivorous ⁸	terrestrial, arboreal	LC	West Africa	1/0
Anura, Limnodynastidae	<i>Limnodynastes peronii</i>	unknown	lentic: benthic ⁹	primarily herbivorous ⁹	terrestrial, aquatic, arboreal	LC	Eastern Australia	1/0
Anura, Limnodynastidae	<i>Limnodynastes tasmaniensis</i>	unknown	lentic: benthic ¹⁰	primarily herbivorous ¹⁰	terrestrial, aquatic, arboreal	LC	Australia	1/0
Anura, Pipidae	<i>Xenopus tropicalis</i>	ZWY	lentic: suspension feeder	primarily herbivorous	aquatic	LC	The range covers coastal areas and adjacent regions of West Africa	20/6
Anura, Pipidae	<i>Xenopus laevis</i>	ZW/ZZ	lentic: suspension feeder	primarily herbivorous	aquatic	LC	Sub-Saharan Africa (native range)	61/28
Anura, Ranidae	<i>Glandirana rugosa</i>	XX/XY or ZW/ZZ	lentic: suspension feeder ¹¹	primarily herbivorous ¹¹	terrestrial, aquatic	LC	Japan, Northern and Southwestern Korea, Northeastern China	4/0
Anura, Ranidae	<i>Indosylvirana caesari</i>	unknown	no data	no data	terrestrial, aquatic ¹²	unknown	India	1/0
Anura, Ranidae	<i>Lithobates catesbeianus</i>	XX/XY, observed TSD	lentic: benthic	primarily herbivorous	terrestrial, aquatic	LC	North America; introduced: many parts of South America, Europe, Asia	4/0

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TABLE 1 (Continued) Nomenclature, systematic position, sex determination system (GSD – genetic sex determination, TSD – temperature-dependent sex determination) and ecological traits of species of amphibians in experimental studies on the impact of endocrine-disrupting compounds on sex and sex-related traits with external exposition to compound. Please note that some studies used more than one species.

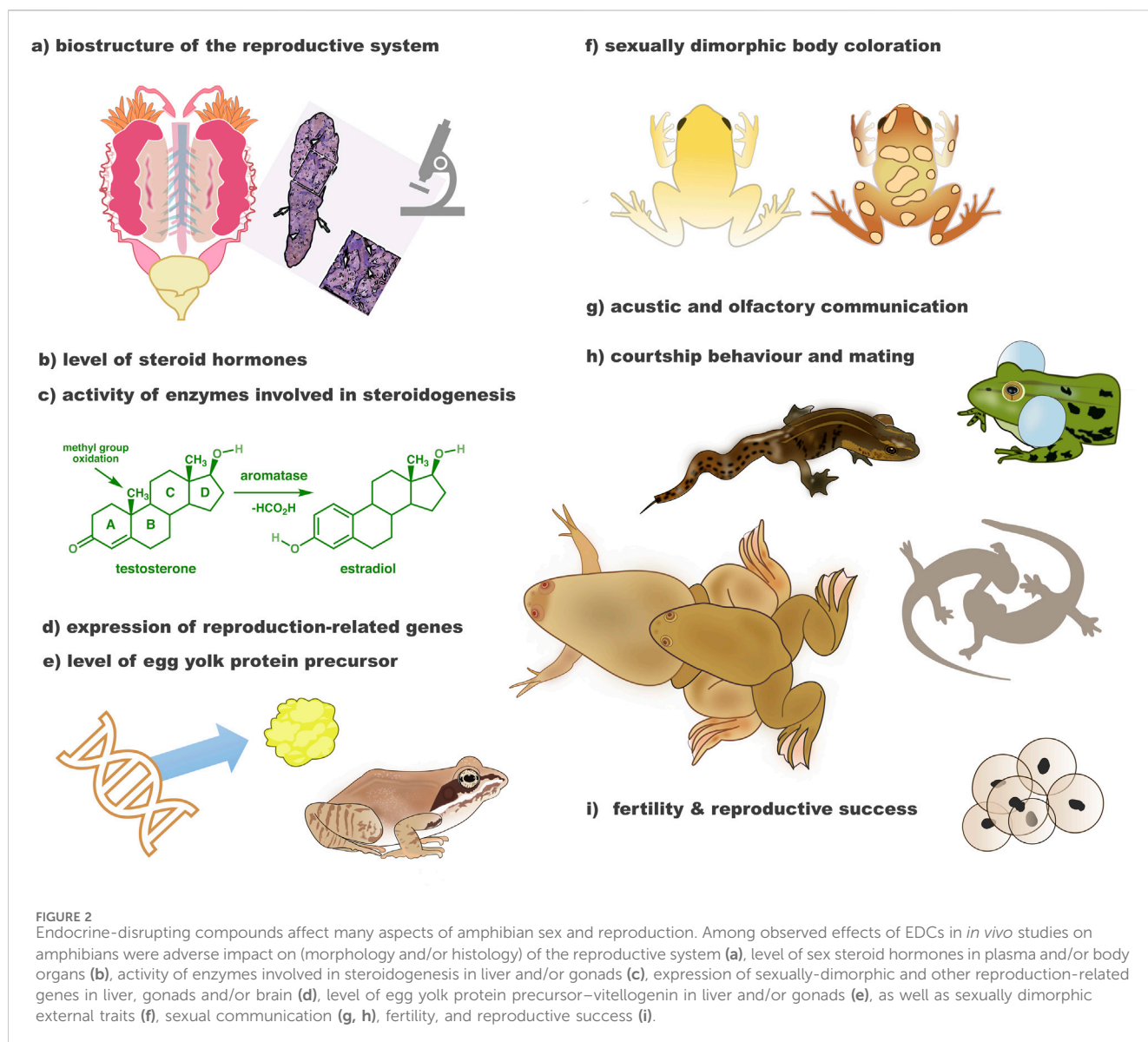
Order, Family	Latin species name	Sex determination system	Ecology of larvae	Diet of larvae	Ecology of adult individuals	IUCN Red List Status Account	Geographical distribution	Number of studies on larvae / on post-metamorphic forms
Anura, Ranidae	<i>Lithobates clamitans</i>	XX/XY	lentic/lotic: benthic	herbivorous - detritivorous	terrestrial, aquatic	LC	North America	1/0
Anura, Ranidae	<i>Lithobates pipiens</i>	XX/XY	lentic: benthic	herbivorous - detritivorous	terrestrial, aquatic	LC	North America	11/1
Anura, Ranidae	<i>Lithobates sphenoccephalus</i>	XX/XY	lentic: benthic	primarily herbivorous	terrestrial, aquatic	LC	North America	1/0
Anura, Ranidae	<i>Lithobates sylvaticus</i>	XX/XY, observed TSD	lentic: benthic	herbivorous - detritivorous; carnivorous	terrestrial, aquatic	LC	North America	7/1
Anura, Ranidae	<i>Pelophylax kl. esculentus</i>	unknown (hybridogenetic species; hemiclinal mode of reproduction)	lentic/lotic: benthic	primarily herbivorous	terrestrial, aquatic	LC	Widespread in Europe, range extends west to France, east to Russian Federation, north to Scandinavia, south to the Balkans. Introduced to Spain and United Kingdom	0/1
Anura, Ranidae	<i>Pelophylax nigromaculatus</i>	XX/XY	lentic/lotic: benthic	primarily herbivorous	terrestrial, aquatic	NT	East Asia, Southern part of Russian Federation	7/5
Anura, Ranidae	<i>Pelophylax perezi</i>	XX/XY	lentic/lotic: benthic	herbivorous - detritivorous	terrestrial, aquatic	LC	Iberian Peninsula, South of France	1/0
Anura, Ranidae	<i>Rana chensinensis</i>	unknown	lentic/lotic: benthic	herbivorous - detritivorous	terrestrial, aquatic, arboreal	LC	East Asia, south-east part of Russian Federation	1/2
Anura, Ranidae	<i>Rana dalmatina</i>	XX/XY, observed TSD	lentic: benthic ¹³	herbivorous - detritivorous ¹³	terrestrial, aquatic, arboreal	LC	Widespread in Europe, range extends north to the Sweden, Finland and Poland, west to the France, east to the Ukraine; introduced in Belgium.	3/0
Anura, Ranidae	<i>Rana temporaria</i>	XX/XY, observed TSD	lentic: benthic	herbivorous - detritivorous	terrestrial, aquatic, arboreal	LC	Widespread in Europe, range extends west to United Kingdom and Ireland, east to Russia, north to Scandinavia, south to Spain and Italy.	3/0
Anura, Rhacophoridae	<i>Polypedates cruciger</i>	unknown	lentic: benthic ^{14,15}	herbivorous, zooplanktivorous ^{14,15}	terrestrial, arboreal	LC	Sri Lanka	1/0
Urodela, Ambystomatidae	<i>Ambystoma tigrinum</i>	ZW/ZZ	lentic	carnivorous	fossorial, terrestrial, aquatic	LC	North America	1/0

(Continued on following page)

TABLE 1 (Continued) Nomenclature, systematic position, sex determination system (GSD – genetic sex determination, TSD – temperature-dependent sex determination) and ecological traits of species of amphibians in experimental studies on the impact of endocrine-disrupting compounds on sex and sex-related traits with external exposition to compound. Please note that some studies used more than one species.

Order, Family	Latin species name	Sex determination system	Ecology of larvae	Diet of larvae	Ecology of adult individuals	IUCN Red List Status Account	Geographical distribution	Number of studies on larvae / on post-metamorphic forms
Urodela, Cryptobranchidae	<i>Andrias davidianus</i>	ZW/ZZ, observed TSD	lotic	carnivorous	aquatic	CR	China, introduced: Taiwan	1/0
Urodela, Salamandridae	<i>Lissotriton boscai</i>	XX/XY	lentic/lotic	carnivorous	terrestrial, aquatic	LC	Portugal, Spain	0/1
Urodela, Salamandridae	<i>Lissotriton helveticus</i>	XX/XY	lentic/lotic	carnivorous	terrestrial, aquatic	LC	Western Europe, northern parts of Portugal and Spain	0/3
Urodela, Salamandridae	<i>Notophthalmus viridescens</i>	unknown	lentic/lotic	carnivorous	terrestrial, aquatic	LC	North America	0/1
Urodela, Salamandridae	<i>Pleurodeles waltl</i>	ZW/ZZ, observed TSD	lentic/lotic	carnivorous	terrestrial, aquatic	LC	Morocco, Portugal, Spain	2/0

Data on sex determination systems was sourced from the HerpSexDet database (Nemesházi and Bókony, 2023). Data on species ecology was primarily obtained from AmphibiaWeb (2024) and the AmphiBIO database (Oliveira et al., 2017). For specific cases, additional sources were used and are indicated as follows: ¹Duport Bru, 2020; ²Traijitt et al. 2021; ³Khan, 2018; ⁴Salo and Solania, 2022; ⁵Padhye et al. 2017; ⁶Johnson and Christiansen, 1976; ⁷Escoriza, 2014; ⁸Viertel et al., 2007; ⁹Barker et al., 1995; ¹⁰Peterson and Boulton, 1999; ¹¹NatureServe Explorer, 2023; ¹²Sayyed, 2015; ¹³Ćirković et al., 2023; ¹⁴Meegaskumbura et al. 2010; ¹⁵Bowatte et al, 2013.



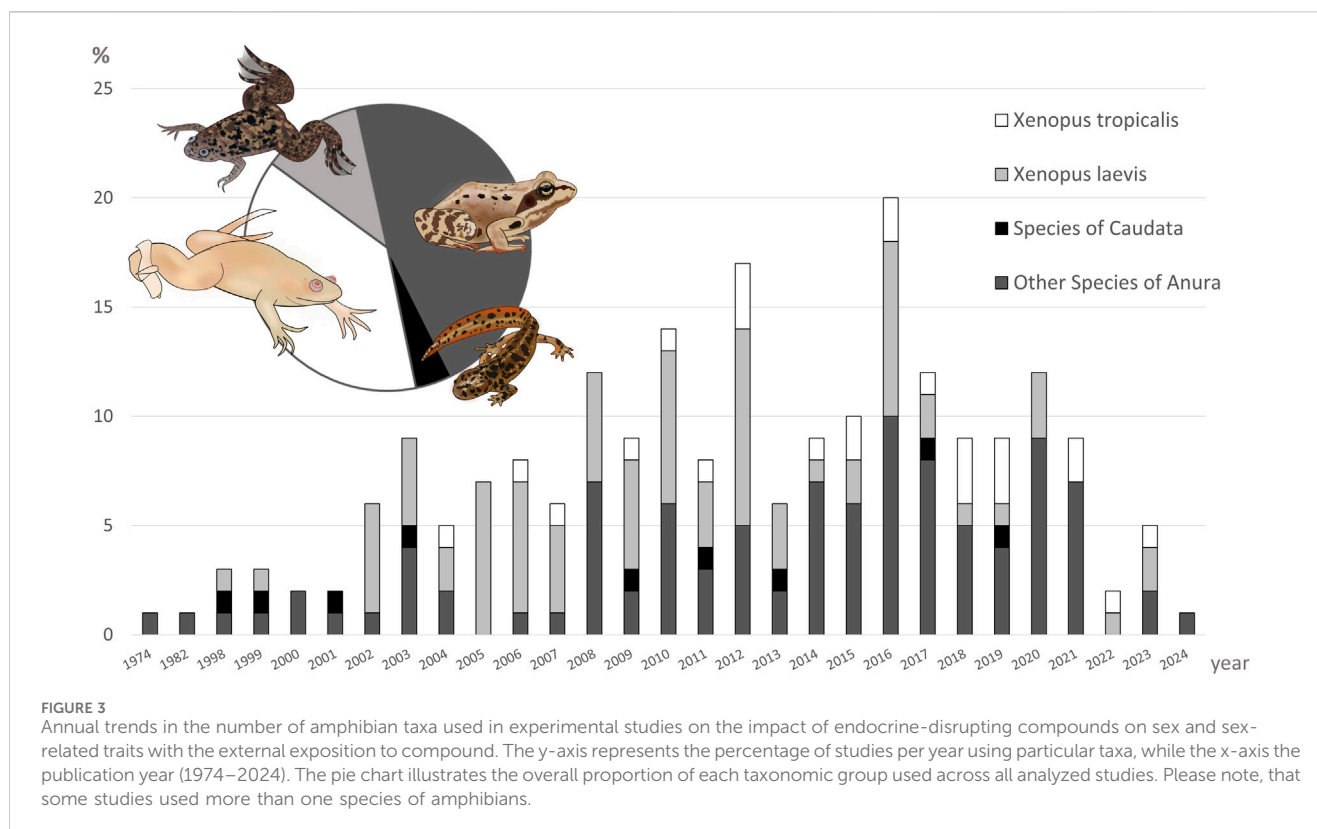
reproductive system caused by EDCs, we use the nomenclature proposed by Lutz et al. (2008).

3 Results

We identified 241 experimental studies on the impact of EDCs on sex and sex-related traits of amphibians, from which 214 were *in vivo* studies. The majority of the experimental works were performed on developmental, aquatic forms of amphibians (see Table 1). In the Supplementary Information, detailed information about found experiments with waterborne exposition (90,19% of all *in vivo* studies) of amphibians to EDCs, including time of exposition, life stages, concentrations of EDCs, and the most significant results can be found (Supplementary Table S1). Among *in vivo* experiments, we identified only two articles in which the compound was ingested by animals (Tang et al., 2020; Ziková et al., 2013). In some, mostly older studies, amphibians were

exposed to EDCs via injections or intraperitoneal implants (9,81% of all *in vivo* studies), and we consider them to be generally less environmentally relevant. Full lists of studies with these routes of exposition to EDCs (Supplementary Table S2) as well as *in vitro* and *ex vivo* studies (Supplementary Table S3), and species used in them, are included in the Supplementary Information (Supplementary Data Sheet S1). Moreover, in the Supplementary Information, we attach a table with abbreviations and IUPAC (International Union of Pure and Applied Chemistry) nomenclature of reviewed EDCs. (Supplementary Table S4). The most significant, recognized effects of EDCs on sex and sex-related traits of amphibians in the researched literature are presented in Figure 2.

We found that *in vivo* studies externally exposing amphibians to EDCs included 45 species (Table 1), constituting 0,52% of all species of amphibians known to date (31.12.2024). The two most frequently used species, from the same family Pipidae, account for almost half (49,78%) of all researched species (Figure 3).



4 Discussion: are model species the best choice?

4.1 The overreliance on *Xenopus* spp.

The African clawed frog *X. laevis* is still perceived as an ideal model for studying the effects of endocrine-disrupting compounds. It is due to its quick somatic and sexual development, the well-established knowledge about life stages, and available methods of genetic sex determination (Cong et al., 2006; Huang et al., 2005; Oka et al., 2006; Yoshimoto et al., 2008). It should be noted that *Xenopus laevis* is an evolutionarily conserved lineage, having diverged nearly 17.5 million years ago (Furman et al., 2015). While, for example, the American toad *Anaxyrus americanus* has existed for only 1.3 million years (Masta et al., 2002). The evolutionary conservation of *X. laevis* contributes to its utility as a model for human biology because it implies stability in its genetic and developmental pathways over millions of years. This stability makes it easier to study fundamental biological processes, such as cell division, gene regulation, and embryonic development, that are shared between humans and *X. laevis*. Its conserved nature ensures that findings in *X. laevis* are often applicable to humans, providing a reliable system for studying evolutionarily shared mechanisms (Blum and Ott, 2018; Gao and Shen, 2021; Nenni et al., 2019).

However, evolutionary conservation of *X. laevis* may limit its representativeness for amphibian biology. Its long divergence time means that *X. laevis* represents a unique and relatively ancient lineage compared to other amphibians, which may not well reflect the evolutionary and ecological traits of most of the contemporary

species (Roelants et al., 2007). Another commonly used model closely related to *X. laevis* is the western clawed frog *Xenopus tropicalis*, which shares its advantages for toxicological research (Olmstead et al., 2010). Unfortunately, it does not add sufficient variance to account for responses to toxic substances (Cannatella, 2015; Robert and Cohen, 2011). Both species from the family Pipidae belong to the basal anurans (suborder Mesobatrachia), constituting less than 5% of currently known species (together with Archaeobatrachia), while most modern Anura belong to Neobatrachia. Therefore, the diversity of amphibians is still poorly represented in laboratory research (Wake and Koo, 2018).

The most commonly used species in ecotoxicology research are easy to obtain, common, and widely distributed (Schiesari et al., 2007), which is also evident in our study. Species bias in studies on endocrine disruptors may have significant consequences for the conservation of amphibians. The species most threatened by pollution with EDCs may be the ones difficult to obtain, living in areas with little interest and investment in ecotoxicological research (Schiesari et al., 2007). Another issue is the origin of animals used in the studies. Model species are usually obtained from laboratory populations with limited genetic variance and do not undergo natural selection like wild populations. They can also be a subject of unintentional selection for traits supporting survival in captivity conditions, of which researchers using such “domesticated” lines are not always aware. It has been shown that wild populations may develop tolerance to pollutants in the environment. Consequently, they can be less sensitive to the harmful effects of some toxicants than animals of the same species bred for generations in controlled, laboratory conditions (Romero-Blanco and Alonso, 2022).

4.2 Amphibians differ in sensitivity to EDCs

Results obtained from studies on non-model species often contradict those from studies on model ones. For example, significant differences in sensitivity of species to the effects of exogenous 17 β -estradiol (E2) were found. E2 is widely present in surface waters, as it is excreted into the environment by humans and farm animals. In water-borne exposition studies, E2 was found to have a feminizing impact on morphology and histology of gonads in *X. laevis* (Hu et al., 2008; Lutz et al., 2008; Oka et al., 2006), the Indian skipper frog *Euphylyctis cyanophlyctis* (Phuge and Gramapurohit, 2015) and the Japanese wrinkled frog *G. rugosa* males (Ohtani et al., 2000; Oike et al., 2020). Moreover, exposition through the larval period to different concentrations of E2 caused female-biased to 100% female sex ratio in *X. laevis* (Bögi et al., 2002; Hu et al., 2008; Lutz et al., 2008; Oka et al., 2006), *E. cyanophlyctis* (Phuge and Gramapurohit, 2015), the common toad *Bufo bufo* (Petrini and Zaccanti, 1998), and *Glandirana rugosa* males (Ohtani et al., 2000; Oike et al., 2020), which suggested cases of sex reversal.

However, in the green frog *Lithobates clamitans*, injections with E2 for a long period did not significantly affect the sexual development of male and female tadpoles (Mintz et al., 1945). Also in the common reed frog *Hyperolius viridiflavus* exposition to E2 did not have any impact on gonadal morphology, even when a dose of E2 was very high (Richards, 1982), and in an outdoor study of the Blanchard's cricket frog *Acris blanchardi* E2 was found to have minimal effect on sexual development (Windle et al., 2021). Another study comparing the impact of E2 on the *X. laevis*, the European fire-bellied toad *Bombina orientalis*, the yellow-bellied toad *Bombina variegata*, the European tree frog *Hyla arborea*, and the European green toad *Bufo viridis*, found significant differences in the extent of induced changes within gonads of these species (Piprek et al., 2012).

More examples of differences in sensitivity to EDCs can be found in studies using a bacteriostatic compound triclosan (TSC), widely present in the environment as a residue from, e.g., personal care products. In the dark-spotted frog *Pelophylax nigromaculatus*, exposure to TSC during larval development caused a male-biased sex ratio, abnormalities within the gonads, and changed expression of reproduction-related genes (Chen et al., 2018). In comparison, TCS did not significantly impact sexual development and gonadal differentiation in *X. laevis* (Fort et al., 2017) or the expression of reproduction-related genes in adult *X. tropicalis* (Regnault et al., 2016).

Significant differences in sensitivity between species were also noted in other studies, exposing amphibians to a synthetic estrogen ethinylestradiol (Tamschick et al., 2016b; Tamschick et al., 2016c), plasticizer bisphenol A (Tamschick et al., 2016a), nonylphenol, compound widely used in surfactants and detergents (Mackenzie et al., 2003) and a fungicide agent ethylenethiourea (Phuge et al., 2021).

Differences in sensitivity to EDCs between species could be largely due to various sex determination and differentiation mechanisms, as well as physiological and ecological differences, which we discuss below.

4.3 Differences in sex determination and differentiation in amphibians

The sexual determination system for most species of amphibians is still unknown. The limited data we have,

however, show a huge variety of sex determination systems in this group of vertebrates (Nakamura, 2009). In this context, it becomes obvious that studying the effects of EDCs on only model species (such as *X. laevis* and *X. tropicalis*) is not enough to identify the true impact of these compounds on a huge variety of modern amphibian forms.

4.3.1 Genetic sex determination

Although it is generally accepted that the sex of amphibians is determined genetically, for most amphibian species, morphologically distinguishable sex chromosomes are not identified. Until now, the sex chromosome system has been recognized only for about 2% of the species (Bullejos et al., 2024). Data on sex-determining genes and gene regulatory networks involved in sexual differentiation are also limited (Nakamura, 2009; Ruiz-García et al., 2021; Kuhl et al., 2024).

No universal sex-specific loci in amphibians were found, and at this moment, genetic determination of sex is possible only for a few species (Olmstead et al., 2010; Yoshimoto et al., 2008). It is particularly striking when considering the diversity of this taxonomic group. To date (31.12.2024), 8,816 amphibian species have been identified, and new species are discovered every year (AmphibiaWeb (2024)).

However, even this limited knowledge about genetic sex determination systems in amphibians shows a huge variability and plasticity within the group (Dufresnes and Crochet, 2022; Ma and Veltsos, 2021). The male (XX/XY) or female (ZZ/ZW) heterogametes in amphibian species can be found, as well as novel systems of chromosomal sex determination. Examples are the 00/W0 sex chromosome system in the Hochstetter's frog *Leiopelma hochstetteri* (Green, 1988), or the multiple sex chromosomes (=XXAA~XXAY ~ type) system in the Puerto Cabello robber frog *Strabomantis biporcatus* (Schmid et al., 1992). Notably, in some phylogenetic lines of amphibians, the events of switching chromosomal sex determination systems occurred (Evans et al., 2012).

Variations of sex chromosomes have been identified even within a single species. *Xenopus tropicalis* is an interesting example, as in this species, three homomorphic sex chromosomes (Y > W > Z) were identified. As a result, males of *X. tropicalis* can possess ZZ, YZ, or YW chromosome combinations, while in females, ZW or WW chromosome sets can be found. Additionally, both sexes can be either heterogametic or homogametic. It should be taken into account in the studies on EDCs, as depending on the chromosome sets and types of gametes of parents, the sex ratio obtained in the offspring may differ from the conventionally assumed 1:1 (Furman et al., 2020; Roco et al., 2015). Another, somewhat extreme example, is the Japanese wrinkled frog *G. rugosa* the 6 geographic variants, with both XX/XY and ZZ/ZW sex chromosome systems, were found (Miura, 2007; Ogata et al., 2003; Oike et al., 2017; Mawaribuchi et al., 2023).

There are pieces of evidence that the sex determination system may impact the sensitivity of amphibians to hormonal compounds. *Glandirana rugosa* frogs from populations with novel chromosome sex determination systems were found to have lower sensitivity to hormonally induced gonadal sex reversal and the associated gene expression pattern (Miura et al., 2016). In theory, they could be less prone to the impact of EDCs. Thus, switching sex determination systems can have serious consequences for the survival of amphibian

populations. According to the theory of “asymmetrical sex reversal”, homogametic sexes are more prone to phenotypical sex reversal and require weaker stimuli to undergo it (Nemesházi and Bókonyi, 2022).

4.3.2 Impact of steroidal sex hormones

Steroidal hormones play a crucial role in sex determination in amphibians (Hayes, 1998; Nakamura, 2013; Zaborski, 1986). They are also involved in the development and maintenance of sex accessory structures and secondary sexual characteristics in these animals (Harvey and Propper, 1997; Iwasawa and Kobayashi, 1974; Norris, 2011a). EDCs mimic steroidal hormone effects in sex determination, making amphibians extremely sensitive to these compounds. However, the role of steroidal hormones in sex determination and differentiation varies among species (Mali and Gramapurohit, 2016), which can be another reason for a species-specific sensitivity to the endocrine-disrupting compounds in the environment.

4.3.3 Impact of temperature on the sex of amphibians

The available evidence suggests that no amphibian species has exclusively temperature-dependent sex determination, although some of them retain sensitivity to it (Hayes, 1998; Nakamura, 2009; Ruiz-García et al., 2021). Examples are some Urodela species: the Edough ribbed newt *Pleurodeles poireti* (Dournon et al., 1984), the iberian ribbed newt *Pleurodeles waltl* (Dournon et al., 1990; Zaborski, 1986), as well as Anura: the wood frog *Lithobates sylvaticus* (Lambert et al., 2018), the Dybowskii's frog *Rana dybowskii* (Xu et al., 2022), the agile frog *Rana dalmatina* and the chinese edible frog *Hoplobatrachus rugulosus* (Mikó et al., 2021; Tang et al., 2020). For such species, outcomes of exposure to EDCs may differ depending on the interacting temperature. In the environment, amphibians are simultaneously subjected to many factors that can shape their phenotypical sex. In this context, the potential role of temperature in sex determination should not be overlooked.

4.3.4 Type of gonadal differentiation

In addition to a huge variety of sex determination systems, amphibian species differ in patterns of gonadal differentiation and rate of sexual development. It may significantly impact their sensitivity to endocrine disruptors, the time frame in which EDCs can affect their phenotypical sex, and the outcome of exposure to EDCs (e.g., feminization, masculinization, or none).

In amphibians can be found bipotential gonads. The differentiation of them may be categorized into three types. In most species, bipotential gonads develop directly into ovaries or testes (differentiated type). In some anuran species, however, the timing of differentiation of gonads in males and females is different, with testes developing from bipotential gonads much later than ovaries (semi-differentiated type). In this type of differentiation, some authors mistakenly recognize bipotential gonads in males as ovaries (explained by Ogielska, 2009). In rare cases, another type of gonadal differentiation can be found, in which in genetic males and females bipotential gonads first develop into ovaries, and then, in males, transform into testis (undifferentiated type) (Gramapurohit et al., 2000; Ogielska, 2009; Tanimura and Iwasawa, 1989).

Importantly, even populations of the same species may differ in the timing/type of gonadal differentiation (differentiated vs. semi-differentiated type) (Rodrigues et al., 2015). Studies on Urodela

suggest that they undergo direct differentiation from bipotential gonad into ovary or testis (Dumond et al., 2008), although knowledge on gonadal differentiation in this group of amphibians is scarce.

4.3.5 Rate of gonadal differentiation

Amphibians also differ in the timing and rate of gonadal development and differentiation (for the review see Ogielska and Kotusz, 2004), with some having extremely slow gonadal differentiation in comparison to others (Piazza et al., 2023). The effects of EDCs on sex and sex-related traits may strongly depend on the type and timing of gonadal differentiation in species. *Xenopus laevis*, with direct and synchronized differentiation of bipotential gonad into ovaries or testis, may not be universally representative of the whole group of amphibians.

Data from experimental studies show that species with short ovarian development time, e.g., the Southern leopard frog *Lithobates sphenoccephalus* (Storrs and Semlitsch, 2008), are more susceptible to the impact of EDCs than others. Species with quick sexual differentiation during the aquatic phase of life can be more prone to adverse effects of EDCs in surrounding water than species with slow sexual differentiation, extended to the post-metamorphic, terrestrial life stages. Worth noting, temperature can also have a significant impact on the rate of gonadal differentiation (Burraco et al., 2023).

4.3.6 Mixed-sex amphibians: norm in some species?

In many studies in which amphibians were exposed to EDCs, the presence of mixed-sex gonads (which contain both ovarian and testicular tissue) (Howe et al., 2004), intersex gonads (both testes and ovaries as separate structures in one individual) (Hayes et al., 2002), testicular oocytes (oocytes within testicular tissue) (Jeganathan and Rajakaruna, 2021) or completely sex reversed individuals (e.g., genetic males with regular, female gonads) was observed (Oka et al., 2006). However, there is a question: To what extent are these gonadal changes a natural phenomenon?

For example, mixed-sex individuals and testicular oocytes were observed in both experimental and control groups in several studies regarding impact of pesticide atrazine (Brande-Lavridsen et al., 2008; Coady et al., 2004; Jooste et al., 2005; Orton et al., 2006; Preez et al., 2008; Storrs-Méndez and Semlitsch, 2010) or EE2 (Brande-Lavridsen et al., 2008). Existences of testicular oocytes, mixed-sex or sex-reversed individuals in amphibian populations come also from field studies (Alho et al., 2010; Bókonyi et al., 2021; Lambert et al., 2019; Nemesházi et al., 2020; Preez et al., 2009; Reeder et al., 1998; Xu et al., 2022). Especially testicular oocytes, in many studies regarded as a pathology, may, in fact, be a natural phenomenon in some species or populations (Preez et al., 2009; Storrs-Méndez and Semlitsch, 2010). In some cases, observations of abnormal gonads in control groups could be, however, due to mistakes in experimental design (Hayes, 2005).

Some studies suggest that mixed-sex or intersex stages of gonads may be expected for some species (with semi-differentiated or undifferentiated type of gonadal development). Especially for toads (Bufonidae), not all cases of mixed-sex or intersex individuals should be perceived as anomalies. In Bufonidae rudimental hermaphroditism can be found. Adult males have Bidder's organs—undeveloped ovaries - located anterior to the

testes, in which oogenesis is still active, but oocytes do not reach maturity or are reduced. The exact function of the Bidder's organ is unknown (Petrini and Zaccanti, 1998). Additionally, differentiation of the bipotential gonads into testes in toads is delayed (retarded type of differentiation) (Storrs-Méndez and Semlitsch, 2010). As we underlined in the above section, *Differences in sex determination and differentiation in amphibians*, to accurately identify mixed-sex and intersex individuals, comprehensive knowledge of gonadal development, timing, and subsequent stages in a particular species is crucial.

4.4 Ecological differences

4.4.1 Aquatic vs. biphasic species

Apart from genetic differences between taxa, species also differ in ecology (Table 1), which can impact the main routes of exposure to EDCs in the environment. Most of our knowledge about the impact of EDCs on amphibians comes from studies with aqueous exposure (Slaby et al., 2019). The widely used standardized amphibian toxicity test, FETAX, using *X. laevis*, is based on exposure via water (Testing and Materials, 1998). However, the water environment may not be the primary route of exposure to pollutants for most species of amphibians (Brühl et al., 2013; Purucker et al., 2023). *Xenopus* spp., as fully aquatic species, do not represent the majority of amphibians with biphasic life cycles, which transition between aquatic and terrestrial habitats.

This transition exposes animals to additional stressors, such as pesticide contamination on land, different temperatures, and greater exposure to UV light (Nolan et al., 2023). Moreover, biphasic amphibians by bioaccumulation during the aquatic stage can transfer pollutants such as microplastics from the aquatic environment to the terrestrial one, thus contributing to the dissemination of them in the ecosystem (Park and Kidd, 2005; Szkudlarek et al., 2023). While the *Xenopus* spp. remain a convenient laboratory organism, its life history fails to capture the complexity of biphasic species' exposure to environmental stressors and processes occurring during the transition from the aquatic stage to the terrestrial one. To obtain a more accurate understanding of the impact of EDCs on amphibians, ecotoxicology studies must incorporate biphasic species (Nolan et al., 2023).

4.4.2 Terrestrial exposure

Terrestrial stage of life may be particularly problematic for amphibians living in agricultural areas, where they are exposed to significant quantities of endocrine-disrupting pesticides and fertilizers via contact with polluted soil and overspraying (Brühl et al., 2011; Churko et al., 2024; Cruz-Santiago et al., 2023; Fryday and Thompson, 2017). Pesticides, such as atrazine, may be absorbed by the highly permeable skin of amphibians, especially on the ventral side, and accumulate in the body (Purucker et al., 2023; Quaranta et al., 2009). This has been confirmed in the studies on, e.g., *Anaxyrus americanus* (Méndez et al., 2009), the juvenile barking tree frog *Dryophytes gratiosus*, and the southern leopard frog *Lithobates sphenoccephala* (Belden et al., 2010).

However, species differ in their risk of exposure due to behavioral and ecological traits (Lenhardt et al., 2015). Nocturnal

or fossorial species that remain underground during the day may avoid direct contact with contaminated surfaces, unlike diurnal, surface-active species (Adams et al., 2021; Belden et al., 2010).

Similarly, habitat use—particularly the proportion of time spent in terrestrial versus aquatic environments—modulates the likelihood of EDC exposure (Swanson et al., 2018). In this context, an interesting question is how the risk of exposure to EDCs differs between species with various overwintering strategies of adult forms. For example, species wintering in polluted water bodies in bottom sediment, where EDCs are accumulated (Fuentes et al., 2014; Swanson et al., 2018), are potentially more prone to exposure to EDCs than the species wintering on land.

4.4.3 Skin structure and physiology

Species ecology is closely tied to interspecific differences in skin structure and physiology, which influence both exposure to, and sensitivity to EDCs. Amphibian skin is not only the principal organ for respiration and osmoregulation but also the main route of chemical uptake from the environment (Quaranta et al., 2009). It varies in permeability, keratinization, glandular structure, and lipid content depending on species, developmental stage, and habitat (Hillyard et al., 2008). For example, toads living in arid regions have thicker, more keratinized skin to reduce water loss (Akat et al., 2022), which may also limit absorption of waterborne or soil-based pollutants.

In aquatic larvae, the skin is typically thin and highly vascularized, optimized for cutaneous respiration but also highly vulnerable to aqueous contaminants (Duellman and Trueb, 1994; Fox, 1977). As in some species larvae metamorphose into terrestrial juveniles or adults, keratinization increases to prevent desiccation—yet dermal absorption, especially of lipophilic compounds, remains a key exposure route (Akat et al., 2022). These physiological traits, coupled with their dual aquatic-terrestrial lifestyle, make amphibians sensitive bioindicators of environmental contamination (Kloas et al., 2006), but also demand a broader, more species-diverse approach in toxicological research.

Additionally, it is important to note that some EDCs are airborne (Annamalai and Namasivayam, 2015) and may reach amphibians through less-studied routes such as inhalation or direct dermal absorption from the atmosphere. This pathway remains largely unexplored in amphibians and its potential significance should not be overlooked. To our knowledge, the inhalation or dermal absorption of airborne EDCs and their effects have not yet been studied in amphibians, representing a critical gap in ecotoxicological research.

4.4.4 Feeding strategies

Additionally, it is worth considering the ecological and physiological differences in relation to feeding strategies. Notably, *Xenopus* spp. tadpoles are highly specialized filter feeders, primarily consuming suspended organic particles and zooplankton from the water column (Wassersug, 1996). In contrast, the majority of anuran tadpoles feed by grazing on biofilms and scraping detritus or algae from submerged surfaces using keratinized mouthparts (McDiarmid and Altig, 2000). The key differences in larval feeding strategies are summarized in Table 1.

These differences in feeding modes can substantially affect the route and intensity of EDCs exposure, as shown in fish (Fan et al., 2019; Müller et al., 2020). Filter-feeding larvae may be particularly vulnerable to dissolved or particle-bound EDCs suspended in the water, while surface-grazing tadpoles may ingest pollutants accumulated in periphyton or sediment layers. To our best knowledge, this hypothesis has not been tested yet.

Similar considerations apply to adult amphibians. Species feeding on aquatic invertebrates may remain more vulnerable to waterborne or sediment-associated pollutants (Veseli et al., 2022) whereas terrestrial-feeding species may primarily encounter EDCs present in, e.g., soil-dwelling invertebrates (Kwak and An, 2021). Moreover, trophic level may influence susceptibility: animals feeding on vertebrates or high trophic-level invertebrates may experience biomagnification of certain lipophilic EDCs (Mizukawa et al., 2009; Previšić et al., 2021; Ruhí et al., 2016).

Altogether, both the habitat and dietary preferences of amphibians—during larval and adult stages—contribute to interspecific variation in EDCs exposure and likely influence also physiological susceptibility to chemicals. These ecological and behavioral factors should be taken into account when assessing the environmental risks posed by EDCs across different species.

4.5 Species with unique life history traits

Among anuran amphibians, unique species with direct development, without an intermediate, morphologically distinct, free-living larval phase can be found (Callery et al., 2001). These direct developers, e.g., the Puerto Rican tree frog, *Eleutherodactylus coqui*, still undergo a thyroid hormone-dependent, rapid metamorphosis before hatching. It is interesting how EDCs present in the environment can influence the somatic and sexual development of such species. Are they less or more prone to the effects of endocrine disruptors?

The risk and consequences of exposure to EDCs are also unknown for Caecilians. Only about 5% of all described species of Caecilians have been studied in the topic of reproductive biology (Gomes et al., 2012). Very little is known about sex determination and gonadal development (Serrano-Perez and Ramírez-Pinilla, 2021), and almost nothing about the hormonal system in Caecilians (Brun et al., 2023; Norris, 2011b). Based on the study of two species, the Tenmalai caecilian *Gegeneophis ramsawamii* and *Gegeneophis* sp. (unspecified species), it is stated that Caecilians represent male heterogamety (XX/XY) (Govindappa and Venkatachalaiah, 2005). Because of the fossoriality of these animals, it is hard to observe them in nature and study and identify the environmental risks they are prone to.

Interestingly, some species of Caecilians and Urodela are ovoviviparous, or viviparous, with “pregnancy” controlled by hormones (Exbrayat, 1992). We already know that for many hydrophobic EDCs, the primary exposure route may be via maternal transfer (Kadokami et al., 2004; Liu et al., 2020). Field studies suggest that some EDCs can be transferred to eggs in oviparous amphibians (Du et al., 2019; Guan et al., 2024; Hopkins et al., 2006; Kadokami et al., 2004; Wu et al., 2009). However, to the best of our knowledge, there is currently no data on the impact of EDC on embryos in ovoviviparous and viviparous amphibians. Some clues may

come from the study on reptiles (Squamata), where gravid females of northern watersnakes *Nerodia sipedon*, exposure to atrazine by ingestion altered the sex ratio of offspring, skewing it toward males (Neuman-Lee et al., 2014).

Worth noting, in all known oviparous species of Caecilians, as well as some species of Urodela, such as *Pseudoeurycea juarezi*, and Anura, such as the Rosenberg’s treefrog *Boana rosenbergi*, parental care of offspring can be found (Gomes et al., 2012). It can be suspected that all these processes are, to some extent, controlled by reproductive hormones and possibly can be disrupted by external disruptors.

4.6 Differences in study design

The ecological characteristics of species significantly shape the optimal design of experimental setups to deliver truly environmentally relevant data. In *in vivo* waterborne exposure experiments, methodologies vary, including the flow-through water system, which maintains relatively stable concentrations of contaminants and effectively removes their metabolites or degradation products (Pickford et al., 2003). In contrast, the static renewal system allows for the accumulation of these substances within the water tank, potentially influencing experimental outcomes, especially if these by-products possess endocrine-disrupting properties (Hayes et al., 2003). Flow-through systems may more accurately mimic the environmental conditions of species that inhabit flowing waters. However, this setup might not be entirely suitable for species residing in stagnant water bodies, for example, ephemeral ponds, like puddles or phytotelmata. Moreover, natural aquatic environments often contain sediments and organic matter that can bind significant amounts of EDCs and their metabolites, thereby reducing their concentration in the water, which suggests that the real exposure levels to EDCs for amphibians in wild conditions could differ from those in controlled settings (Fuentes et al., 2014). Worth noting, the impact of the presence of sediments in water tanks may vary between amphibian species with benthic or pelagic specialized larvae (Table 1).

For investigating the complicated interactions between contaminants, their metabolic and degradation by-products, and various environmental factors, outdoor mesocosms offer a viable solution (Windle et al., 2021). Despite the challenges associated with their use in ecotoxicological studies, mesocosms provide conditions that closely resemble those in natural static water bodies. They incorporate multiple ecosystem components and account for additional stressors such as temperature (Tang et al., 2020), UV light (Croteau et al., 2009), pathogens (Paetow et al., 2023; Salla et al., 2024b) or predators (LaFiandra et al., 2008). Even more realistic conditions than mesocosms offer experimental lakes (Park and Kidd, 2005), which are, though, not a widely available solution.

5 Summary

In conclusion, amphibians are notably vulnerable to endocrine-disrupting compounds (EDCs), which pose a significant threat to their

survival and highlight their utility as sensitive bioindicators of environmental pollution. Almost half of the EDCs studies focus on just two amphibian model species, failing to adequately represent the group's diversity. This is particularly true for Caecilians and Urodela, where data on EDC impacts are scarce.

As shown in the previous study of Schiesari et al. (2007), there is a significant biogeographic bias in the general field of ecotoxicology of amphibians. The vast majority of research in this field uses few, common species with the least-concern threat status, primarily found in temperate zones of the northern hemisphere (Table 1). This bias may be due to the concentration of ecotoxicological researchers in these regions and the reliance on traditional, generic ecological risk-assessment methodologies. It should be noted, however, that our review took into account only publications written in English, which could potentially create a research bias (Chowdhury et al., 2022; Konno et al., 2020).

Nevertheless, our review underlines the need to recognize the natural diversity within amphibians in ecotoxicological studies. Alternative model species, representative of diverse taxonomic groups, should be considered (Salla et al., 2024b; Wagner and Viertel, 2017). Whenever possible, the effects of EDCs should be examined on regionally occurring species, under conditions that closely mimic their natural habitats. In studies on native species, non-invasive methods of sex identification and assessing endocrine disruptions are particularly useful (Orton et al., 2023) but still require development. Studies using non-model species in complex experimental setups may be challenging. However, intentional variations in ecotoxicological experiments may help gain a realistic view of the EDC's impact on amphibians in nature (Mikó et al., 2017; Romero-Blanco and Alonso, 2022) and contribute to their protection.

Author contributions

MF: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review and editing. MK: Conceptualization, Writing – review and editing. KS: Supervision, Writing – review and editing. PT: Conceptualization, Methodology, Supervision, Writing – review and editing.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2025.1556788/full#supplementary-material>

SUPPLEMENTARY TABLE S1

Summary of the *in vivo* studies on the impact of endocrine-disrupting compounds on sex and sex-related traits in amphibians with waterborne exposition, including the type of compound, species studied, developmental stage, time of exposition and the most significant results.

SUPPLEMENTARY TABLE S2

Summary of the *in vivo* studies on the impact of endocrine-disrupting compounds on sex and sex-related traits of amphibians with alternative routes of exposition.

SUPPLEMENTARY TABLE S3

Summary of the *in vitro* and *ex vivo* studies on the impact of endocrine-disrupting compounds on sex and sex-related traits of amphibians.

SUPPLEMENTARY TABLE S4

Nomenclature of all chemical compounds and commercial products mentioned in the manuscript and Supplementary Information.

SUPPLEMENTARY TABLE S5

Nomenclature and taxonomy of all species of amphibians mentioned in the manuscript and in the Supplementary Information.

SUPPLEMENTARY DATA SHEET S1

List of references cited only in the Supplementary Materials.

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