# Chapter 4


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Caitlin Gabor, Texas State University, obtains hormones excreted in the water from tadpoles while in the field. Here she is cleaning containers before placing fresh water and tadpoles into the cups. At which point she will agitate the tadpoles/cups all once for one minute every third minute for an hour. By doing this she can measure the glucocorticoid profile of tadpoles at three time points sequentially as an indication of their stress profile - at baseline, stress response (agitation), and then a third for recovery from the stressor. © Jaime Bosch

# Chapter 4

## Ecotoxicology: amphibian vulnerability to chemical contamination

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### Abstract

Amphibian populations are routinely exposed to chemical contaminants in their habitat because contamination is pervasive in industrial, residential, and agricultural areas; contamination moves to remoter regions through aerial drift, runoff, food webs via bioaccumulation and biomagnification, and the water cycle, resulting in contaminant exposure in all natural systems. Exposure to upwind agriculture has been one of the few causal factors linked to amphibian population declines across a large geographic area, yet expected environmental exposures are often below lethal thresholds, suggesting that interactions with other natural and anthropogenic factors may be the key avenue through which contaminants elicit impacts on individuals and populations. Recent data reveal that direct exposure to contaminants can alter physiology or gene expression, causing long-lasting effects that go beyond the exposure period, in some cases even extending across generations. In their natural habitats, amphibians must cope with several biotic (competitors, predators, and pathogens) and abiotic stressors (temperature, precipitation and other environmental conditions). Anthropogenic stressors, such as habitat alteration/degradation, pollution and climate change, provide an additional challenge to these species. Evidence suggests that the presence of multiple stressors increases the likelihood that contaminants will cause effects on amphibians and their populations, potentially increasing their extinction risk. While some contamination is perhaps unavoidable in a human-dominated globe, there are ways to reduce exposure to contaminants, such as managing their release and use, creating biological buffers from areas of exposure, and implementing better policies that protect natural systems. Managing the risk of contaminants to amphibians will require a concerted effort among scientists, policymakers, local communities, landowners, and other stakeholders around the world to protect amphibians and the natural systems of which they are part.

### Introduction

On a planet where over 6 billion pounds of active ingredient pesticides are sold each year (Atwood & Paisley-Jones, 2017) and where an estimated 90–100,000 chemicals are released into the environment from agricultural and industrial activities (Holt, 2000), chemical contaminants are widespread and found in every environment. Contamination from pesticide pollution alone is widespread with 64% of agricultural lands at risk to exposure to more than one contaminant (Tang et al., 2021). Further, there is a high overlap between areas prone to pesticide exposure and high-biodiversity regions, particularly in South Africa, China, India, Australia, and Argentina, although the risk is global (Tang et al., 2021). These contaminants can be detected above and below ground, posing a threat to living organisms through direct exposure and indirect routes via water systems and food webs. Early reports of amphibian population declines (Wake, 1991) posited that contaminants could play an important role in declines and approximately 30% of globally threatened amphibians are affected by pollution (Baillie, Hilton-Taylor & Stuart, 2004).

A recent assessment on our progress elucidating the causes of amphibian declines (Green et al., 2020), however, did not explicitly include contaminants. Yet, of the many attempts to look for causal factors, contaminants have been one of the few statistically linked to declines: Upwind pesticide use has been associated with amphibian population declines in California, USA across numerous studies (Davidson, 2004; Davidson & Knapp, 2007; Davidson, Shaffer & Jennings, 2001, 2002). Further, California is one of the places with the best records for pesticide use and application rates, making it one of the areas more likely to find associations if they existed. Yet, directly linking contaminants to declines is difficult (Bradford et al., 2011; Davidson, Stanley & Simonich, 2012; Grant et al., 2016, 2020) given that environmental concentrations are often below known effect thresholds, contaminant effects can appear years after exposure, the types of contaminants used change over time, testing often occurs long after a contaminant is used, peak concentrations that cause effects may occur well before testing, break-down products may have different toxicity, and demographic data on amphibians is scarce (Conde et al., 2019). Additionally, the sheer number of contaminants found in environments (Smalling et al., 2012) and the temporal and spatial variation in application make pinpointing contaminants as a driver of amphibian declines problematic. Indeed, despite chemical innovation that has led to a diversity of novel products (e.g. PFAS [perfluoroalkyl and polyfluoroalkyl substances], antimicrobials, microplastics; Kumar, Borah & Devi, 2020), our current understanding of the role of contaminants on amphibian declines stems from work on selected pollutants (Egea-Serrano et al., 2012). However, population viability analysis by Willson et al. (2012) demonstrated how contaminants that impact larval and juvenile survival can increase the risk of local extirpation, suggesting that understanding the effects on key life stages can be important for predicting population consequences. For all of these reasons, determining cause-effect linkages is challenging even if contaminants were a central causative factor in declines.

Despite the risk of chemical contaminants to amphibians, the initial concern that amphibians may be more sensitive to contaminants than other vertebrates because of their permeable eggs, skin, and gills (Bishop & Pettit, 1992), has not been supported (Bridges et al., 2002; Kerby et al. , 2010). Larval amphibian susceptibility to contaminants is roughly similar to that of fish (Glaberman, Kiwiet & Aubee, 2019; Ortiz-Santaliestra et al., 2018), although variation exists within and between species and taxonomic groups (Bridges & Semlitsch, 2000), which can change with repeated exposure (Hua, Jones & Relyea, 2014; Hua, Morehouse & Relyea, 2013). Assessment of contaminant risks could also vary across biogeographical regions, but most research has focused on species in the Northern Hemisphere, which biases research toward certain types of contaminants, species with complex life cycles, and a narrow set of life history traits (Schiesari, Grillitsch & Grillitsch, 2007). Nevertheless, amphibians are susceptible to environmental contaminants (Baker, Bancroft & Garcia, 2013), and contaminants could pose an important threat to amphibian populations in the wild (Willson et al., 2012).

Collectively, while substantial progress has been made in past decades, the major goals of this chapter are to highlight research gaps, suggest key research directions towards the goal of continuing to understand amphibian vulnerability to chemical contamination, and identify actions to mitigate and reduce the effects of contamination on amphibian communities. In 2007, contaminant risks were assessed and reviewed by the IUCN working group

(Gascon et al., 2007) and recommendations were updated in 2015 (Wren et al., 2015), which noted the potential for contaminant exposure risks to amphibians in ways that may be more obvious (mortality) to more subtle (endocrine disruption, impacts on fertility, reduced overwinter survival). These assessments and others have noted that the most serious threat to amphibians from contaminants is their potential to interact with other factors like habitat loss and degradation, novel diseases, climatic changes, exotic invasive species, and natural factors like predators/parasites and competitors (Carey et al., 2001; Grant et al., 2016; Hayes et al., 2006). The data have come to support this supposition in the last decade (e.g. Davis et al., 2020; Rohr et al., 2008a; Rumschlag & Rohr, 2018). Contaminants can change community composition, which can alter critical life history traits and alter susceptibility to abiotic and biotic factors, and serve as a physiological stressor, which can influence the susceptibility to other environmental stressors and the likelihood for interactive effects.

Because current research suggests the important role of contaminants as both an additive (i.e. combined effects equal the sum of the effects of each factor alone) and interactive factor in natural systems, the potential for interactions between expected and observed environmental concentrations of contaminants and other factors is the focus of our review here. The objectives of this chapter are to **1)** review key ecotoxicological research not addressed in previous IUCN assessments, **2)** identify gaps in amphibian ecotoxicology knowledge, **3)** evaluate the priorities for future amphibian ecotoxicology research, and **4)** provide effective and strategic conservation recommendations to mitigate contaminant risks to amphibians.

## Contaminant risks

### Types of chemical risks to amphibians

Amphibians are vulnerable to toxicants and pollutants from several sources (Figure 4.1) and very different chemical natures, which have been reviewed extensively elsewhere (e.g. Sparling et al., 2010; Thambirajah et al., 2019) and which are summarised here briefly. Industrial and agricultural chemicals likely constitute the most pervasive type of chemicals to which amphibians are exposed, as they contaminate soils and the water bodies that amphibians use as primary breeding habitats. These substances cause direct damage to larval and adult amphibians through poisoning, endocrine disruption, or other means of physiological impairment. Some of these substances are highly persistent in the natural environment and amenable to bioaccumulation, consequently remaining a grave concern even long after their use is stopped or legally banned. Insecticides (e.g. DDT, carbaryl, deltamethrin, parathion, rotenone, esfenvalerate, 3-trifluoromethyl-4-nitrophenol, endosulfan, endrin, toxaphene) and herbicides (glyphosate, atrazine, acetochlor, triclopyr, paraquat) pose a major threat to amphibians, given the frequent and extensive use of them worldwide. Phosphorus and nitrogenous compounds widely used as fertilisers in agricultural fields (e.g. nitrates, nitrites, ammonia, humic acid) often spill over to aquatic habitats, also decreasing survival and otherwise affecting larval development of amphibians. Similarly, secondary salinization of freshwater systems, which has increased over the past several decades due to human activities such as agricultural irrigation, coastal flooding, and the application of road salts (Cañedo-Argüelles et al., 2016; Saumure et al., 2021) can result in direct mortality of freshwater species leading to deleterious outcomes for wildlife populations (Hintz & Relyea, 2019). Other contaminants derived from industrial activity are also a common concern for the well-being of amphibians, from flame retardants to chemicals used in the manufacture of plastics and resins. These include substances such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), bisphenol A (BPA), tetrabromobisphenol A (TBBPA), dioxins, genistein, furans, perfluorooctanesulfonate (PFOS), perchlorates or phthalates. Another group of toxicants derived from industrial and mining activities are metals, metalloids, and nanoparticles, including arsenic, boron, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, or zinc. Petroleum oil products

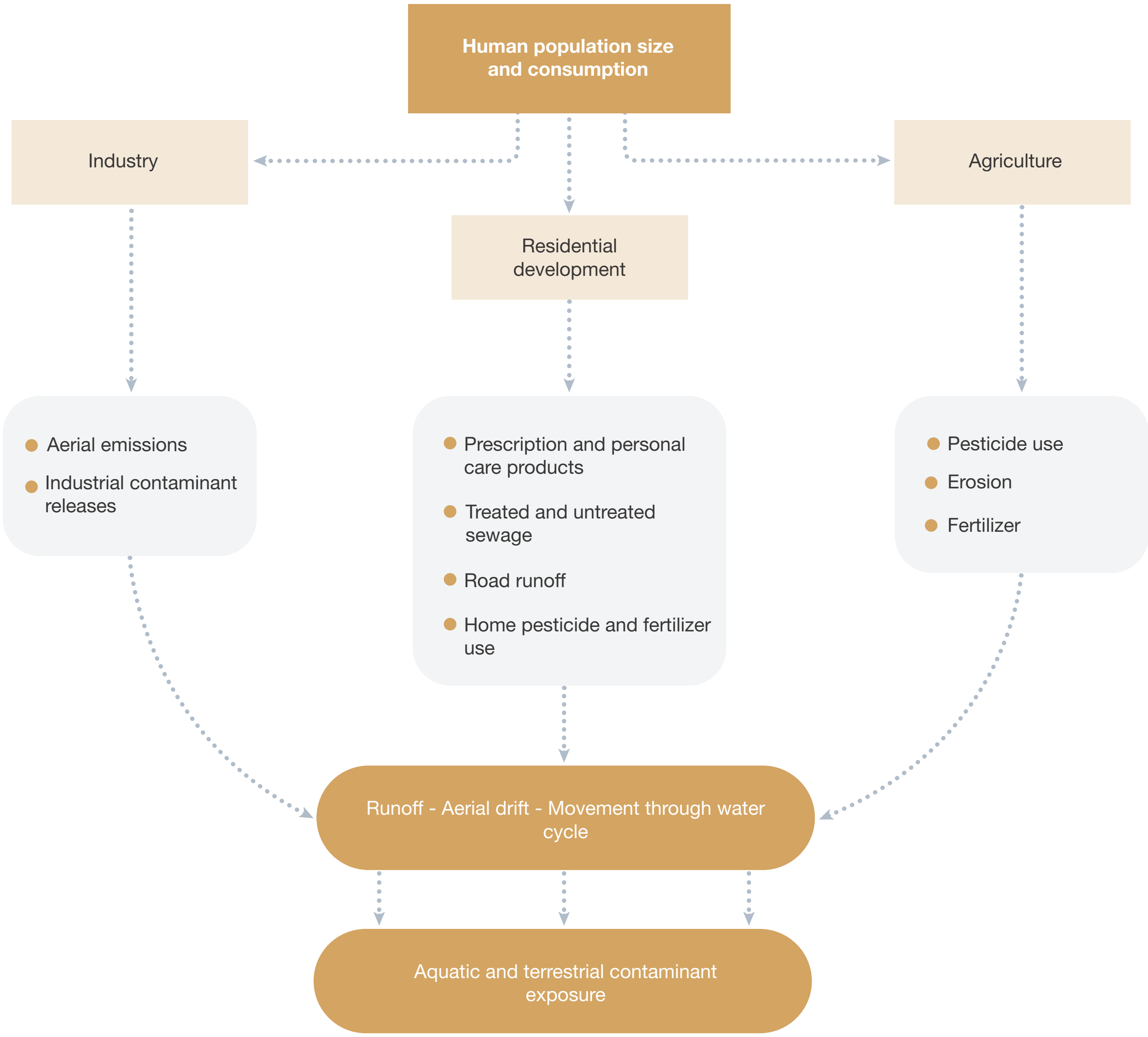


**Figure 4.1**: Human population size and consumption drives the industrial, residential, and agricultural footprints on the landscape that can contribute to chemical contamination of aquatic and terrestrial ecosystems. Source: Developed by Michelle D. Boone and Jessica Hua.

can be often spilled to water bodies, and both their polycyclic aromatic hydrocarbons and the naphthenic acid represent direct threats to amphibians. Pharmaceutical and personal care products are additional sources of chemical pollution that raise concern, particularly considering that methimazole, ibuprofen, estrogen, propylthiouracil, ethylenethiourea, triclosan, and triclocarban, all can interfere with amphibians' endocrine pathways. In the end, chemical contaminants of diverse sources and types move through water in natural and human-made systems, making amphibians vulnerable to exposure to pollution during their life cycles.

## Generalizable toxicity across classes, types, and modes of action of active ingredients

Predicting the effects of the thousands of environmental contaminants is enormously challenging because of the diverse array of contaminants to which ecosystems are exposed. Although basic toxicological data are available for a few model organisms, the ecological ramifications of exposure for most contaminants are not clear. Predicting responses in natural systems, however, is critical so that effects of exposure can be reasonably estimated for regulatory purposes—and such predictions are possible. An important means to anticipating community- and ecosystem-level effects can be coarsely achieved by using an active ingredient's chemical class, mode of action, and/or type (e.g. herbicide, insecticide, metal) to make predictions concerning the potential influence on natural systems.

By considering a contaminant through a categorical lens, some general principles can be reached. For example, Boone (2008) evaluated if combinations of insecticides with a different or the same mode of action were more or less likely to have additive or nonadditive effects on metamorphosis; in this study, aquatic environments containing two insecticides that were acetylcholinesterase inhibitors were more likely to have nonadditive effects than if the two insecticides had a different mode of action. Such approaches can improve our ability to anticipate effects of chemical mixtures, which are common in environments. Further, for contaminants that are well studied like the insecticide carbaryl (e.g. Boone et al., 2004, 2007; Zippel & Mendelson, 2008), the herbicides atrazine (Rohr & McCoy, 2010) and glyphosate (e.g. Relyea, 2005), and the metal mercury (e.g. Bergeron et al., 2011), the effects found in an array of studies from lab to field for these contaminants can offer insight for the ecological effects of contaminants with a similar mode of action or of a similar type/characteristic if we know that contaminants from similar classes and types have similar effects.

Data are beginning to suggest that chemical types and classes do have generalizable consequences. To evaluate chemical classes, Shuman-Goodier and Propper (2016) found effect sizes for swim speed and activity in fish and amphibians were similar for contaminants within the same chemical class. Using a meta-analysis, Egea-Serrano et al. (2012) determined that types of contaminants had different effect sizes across amphibian responses, suggesting that some contaminant types were more likely to have negative effects. Kerby et al. (2010) compared the sensitivity of amphibians via LC50s (lethal concentration of 50% of the population) with other taxonomic groups to contaminants based on chemical class and found amphibians had moderate to low sensitivity to pyrethroid, carbamate, organophosphate, and organochlorine pesticides; heavy metals; and inorganics relative to other groups; however, amphibians appeared to have higher sensitivity to phenols than other taxa. Evaluating sensitivity by chemical class or type is a useful way to infer contaminant categories that may be of more concern than others. Rumschlag et al. (2019) found that pesticides with the same chemical class or type (e.g. insecticide or herbicide) had similar impacts on amphibian host-trematode parasite communities, and Rumschlag et al. (2020) demonstrated that community structure and ecosystem function were impacted similarly based on a pesticide's effect through direct and indirect pathways. These studies suggest that based on class or type, we can expect some generality to contaminant effects, and we should be able to predict more complex ecological outcomes in systems based on direct effects at different trophic levels. These approaches offer a means of understanding contaminant impacts in natural systems so that we can minimise contaminant effects that can directly and indirectly impact species of concern, like amphibians, even without exhaustive studies for each particular contaminant.

### Direct effects

- **Physiological**

  Extensive research has found that contaminant exposure at ecologically relevant concentrations can impact amphibian physiology in a myriad of important ways, from non-monotonic (a dose-response relationship characterised by a U-shaped or inverted U-shaped curve across increasing doses; Lagarde et al., 2015) modulation of stress hormones like corticosterone (Larson et al., 1998; McMahon et al., 2011), to altered cardiac function (Jones-Costa et al., 2018; Palenske, Nallani & Dzialowski, 2010), to the disruption of endocrine axes (including the feedback loops between hypothalamic-pituitary-adrenal axis or hypothalamic-pituitary-thyroid components of the endocrine system; Thambirajah et al., 2019; Trudeau et al., 2020), to immunomodulation (e.g. Forson & Storfer, 2006; McMahon et al., 2011), to impaired neuronal function (Sparling et al., 2010) or altered metabolism (Burraco & Gomez-Mestre, 2016). Moreover, contaminants have also been shown to be genotoxic (Maselli et al., 2010; Patar et al., 2016), and the damage caused to the DNA may potentially affect gene expression and lead to mutation-based diseases. One of the most commonly used pesticides in North America, the herbicide atrazine, has been shown to reduce size at metamorphosis, diminish immune function, and modulate gonadal morphology, impacting spermatogenesis and sex hormone production (Hayes et al., 2002; Rohr & McCoy, 2010; Vandenberg et al., 2012). Indeed, atrazine exposure can cause feminization in genetic male frogs (Hayes et al., 2002; Hayes et al., 2010; Rohr & McCoy, 2010), altering their overall fitness. Chlorothalonil, one of the most commonly used synthetic fungicides in North America, impacts immune response and degrades tadpole liver tissue in a non-monotonic fashion (McMahon et al., 2011). The severity of impact of contamination on amphibian physiology is also altered by timing of exposure (e.g. Rohr et al., 2013). Early life exposure is often, but not always, more detrimental than late life exposure. Additionally, there is evidence that the impact of contaminant exposure on physiology impacts the successive generations, as well. For example, male *Xenopus tropicalis* exposed to pesticides had reduced fertility due to endocrine disruption, were smaller in size, and their offspring had decreased plasma glucose levels (Karlsson et al., 2021). Many studies with amphibians do not examine physiological responses, but for those that do, effects appear to be commonplace (Thambirajah et al., 2019), suggesting biochemical changes that can have long-term effects are an important avenue for future research.

- **Carryover effects**

Exposure to a contaminant has the potential to result in acute effects; understanding those effects and their ramifications can help managers minimise or mitigate the consequences. Yet even more pernicious are the effects that have consequences well after exposure, making short-term toxicity studies less useful in predicting effects in natural systems; further, effects that occur well after exposure make establishing cause-effect linkages challenging. Long-term effects stemming from conditions earlier in life are carryover effects. Carryover effects can occur when a contaminant has an obvious short-term effect with the consequences persisting or when a contaminant has no observed effect at exposure with impacts appearing later in life after exposure has ended (O'Connor et al., 2014).

For instance, if contaminant exposure results in smaller size at metamorphosis in amphibians, then future fecundity, time to reproduction, and survival in the terrestrial environment (i.e. fitness) can be impacted (e.g. Altwegg & Reyer, 2003; Chelgren et al., 2006; Earl & Whiteman, 2015; Scott et al., 2007) even though contaminant effects may have been acute. Many contaminants affect endpoints correlated with fitness, through either direct chemical effects or indirect effects through changes in the food web (e.g. Relyea & Diecks, 2008). It follows that any contaminant that alters these critical endpoints have a higher probability of impacting future responses via carryover in ways that affect populations. Currently, studies that have followed amphibians after contaminant exposure early in development have found that carryover effects from acute exposures can have lasting effects on terrestrial growth and overwintering for some species and not for others (Boone, 2005; Distel & Boone, 2010).

Carryover effects from contaminant exposure in early life can also appear later in life despite no apparent effects immediately after exposure via altered physiology, behaviour, or gene expression (O'Connor et al., 2014). For instance, while negative chemical effects were not apparent in anurans reared in wastewater treatments relative to controls, terrestrial growth was reduced for those from wastewater, suggesting a metabolic cost of exposure was not apparent until later in development (Zeitler, Cecala & McGrath, 2021). Similarly, Rohr and Palmer (2005) found that the herbicide atrazine unexpectedly increased terrestrial desiccation risk in salamanders through altered activity months after larval exposure. Delayed effects, like acute ones, are important because they can reduce survival, fertility, and growth; therefore, delayed carryover effects are critical to understand.

Contaminants that result in biochemical changes, such as changes in hormones for example thyroid hormones (Thambirajah et al., 2019), stress hormones (Bókony et al., 2021; Davis et al., 2020), sex hormones (Hayes, et al., 2010), or gene expression (e.g. Hinther et al., 2011; Zhang et al., 2019) may be more likely to have carryover effects. Carryover effects appear to be a common, under-studied consequence of contaminant exposure (Bergman et al., 2013; Edwards & Myers, 2007). Surprisingly, some carryover effects are positive: prior exposure to a contaminant can lead to greater tolerance to other stressors later in life, potentially through induction of a generalised stress response (Billet & Hoverman, 2020; Hua, Morehouse & Relyea, 2013). However, general patterns have not yet been identified.

Carryover effects can also include those that cross generational boundaries—an area of research that offers many opportunities for discovery, given that the currently available data are quite limited. In particular, endocrine-disrupting chemicals (including phthalates, bisphenol A, microplastics pharmaceutical and personal care products, and persistent environmental contaminants like PCBs) are likely to have transgenerational impacts (Brehm & Flaws, 2019; Schwindt, 2015; Zhou et al., 2020). For instance, Karlsson et al. (2021) demonstrated that exposure of males to an anti-androgenic pesticide (linuron) resulted in effects across two generations in anurans. Additionally, maternal mercury exposure in anurans had negative effects on growth and survival in the next generation of tadpoles through maternal transfer of mercury (Bergeron et al., 2011), suggesting that contaminants that bioaccumulate in breeding females may have the potential to cross generational boundaries. Similarly, breeding pairs from agricultural and urban ponds with high concentrations of endocrine-disrupting pesticides (Bókony et al., 2018) produced tadpoles and juveniles with lower growth rates and development. Endocrine disruption caused by pesticide exposure can affect subsequent unexposed generations, for at least two generations (Karlsson et al., 2021). Although there are few studies examining transgenerational impacts, current knowledge suggests that such effects may be common.

Carryover effects are understudied in amphibian ecotoxicology (as well as more broadly), and they have the potential to impact population health and persistence through time (O’Connor & Cooke, 2015). While we have a good understanding of the consequences that follow for some responses (e.g. effects on time and size at metamorphosis; early life stress hormones), species variation may still undermine broad generalisations, which could become predictable with more study (Earl & Whiteman, 2015). Making cause-effect linkages remains a major challenge for contaminants that have carryover effects and calls for studies across the life cycle and through multiple generations.

### Indirect effects

Given that freshwater systems are among the most biodiverse in the world (Dudgeon et al., 2006), predicting the cumulative effects of contaminants on amphibians is hampered by the myriad of possible indirect effects, mediated through and compounded by species interactions and food web structures. Despite the magnitude of the threat that contaminants impose on amphibians and freshwater systems (Bernhardt, Rosi & Gessner, 2017; Burton et al., 2017), indirect effects of contaminants are often overlooked by research communities and funding agencies. Classic toxicological lab-based experiments have documented scores of contaminants that can cause acute toxicity to organisms (Sparling et al., 2010), but they fail to predict complex suites of effects that can occur when contaminants enter freshwater systems (Bernhardt et al., 2017; Gessner & Tlili, 2016; Rohr, Salice & Nisbet, 2016). Contaminant-induced changes in behaviour, competition, and predation/grazing rates can lead to changes in abundance, richness, and/or composition of community members (Fleeger, Carman & Nisbet, 2003; Hillebrand & Matthiessen, 2009), which can impact amphibians via bottom-up and top-down trophic cascades (Fleeger et al., 2003; Hillebrand & Matthiessen, 2009). Advancements in replicated, field-based in situ, and mesocosm studies have offered a way to incorporate the complexity of multitrophic communities, so that the cumulative effects of contaminants on amphibians can be better evaluated.

Bottom-up indirect effects of contaminants alter food resources of amphibians. In the larval environment, alterations to algae can influence the survival and development of tadpoles. For instance, contaminants, including coal ash, fungicides, and herbicides, can decrease the abundance or alter the composition of phytoplankton and periphyton (Brock, Lahr & van den Brink, 2000; McMahon et al., 2012; Rowe, Hopkins & Coffman, 2001; Rumschlag et al., 2020). Top-down effects of contaminants alter the community of amphibian predators. Insecticides can reduce survival of predators (Schäfer et al., 2011), which can benefit amphibian larval survival and growth through a predator release (Rumschlag et al., 2020). Amphibian behaviour can also be directly impacted by contaminants, which can indirectly lead to altered predator-prey interactions. Sublethal concentrations of contaminants, including copper and insecticides, can reduce tadpole activity, increase rates of abnormal swimming, reduce escape responses, or inhibit detection of predator cues by tadpoles, leading to increased predation risk (Hayden et al., 2015; Polo-Cavia, Burraco & Gomez-Mestre, 2016; Sievers et al., 2019).

Contaminant-driven bottom-up and top-down effects can also alter transmission of parasites in amphibian populations by altering parasite exposure risk. For instance, in amphibian-trematode systems, triazine herbicides, organophosphate insecticides, and nutrients are linked with increases in snail abundance (first intermediate host) and thus trematode exposure, through increases in snail resources (periphytic algae, bottom-up effect) and changes to predator dynamics (top-down effect; Johnson & Chase, 2004; Rumschlag et al., 2019). In an amphibian-chytrid system, effects of contaminants on parasite exposure and load can be non-monotonic (McMahon, Romansic & Rohr, 2013), demonstrating complexity in predicting effects of contaminants on parasite transmission.

Indirect effects of contaminants on amphibians and other community members have even been linked to ecosystem-level consequences (Halstead et al., 2014). For instance, diverse arrays of insecticides can all lead to increases in primary productivity (through predation/grazing release) and ecosystem respiration through negative effects on larval salamanders and other zooplankton predators, which change zooplankton abundance and composition (Rumschlag et al., 2020).

The findings documenting the indirect effects on contaminants on amphibians highlight the need for a large-scale perspective in terms of ecology, community composition, and time. Amphibians do not experience chemical exposure in isolation, and therefore holistic research on the indirect effects of exposure is needed to understand the net ecological impact.

### Evolutionary effects of contaminants

The call to incorporate evolutionary perspectives in our understanding of amphibian conservation and mitigation of amphibian declines was clearly articulated more than a decade ago (Blaustein & Bancroft, 2007). Indeed, since then, we have amassed ample evidence suggesting that amphibians can adapt in response to novel environmental conditions generated by pollutants (Brady, 2012; Cothran, Brown & Relyea, 2013; Homola et al., 2019; Hua et al., 2015), although the ability to adapt depends upon the presence of resistant genotypes in the population.

Additionally, in the last 15 years, our understanding of the various adaptive mechanisms driving responses to pollutants has markedly improved. For example, endocrine flexibility is a crucial coping mechanism in response to anthropogenic environmental change. Generally, corticosterone, the main amphibian glucocorticoid associated with the hypothalamic-pituitary-interrenal axis (HPI axis), is predicted to be elevated with exposure to pollutants and other environmental stressors (Bókony et al., 2021; Forsburg, Guzman & Gabor, 2021; Gabor, 2018; Gabor et al., 2018; Goff et al., 2020; Hopkins, Mendonça & Congdon, 1997; Tennessen et al., 2018). Yet not all populations (mostly endotherms) show elevated glucocorticoids in urbanised populations (Injaian et al., 2020; Murray et al., 2019). Further, Bókony et al. (2021) found that tadpoles of *Bufo* from anthropogenic and natural habitats that were reared in common garden experiments had higher baseline corticosterone-release rates in urban ponds; however, tadpoles from urban and agricultural ponds showed an adaptive response by responding to stressors with a greater stress-induced change than tadpoles from natural habitats, indicating that tadpoles from anthropogenic sites had a more efficient negative feedback (return to baseline). Collectively, these findings indicate the complexity of mitigating amphibian declines and suggest that more mechanistic studies may aid in exposing alternative methods for minimizing the amphibian response to contaminants by decreasing application rate, changing the timing, or using different contaminants, even when the contaminants cannot be removed.

While the adaptive response to pollutants provides an optimistic perspective to amphibian populations facing contaminant exposure, recognition that these adaptations can lead to costs is growing; examples include a reduction of fitness (Brady, 2012; Brady et al., 2019; Hua et al., 2015; Semlitsch, Bridges & Welch, 2000), and absence of protective co-tolerance effects to pollutants or natural stressors like predators and pathogens (Hua et al., 2016; Hua et al., 2013; Hua et al., 2013; Jones et al., 2021; Rumschlag et al., 2020). A number of advances in techniques to assess the evolutionary effects of contaminants on amphibians have been made, including traditional toxicity assays (e.g. time to death – TTD – assays, LC50s) to compare functional traits like tolerance across groups, physiological coping capacity assays that measure stress physiology and capacity to cope with pollutants and environmental change (reviewed by Narayan et al., 2019), and community metabarcoding to study diversity of amphibian microbiomes, which has applications in disease mitigation and captive breeding for translocation purposes (Ficetola, Manenti & Taberlet, 2019).

Despite the growth in our understanding of evolutionary effects of contaminants on amphibians, few studies have directly implemented evolutionary principles and evaluated these efforts to inform and facilitate amphibian conservation. Future work should consider designing and testing conservation strategies based on our understanding of evolutionary effects of pollutants on amphibians. These may include selective breeding, introduction of adaptive variants through translocations, ecosystem interventions aimed at decreasing phenotype–environment mismatch, or genetic engineering (Pabijan et al., 2020). Some challenges to consider include whether we should expose amphibians in captive breeding to stressors that can help habituate the HPI axis and/or promote coping with unpredictable environments that they will experience if they are reintroduced to the wild. Similarly, we need to evaluate if we should engineer husbandry conditions that are similar to those in the wild to improve management outcomes (i.e. bioaugmentation techniques to initiate the establishment of healthy skin microbiotas in captive hellbenders prior to release; Kenison, Hernández-Gómez & Williams, 2020).

While evolutionary responses may protect some amphibian populations from the effects of pollutants, other populations may not respond rapidly enough to cope with the pace of pollutant contamination even if genetic variation in resistance/tolerance exists in the population (Pabijan et al., 2020). Therefore, looking ahead, integrating evolutionary findings from the past 15 years to develop and directly test evidence-based evolutionary principles to protect the most vulnerable amphibian populations will be imperative to our amphibian conservation efforts.

## Interactions of contaminants with other environmental factors

While contaminants alone and in mixtures have been put forward as a potential cause for amphibian population declines and while contaminants can theoretically cause local extinction (Willson et al., 2012) or serve as habitat sinks (e.g. coal ash, Rowe et al., 2001), the interactive effects of contaminants with other natural and anthropogenic factors has long-been anticipated to result in deleterious effects (Blaustein et al., 2011; Carey & Bryant, 1995; Hayes et al., 2010).

**With habitat degradation and alteration**

- **Land-use/land-cover increases the risks of contamination:**

  Conversion of habitats to agriculture, residential, developed, and sub/urban lands can lead to increased contaminant exposures in the aquatic and terrestrial habitats used by amphibians (Sievers et al., 2018), which can directly affect amphibians, and which can alter and degrade the quality of the habitat in ways that create the potential for multiple stressors. While contaminant exposure in the environment is pervasive in protected areas with low human impact to areas of agricultural and industrial activity (Battaglin et al., 2016; Bókony et al., 2018; Hageman, 2006; van Dijk & Guicherit, 1999), the likelihood of exposure is greater in some areas. Contaminants accumulate in water bodies, making these areas an important exposure pathway for amphibians with complex life cycles or living in areas near streams and wetlands (Battaglin et al., 2016; Bókony et al., 2018). Further, greater likelihood of contaminant exposure exists in aquatic habitats with concentration increasing dramatically for single contaminants and chemical mixtures (Anderson et al., 2013; Battaglin et al., 2016; Hayes et al., 2006) in both agricultural and protected areas (Sparling et al., 2015; Trudeau et al., 2020). Additionally, some types of agricultural techniques such as surface drainage ditches and subsurface tile drains contribute to habitat loss and transport pesticides, nutrients, and other contaminants into wetland habitats (Blann et al., 2009). Chemical mixtures increase the likelihood of effects (Hayes et al., 2006), which can ultimately reduce offspring fitness in amphibians (Bishop et al., 2010; Bókony et al., 2018; Semlitsch et al., 2000), but which can also lead to pesticide tolerance or resistance (e.g. Cothran et al., 2013; Hua et al., 2015) in ways that alter populations.

- **Contaminants as habitat degradation:**

  Ponds are natural features on the landscape and are often added by people for recreational or aesthetic reasons, or for their ability to remove sediments moving across the landscape or water across impervious surfaces (Davis et al., 2021; Gallagher et al., 2011; Monaghan et al., 2016; Renwick et al., 2005); both natural and human-made ponds are readily used by amphibians. Yet, environmental contaminants in these water bodies represent a form of habitat degradation. Ponds on human-dominated landscapes like golf courses, agricultural areas, parks, or multi-residential properties are more likely to be chemically managed to control algal or plant overgrowth, which can increase exposure risks to amphibians and influence population persistence (Sievers et al., 2018). For instance, golf courses manage water features for aesthetics and are impacted by fertiliser and pesticide runoff with occasional application of chemicals like copper sulphate directly to ponds to reduce algal and plant growth, which can also be toxic to amphibians (Puglis & Boone, 2012). Use of pond dyes has become more common in residential and urban ponds as a means of reducing algal growth; effects have not been found to have direct impacts on amphibian metamorphosis, but such management practices change the food web, reducing algal and zooplankton food resources for amphibians (Bartson et al., 2018; Suski et al., 2018). Chemical exposure that reduces emergent vegetation can also impact the quality of a site for breeding and larval development via reduced cover and increased vulnerability to predators (Shulseet al., 2010), although the direct and indirect consequences can make predicting outcomes difficult (Edge et al., 2020). The effect of contaminants on habitats can alter the quality of habitat, which can have population- and community-level repercussions, and which may not be obvious from traditional toxicological studies (e.g. LC50s in single species tests). Physiological and behavioural studies provide mechanisms for documenting systems in decline, especially in habitats that are experiencing conversion, before environmental stressors can be mitigated (Walls & Gabor, 2019).

While terrestrial buffers are mandated, for instance, in some areas near streams to reduce habitat degradation from nutrient runoff and soil erosion in waterways, they are generally not required around small temporary or permanent ponds often used by amphibians for breeding and larval development. Terrestrial buffers can promote contaminant and nutrient filtering from ponds (Cole, Stockan & Helliwell, 2020; Mayer et al., 2005; Muscutt et al., 1993; Skagen, Melcher & Haukos, 2008) and also serve as key upland habitats for terrestrial species or life stages (Semlitsch & Bodie, 2003). Physical habitat structure may also intercept aerial deposition of contaminants that may physically/directly impact amphibians in terrestrial habitats and can offer a solution to minimise contaminant impacts on water quality and on the species that live there.

**Land-use/Land-cover influences environmental conditions and can interact:**

- **With contaminant exposure**

Land-use/land-cover changes alone have dramatic impacts on populations and communities, and amphibians can be affected by the interaction of habitat characteristics and contaminant exposure in ways that lead to the co-occurrence of environmental characteristics (e.g. Faulkner, 2004; Renick et al., 2015). For instance, loss of surrounding forest habitat can reduce leaf litter inputs and, thus, dissolved organic carbon that attenuates UV radiation; because some contaminants are more toxic in the presence of UV, changes in UV penetration can influence how toxic the same environmental concentration of a contaminant is and directly impact amphibian growth and survival (Puglis & Boone, 2011; Roberts, Alloy & Oris, 2017). Conversion of forest to rangeland can have impacts at a larger landscape scale and can interact with the resulting consequences, which may include: reduction in emergent vegetation in ponds used for egg laying and predator protection of larvae; diminished quality of the terrestrial habitat for juvenile and adult growth and survival; changes in the hydroperiod of the wetland (which may be lengthened for cattle watering or shortened for planting); altered aquatic food webs resulting in changes in food availability and predators abundance; and reduced water quality (Moges et al., 2017; Tilman, 1999). The addition of a contaminant that lengthens larval period in a habitat that has a shortened hydroperiod because of agricultural tiling or draining, for instance, can reduce recruitment of juveniles into the adult population, as Relyea and Diecks (2008) found for anurans reared in drying experimental ponds exposed to the insecticide malathion. Additionally, land use changes that impact water quality may result in algal blooms and higher water temperatures that spur management by land managers or residents. For instance, Goff et al. (2020) found that water quality and land cover type affected the physiological and bacterial diversity of ornate chorus frogs (*Pseudacris ornata*), thus affecting the overall population health. In this way, land-use and land-cover changes can alter a number of abiotic and biotic factors and interact with contaminant exposure to impact development and physiology of individuals, which can have acute and long-term consequences.

The potential for interactive effects of contaminants is illustrated in two field studies. The threatened Jollyville Plateau salamander (*Eurycea tonkawae)* is a fully neotenic stream dwelling species found in central Austin, Texas, USA. This species is on the United States Endangered Species List because of threats from urbanization; indeed, counts of this species declined more in areas with the largest residential development than less developed areas throughout the species range (Bendik et al., 2014). In a follow-up study exploring the mechanisms associated with declines, Gabor et al. (2018) found that in two out of three years, salamanders from streams in more developed watersheds released higher corticosterone (an endocrine hormone associated with the stress axis) than salamanders from populations in preserves. Corticosterone levels were also higher in urban streams than in rural ones. Positive feedback between stream background corticosterone and baseline corticosterone may account for the higher corticosterone release rates found for *E. tonkawae* in urban streams, because amphibians can uptake exogenous corticosterone through their skin (Glennemeier & Denver, 2002). Because urban catchments are associated with septic systems and sewer lines, exogenous corticosterone from these systems plus runoff will continue to plague amphibians within these catchments. Further, Davis et al. (2020) found that salamanders located in agricultural wetlands compared to reference wetlands had higher ranavirus infection loads and higher corticosterone release rates. At the same time, corticosterone release rates were higher in ranavirus infected salamanders. Together, these results indicate that amphibians are being hit by multiple stressors, which likely increase the rates of amphibian declines. These studies show the usefulness of using water-borne corticosterone as one mechanism by which habitat impacts on amphibian population health can be measured in the field.

- **With disease**

Given the important role disease has played in amphibian population declines (Scheele et al., 2019; *also see* Chapter 6) — particularly ranaviruses and the amphibian chytrid fungi (*Batrachochytrium dendrobatidis* [Bd] and *B. salamandrivorans*) — and given that disease pathogens and contaminants are distributed across space while disease outbreaks appear more localised, the potential for disease by contaminant interactions is of critical importance (Blaustein et al., 2018). Because contaminants have a wide range of modes of actions, they have the potential to affect pathogens, hosts, or their interaction, which can alter disease dynamics and could explain the range of observed effects in experiments and natural systems (Blaustein et al., 2018). In experimental studies, the presence of contaminants may not alter the susceptibility of amphibians to a pathogen (as some studies have found, e.g. Buck et al., 2015; Gaietto, Rumschlag & Boone, 2014; Kleinhenz, Boone & Fellers, 2012) or it can increase susceptibility (e.g. Cusaac et al., 2021; Rohr et al., 2013; Wise, Rumschlag & Boone, 2014), and these differences may be attributed to life stage exposure and species/population susceptibility. Field studies find associations between host-pathogen relationships and environmental contamination, although the type of contamination or effect may vary among study systems. For instance, King et al. (2010) found parasite infection risk was greater for anurans in polluted habitats, but risk varied with land cover in the landscape. Battaglin et al. (2016) found that frogs at field sites across the USA were more likely to be positive for Bd at sites with higher fungicide concentrations in water and sediments, and with more dissolved organic carbon, total nitrogen, and phosphorus in the water. Reeves et al. (2017) found Bd zoospore abundance was negatively associated with neonicotinoid concentration in wetlands in Iowa, USA.

Rumschlag and Rohr (2018) found herbicide use was associated with low Bd infection prevalence in larval aquatic habitats and high infection prevalence in post-metamorphic terrestrial habitats. Further, populations exposed to salt runoff had slightly more frequent ranavirus-related mass mortality events, more lethal infections, and 117-times greater pathogen environmental-DNA (Hall et al., 2020). Generally, the presence of contamination in environments is associated with increased likelihood of pathogen/parasite infections in some systems in ways that are not currently predictable.

Anticipating how contaminants will impact pathogen-amphibian dynamics is difficult because underlying mechanisms determining these interactions are not well understood, because non-monotonic responses result with exposure to some contaminants (e.g. endocrine disruptors), and because amphibian populations/species (e.g. Hoskins & Boone, 2017; McMahon et al., 2011, 2013; Rohr & McCoy, 2010) and pathogens (e.g. Bd; McMahon et al., 2011) vary in response to contaminants. Yet, a promising research avenue for predicting pathogen-contaminant interactions is the examination of contaminant effects on immunomodulation (Hayes et al., 2006; McMahon et al., 2011) and on antimicrobial skin peptides or other defences that can prevent infections (McCoy & Peralta, 2018; Rollins-Smith et al., 2002). For instance, Davidson et al. (2007) found that an insecticide impacted the ability of anuran skin peptides to reduce Bd growth in vitro. Because pollution and other environmental conditions can influence the skin and gut microbiomes that can compromise an amphibian's ability to fight disease pathogens, contaminant effects on the amphibian host microbiome are likely an important mechanism influencing disease dynamics (McCoy & Peralta, 2018).

Contaminants can also alter the environment in ways that increase susceptibility to pathogens even if the contaminants themselves do not directly impact amphibians. For instance, Johnson et al. (2007) found that trematode infections were increased in amphibians through eutrophication of systems via nutrient runoff; in this way, contaminants can change the system to favour pathogens and increase infection rates. There are many ways that contaminants can alter the environment through changes in abiotic conditions or physical structure, or in the biotic community that could alter host-pathogen systems. For example, if contaminants can alter the abundance of microscopic aquatic predators that feed on infective stages of trematode parasites or Bd zoospores, they could influence infection prevalence and disease dynamics (Schmeller et al., 2014). Additionally, indirect effects of contaminant exposure can increase disease risk by increasing the abundances of intermediate hosts of pathogens in the environment or through slowing host development in stages especially vulnerable to infection (Halstead et al., 2014; Rumschlag et al., 2019). These interactions can be complex with outcomes mediated by host species, host and pathogen quality, and environmental properties.

Given that disease-causing parasites and pathogens are on the rise (Scheele et al., 2019), determining which factors can increase the likelihood of disease outbreaks is critical; current data suggest contaminants may be an important cofactor, yet there are thousands of chemicals that occur at different concentrations and that have divergent properties, creating a Russian roulette scenario in natural systems. Rumschlag et al. (2019) found that pesticide class predicted effects on trematode parasites and their hosts in aquatic communities, which offered some general conclusions that could be applicable to other areas. Such studies offer a powerful approach that provides predictive power to better shape both management and policy in ways that reduce the likelihood that contaminant exposure will lead to catastrophic disease outbreaks that negatively impact amphibian populations and species.

- **With climate change**

The IPCC (2013) predicts changes in temperature and precipitation patterns across the globe, including shifts in average temperatures and increases in extreme climatic events (Diffenbaugh & Ashfaq, 2010; Schär et al., 2004; *see also* Chapter 3). Understanding how contaminants will impact amphibians in a climate change scenario is a major challenge for amphibian conservation. Temperature can alter amphibian susceptibility to contaminants, but its effects are chemical dependent. Some studies find that higher temperatures can decrease sensitivity to pollutants, such as copper sulphate (Chiari et al., 2015) and atrazine (Rohr, Sesterhenn & Stieha, 2011). In contrast, other studies report that increasing temperature results in greater toxicity, including endosulphan, carbaryl, methomyl and pyrethroid insecticides (Boone & Bridges, 1999; Broomhall, 2002; Lau, Karraker & Leung, 2015; Materna, Rabeni & Lapoint, 1995). It is clear that interactive effects between contaminants and temperature exist and understanding the mechanisms by which pollutants and temperature interact is important (similar to Burraco & Gomez-Mestre, 2016) to develop effective conservation strategies.

Further, climatic instability/unpredictability may also prompt amphibians to experience lower temperatures if reproduction events are prematurely cued (i.e. a false spring; Parmesan, 2007). Exposure to cold temperatures during embryonic stages can negatively affect amphibians by increasing tadpole susceptibility to pollutants (Wersebe et al., 2019). Similarly, phenological shifts that expose breeding amphibians to freezing conditions can have cascading consequences on offspring ability to tolerate pollutants (Buss, Swierk & Hua, 2021).

Contaminants could also alter adaptive traits (morphological, physiological and behavioural) that are crucial for species to cope with climate change. In the past 15 years, our knowledge on amphibian thermal physiology traits has grown significantly (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Katzenberger et al., 2021; Sunday et al., 2014). Contaminant effects on traits related to thermal physiology appear to be species- and chemical-dependent. Katzenberger et al. (2014), for instance, found that the herbicide Roundup® did not affect the critical thermal maximum (CTmax), but it changed the shape of the thermal performance curve; in contrast, Quiroga et al. (2019) found that tadpoles exposed to the insecticide chlorpyrifos showed a significant decline in CTmax but not in CTmin.

Currently, we have insight on how a few chemicals impact amphibians, but the vast majority remains untested and generalisations are difficult. An important and straightforward step would be to determine how toxicity of common contaminants changes with temperature for critical components of the food web (i.e. from reports like Aronson et al., 1998), which would improve our ability to mitigate deleterious effects in ecological systems.

## Priorities in research

Amphibian ecotoxicological research has exploded in recent decades (Sparling et al., 2010) – assessing across scales from basic individual toxicity in the laboratory to ecologically relevant community-level questions in outdoor mesocosms and field enclosures, to landscape-level system questions. While research originally focused on mortality, it has now expanded to include responses across life stages (metamorphosis through to adult life stages), physiological responses such as endocrine and reproductive system modulation, and changes in behaviour, physiology, and genomic expression. Because amphibians are experimentally tractable across life stages, they can serve as models for understanding the effects of contaminants in natural environments. The two key research areas for amphibian conservation related to pollution should focus on issues that will, first, protect populations in the wild that are impacted by contaminants and that will, second, improve regulatory data collection to better protect natural systems.

### Population declines and amphibian conservation

We know amphibian populations are experiencing worldwide declines with no clear global explanation (Grant et al., 2020, 2016) and that contaminants are pervasive (e.g. Battaglin et al., 2016; Gibbs, MacKey & Currie, 2009). To understand the role contaminants play in declines and in systems not experiencing declines, we need to focus on the ecological ramifications of contaminant exposure. We achieve this focus by identifying the important factors that interact with contaminant exposure to impact traits associated with amphibian fitness; these factors likely include habitat change, disease, and climate change, factors which are additional stressors in communities already experiencing naturally occurring competition, predation, and physiological stressors. We need to conduct experiments that examine exposure at multiple time points and that span life stages of diverse amphibian species because of the wide variety of life history strategies utilised by Amphibia. Biases in geography, ecosystems, life stages, and species of study creates a risk that we reach general conclusions that will not be reality-based, particularly given that some species and areas experiencing population declines are not those that have been the most extensively studied (Leaning, 2000; Trimble & van Aarde, 2012). Schiesari et al. (2007) found that while the majority of amphibian declines have taken place in the tropics, most studies were conducted on temperate systems using a small number of mainly temperate species. Hence, biogeographical and taxonomic biases can and should be addressed, at least partially, by including amphibians in routine federal toxicity testing, using native species from around the world.

### Ecotoxicological studies for amphibian conservation

Traditional toxicological studies for regulatory purposes do not explicitly include amphibians, which is problematic given the role contaminants likely play in the amphibian biodiversity crisis, as outlined in this chapter. Yet, traditional toxicological approaches (e.g. LC50s) may not provide us with the information we need to protect this taxonomic group. Short-term studies often do not link exposure effects to critical traits correlated with fitness or to population dynamics, yet they are a good place to begin particularly in systems where there is little baseline data (e.g. many tropical systems). To determine long-term consequences of contaminant exposure, we need studies that examine consequences of exposure across life stages (i.e. carryover effects) and we need to use empirical data to parameterise population models to examine population viability in light of contaminant effects in complex communities (Willson et al., 2012). Linking responses that may happen with exposure (e.g. biomarkers like corticosterone; Gabor et al., 2018) to consequences later in life, offers promise to predict future consequences. Further, natural systems are more complicated and include contaminant mixtures and multiple potential stressors, so studies are needed that incorporate chemical and natural complexity and that can be paired with natural field studies (e.g. Hayes et al., 2003; Rohr et al., 2008a, 2008b), which enable us to make meaningful and powerful inferences. Such experiments can be logistically complicated, yet they are essential to establish cause-effect relationships and to evaluate the likelihood of additive or nonadditive effects. Many regulatory agencies in the US or Europe do not go beyond laboratory studies, but laboratories do not typically mimic systems - mesocosm or field studies are needed to do this (e.g. Halstead et al., 2014) - and when experimental field conditions match natural systems, their results yield predictive power (e.g. Boone et al., 2004; Kidd et al., 2007). Complex ecotoxicology studies will be more easily achieved if chemical classes and types allow predictability, as the data currently suggest (Rumschlag et al., 2019, 2020); for then, a representative chemical can be used to explore interactions with other factors, across life stages, and general conclusions can be made for a suite of contaminants, which will help address the regulatory challenges associated with contaminant testing and regulatory delay.

## Solutions for mitigating contaminant effects: Activities and opportunities

Considering that contaminant effects are well-documented, are associated with amphibian population declines (Davidson et al., 2002), are predicted to interact with other stressors (see above), and are predicted to cause declines when they affect survival (e.g. Willson et al., 2012), there are many reasons to reduce contaminant exposure in natural systems. Hence, stronger federal policies, improved and implemented conservation strategies, and individual actions can reduce the risk of amphibians' exposure to contaminants.

### Policy

Environmental contaminants are pervasive largely because environmental policies (or lack thereof) support this outcome. As such, effective policies are the most important way through which exposure can be reduced. Given that contaminants move through food webs, atmospheric drift, and the water cycle, one or a few countries with poor policies can lead to global distribution of contaminants. However, contaminant release may at times be necessary for society or inevitable to meet national or global needs. The question of policy relates to societal decisions of assessing when benefits justify the environmental and health costs, which can be difficult to answer without adequate scientific evidence and transparent public discussions that are not obfuscated by misleading information from industry (e.g. Oreskes & Conway, 2010).

For instance, the herbicide atrazine increases crop yields by < 6% at best and many reviews suggest average yields improve 1-3% (Ackerman, 2007). Atrazine is known to alter food webs by impacting the lowest trophic levels and, perhaps even more significantly, results in endocrine disruption across taxa (Hayes et al., 2011); however, atrazine's manufacturer works to muddle these results from influencing public policy and regulation in the USA (Boone et al., 2014; Hayes, 2004; Rohr, 2021) by attacking scientists (e.g. Aviv, 2014) and funding/influencing research that disproportionately produces studies showing no effects of atrazine (Hanson et al., 2019; Hayes, 2004). Is this an example of good policy where benefits disproportionately outweigh the costs or an example of the disproportionate influence of industry slowing regulatory processes (sensu Oreskes & Conway, 2010)? For amphibians, the weight of evidence suggests that there are significant costs to this policy that leads to widespread atrazine contamination of aquatic habitats (e.g. Rohr & McCoy, 2010), and the example of the regulatory process of atrazine is exceptional only in that the role of industry to slow the regulatory process has been well documented and publicised. Better policy that limits the role of industry in the experiments used to inform regulatory decisions could lead to better policy in the USA and other nations (Boone et al., 2014).

A policy of precaution, which is more pervasive in Europe, would also decrease the exposure risks to single chemicals and chemical mixtures, both of which increase the probability of biological effects and the interactive effects that result from interactions with other contaminants and environmental factors. However, for precaution to be an option, accurate predictions about how diverse contaminants will affect species and food webs are necessary. Towards this goal, while a wealth of data exists for amphibians and other taxa for a few contaminants, there are thousands of other regulated contaminants for which relatively little data exist. Looking ahead, expanding our understanding to include more contaminants and their potential interactions based on more general chemical properties or classes is an area of research that needs to be greatly expanded to allow informed decision-making or to adequately apply precaution. With more rigorous policy devoid of industrial influences, society and natural systems would reap more benefits from the trade-offs between pesticide use and restraint than they currently do.

**Conservation strategies**

Even in the absence of policies that reduce contaminant release, strategies exist that can diminish the likelihood of exposure or the concentration to which systems are exposed (e.g. Smith & Sutherland, 2014) which influences the direct and indirect consequences experienced by organisms. Terrestrial buffers around aquatic habitats absorb nutrient and chemical contamination in runoff, and slow the rate of movement, which can reduce exposure risk (see above). Policy that requires adequate habitat to surround aquatic environments could have a number of benefits including improved water quality and (potentially) flood control, which would benefit amphibians and a host of other taxa (including humans); however, buffer characteristics will vary across systems and are difficult to standardise (Kuglerová et al., 2014; Luke et al., 2019) with more known about riparian buffers than pond buffers. Terrestrial amphibians and terrestrial life stages are also vulnerable to contaminants (Brühl, Pieper & Weber, 2011; Brühl et al., 2013; James & Semlitsch, 2011), and could benefit from terrestrial buffers around terrestrial habitats.

Societal calls for minimising environmental exposures to contaminants would benefit a host of species, including amphibians and humans. Reducing contaminant use by, for instance, accepting some agricultural losses to pests while using practices that benefit natural pest-predators provides effective and environmentally friendly approaches to achieve pest reduction without chemical pollution. In fact, some research suggests that organic techniques produce yields similar to conventional agriculture without the chemical footprint (Ponisio et al., 2015) and that enhancing the diversity of agricultural systems offers ecosystem services without a loss in yield (Tamburini

**Table 4.1**: A summary of the research gaps highlighted in the 2007 ACAP update and current state of research on each of these gaps. The traffic light colour scheme represents research gaps that have received relatively more attention to less attention in the past 30 years. In the last decades, we have made substantial progress on addressing the research gaps highlighted in the 2007 ACAP. For each of the gaps highlighted in the 2007 ACAP, we highlight areas in need of further investigation (in bold).

| Research gaps from ACAP 2007 | Current status |
|---|---|
| Research is needed that goes beyond traditional toxicity testing by understanding complex chemical mixtures in complicated natural environments. | In the last 30 years, by integrating multiple toxicological techniques (lab to mesocosm to field), we have made substantial progress on understanding the complex direct and indirect effects of contaminants on amphibians. Studies have also worked to understand the interactive effects of complex contaminant mixtures. However, given the multitude of possible contaminant mixtures, we are still missing critical information that will allow us to make predictions about complex chemical mixtures in natural environments. Towards this goal, **future efforts that integrate experimental and predictive modelling efforts remain an important priority.** |
| Few studies have addressed physiological or genetic adaptation to chemical exposure, or how these adaptations to a chemical stressor may influence population persistence or make individuals vulnerable to other factors | In the last 30 years, research has worked to address our understanding of the physiological and evolutionary effects of contaminants as well as costs of responding to contaminants (*see* Physiological effects and Evolutionary effects). However, **we are still missing critical information to allow us to assess how these adaptations may influence population persistence or their relative contribution of mitigating contaminant-induced declines.** |
| We do not understand how contaminants may influence populations through time at multi-generational scales. | In the last 30 years, some efforts have been made to address multi-generational effects of contaminants though **this remains a research gap and this update includes two sections that address this point** (*See* Carryover effects and Evolutionary effects). |
| Examining the interactive effects of contaminants, disease, pathogens, global change, and habitat alteration will be instrumental to planning mitigation measures to thwart declines. | In the last 30 years, addressing interactive effects of contaminants appears to have been a research priority, **but this remains a central gap and major focus of this update** (*see* Interactive effects section). |
| Although much has been learned in recent years about the effects of a few contaminants (e.g. pesticides, coal combustion wastes), little is known about the effects of most other common pollutants on amphibians. | While we have made progress in expanding our understanding to more emerging contaminants (e.g. road salts, PFAS, microplastics, light pollution etc.), **there are many other contaminants that are not well studied. Understanding the impacts of chemical classes is a way to predict the effects of new chemicals that enter the market and is important baseline information that is needed. There is a need to consider not only the direct effects of these various contaminants but also their indirect effects.** |

| Research gaps from ACAP 2007 | Current status |
|---|---|
| Experimental contaminant research has focused almost solely on the aquatic life stage for amphibians | This remains a significant weakness in our understanding of how contaminants influence amphibians. While aquatic exposure remains the most likely site of exposure for amphibians with complex life cycles, there are exposure risks to terrestrial life stages and species. **Research not only remains focused on aquatic life stages but there is geographic bias that should be addressed in future efforts.** |

et al., 2020). Further, reducing the use of contaminants to maintain public gardens and lawns in residential areas could also reduce contaminant inputs into natural systems given that homeowners use 10 times more pesticides per acre than farmers (Meftaul et al., 2020). When the use of chemicals is unavoidable, such as when controlling the vectors of a zoonosis (e.g. *Aedes aegypti*, the mosquito responsible for spreading yellow fever, dengue fever, chikungunya, Zika fever, among others), their application should be accompanied by non-chemical actions (including population education) that add to the desired effect and help reduce the required number/dosage of applications. Prevention of pollution in the first place, particularly given that only a small amount of pesticides even reach pests (Pimentel & Burgess, 2012), is less economically and biologically costly than pollution clean-up.

Ultimately, cutbacks in consumption would reduce pollution associated with industry and development and are steps that individuals can take to reduce their pollution footprint. At the global scale, coordinated and collaborative efforts across intergovernmental agencies, international NGOs, stakeholders in industry, agriculture, government, and society members to reduce the amount of pollution entering natural systems are necessary. Though it is important to emphasise the challenges associated with such proposed concerted efforts due to the disproportionate global distribution of resources and wealth. Holistic approaches that consider strategies for supporting such actions in entities with limited resources or whose economies strongly depend on agriculture or whose political structures limit with regulations associated with environmental impacts remains a central challenge.

To summarise, if stakeholders in industry, agriculture, government, and society members work together to reduce the amount of pollution entering natural systems, amphibians and other species, including humans, are less likely to experience negative consequences of exposure—consequences that often do not reveal themselves for years.

## Conclusions

In the last three decades, we have made substantial progress towards understanding how contaminants influence amphibians and the critical questions we need to address. Notably, we have addressed many priority points highlighted in the 2007 ACAP (Table 4.1). While we have made headway, there remain several research gaps. Of note, continued research is needed to understand the dynamics of how contaminants interact with other important stressors (i.e. habitat degradation, disease, climate change) to influence amphibians in potentially antagonistic, additive, or synergistic ways. Given the sheer number of different contaminants and the potential for diverse contaminant mixtures, an important need remains for predictive models that accurately assess the effects of individual and contaminant mixtures across ecological scales and organisations from molecular and physiological levels to systemic population and community levels. Importantly, this effort will require continued integration of multiple techniques (lab to field), as well as scientists with diverse expertise across biology (molecular to landscape levels). Researchers continue to study and understand the contribution of long-term and multi-generational effects of contaminants on amphibians. Lastly, a concerted effort should be made to address the geographical, ecosystem, and life stage biases that currently favour larval stages in temperate habitats. Addressing research priorities outlined here will allow us to better understand how contaminants influence amphibian declines. Current data indicate that amphibians are exposed to concentrations that elicit several effects (many of which are negative), that these effects are often (at a minimum) additive with other environmental stressors, and that they pose a threat to population viability worldwide. Collaborative work with scientists, policymakers, local human populations, landowners, and other stakeholders could lead to implementation of the best strategies to minimise the impacts on amphibians and the ecosystems at large.

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Sakhalin toad (*Bufo sachalinensis*) eggs © Amaël Borzée


*Alytes obstetricans* tadpoles are placed in clean beakers with 40 ml of water for 60 min to measure baseline glucocorticoids. This can be done in the field (seen here in Asturias, Spain) and repeatedly to measure stress response and recovery from each tadpoles. This species is currently classified as Least Concern. © Caitlin Gabor