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Simulated developmental and reproductive impacts on amphibian populations and implications for assessing long-term effects

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ABSTRACT

Fish endpoints measured in early life stage toxicity tests are often used as representative of larval amphibian sensitivity in Ecological Risk Assessment (ERA). This application potentially overlooks the impact of developmental delays on amphibian metamorphosis, and thereby reduced survival, in amphibian populations constrained by habitat availability. Likewise, the effects of reduced productivity or altered sexual development as a result of chemical exposure are not presented in terms of lower population fecundity in these surrogate tests. Translating endpoints measured in toxicity tests to those that are more representative of amphibian ecology and population dynamics provides a means of identifying how developmental effects result in long-term impacts. Here we compare effects of developmental delay on metamorphosis success in six anuran species and simulate population-level impacts of subsequent reductions in larval survival as well as potential reductions in fecundity as a result of developmental impacts. We use deterministic matrix models to compare realistic combinations of amphibian demographic rates and relative impacts of reduced growth on larval survival and subsequently on population growth. Developmental delays are less detrimental in species with longer and less synchronous larval periods. All six species were most sensitive to changes in first-year survival, and damping ratios were generally a good indicator of resilience to perturbations in both larval survival and fecundity. Further identification of species and population-level vulnerabilities can improve the evaluation of sublethal effects in relevant context for ERA.

1. Introduction

In response to a deficit of standardized toxicological data for amphibian species, empirical data for the most representative species are often substituted in Ecological Risk Assessment (ERA). Typically, early life stage fish tests (e.g., OECD, 1992) are substituted for the aquatic phase of the amphibian life cycle prior to metamorphosis, and the terrestrial stages are represented by bird or small mammal data. Although some studies have assessed chemical toxicity to various amphibian species (Berrill et al., 1998; Bridges and Semiltsch, 2000; Howe et al., 2004; Jones et al., 2009; Relyea and Jones, 2009; Hua and Relyea, 2014), data are generally limited in chemical diversity and often focus on high profile pesticides (e.g., endosulfan, carbaryl, atrazine, glyphosate). While these studies provide additional context about species sensitivity and population vulnerability, they do not follow a uniform approach for comparison among a variety of amphibian species and compounds. When amphibian data are available for newer or less studied chemicals they are generally limited in taxonomic diversity or represent invasive model species (e.g., bullfrog, *Xenopus* spp.). These species are easily bred in the laboratory and ample genetic data are available for identification of molecular-level impacts (Harland and Grainger, 2011; Hammond et al., 2017); however, they differ from many native amphibian species in their sensitivity to toxicants (Birge et al., 2000; Mann and Bidwell, 2000; Fort et al., 2006a) as well as their demography and habitat. Use of surrogates in general introduces uncertainty since species sensitivity and population-level effects of chemical exposure can vary even between closely related species (Bridges and Semlitsch, 2000; Banks et al., 2014) or among populations of the same species (Bridges and Semlitsch, 2000; Cothran et al., 2013; Hua et al., 2013). Likewise, various exposure pathways and diverse life history strategies of amphibians are inadequately captured by their proxies (Johnson et al., 2017).

While larval fish are often sufficiently sensitive to be considered conservative and protective surrogates in both acute and chronic toxicity evaluations using organism-level endpoints (Weltje et al., 2013), these endpoints do not represent long-term effects associated with the unique life cycle and plasticity of amphibian development. In addition to size-based measurements being a poor representation of effects on

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metamorphosis, the most commonly used metrics of chronic toxicity tests are hypothesis test-based endpoints such as No Observed Adverse Effect Concentration (NOAEC) and the Lowest Observed Adverse Effect Concentration (LOAEC). NOAECs and LOAECs are criticized because of their lack of confidence limits, dependence on selected test concentrations, and use of statistical hypothesis testing to determine "no-effect" (Crane and Newman, 2000). Additionally, studies that report only point estimates such as NOAECs and LOAECs are inadequate to estimate toxicity effects in population models, whereas dose-response functions of toxicity effects present this relationship with continuous variables that can be used for model simulations of long-term effects (Kramer et al., 2011). These shortcomings in existing data necessitate approaches to improve population-level assessments of chemical exposure, particularly to amphibians with few representative model species and endpoints. Test protocols that include time to metamorphosis and relevant endpoints for amphibians improve upon data limitations (OECD 2009, 2015). Extrapolation methods based on quantitative adverse outcome pathways could use these endpoints in conjunction with molecular data to estimate toxicity to a variety of species with minimal animal testing. Finally, a framework for population-level assessment (Raimondo et al. in review) would facilitate incorporation of these endpoints in appropriate ecological and management contexts.

Although complex endocrine cues are responsible for developmental processes in both fish and amphibians (Fort et al., 2007; Terrien and Prunet, 2013), mechanisms vary among aquatic taxa (Duarte-Guterman et al., 2014). Evidence of low-dose effects and nonmonotonic response in amphibian species suggest various pathways might be affected by exposure during metamorphosis (Hinther et al., 2010; Vandenberg et al., 2012; Stark et al., 2015). In some cases, developmental processes might be sufficiently altered such that some tadpoles are unable to metamorphose, with sublethal developmental effects effectively resulting in mortality. Wilbur and Collins (1973) describe the probability of metamorphosis as a function of the size and recent growth rate of a tadpole as well as the environmental variability under which the species has persisted. Implicit in this scenario is a range of masses in which metamorphosis will occur and the assumption that below the minimum threshold, transition to the terrestrial juvenile stage is unlikely. Proximate causes of metamorphosis and its plasticity are hormone-driven (Denver, 1997) such that chemical impacts on corticotropin-releasing or thyroid hormones could alter development within environmentally restricted periods. Time to metamorphosis, which may be a function of habitat availability, density dependence, and/or chemical exposure, is the metric we focus on in this study to interpret growth effects in terms of survival and transition to the juvenile stage.

Estimation of growth effects in developing tadpoles is further complicated by habitat constraints (Indermaur et al., 2010) and developmental plasticity of species that are dependent upon ephemeral breeding ponds being inundated for sufficient duration to allow metamorphosis. The duration of the larval stage varies in response to annual precipitation and hydroperiod in some species (Denver et al., 1998; Perotti et al., 2011; Amburgey et al., 2016; cf. Amburgey et al., 2012). Reduced hydroperiod can accelerate development but often with carryover effects of reduced mass, survival, or reproduction (Chelgren et al., 2006; cf. Green and Bailey, 2015). Within a brood, accelerated development has limited compensatory effect for reduced survival associated with shortened hydroperiod (Ryan and Winne, 2001; Amburgey et al., 2016). Repercussions of accelerated development could be seen in reduced survival (Goater, 1994), smaller size at maturity (Smith, 1987), or lower fecundity in smaller females (Woolbright, 1983; Elmberg, 1991).

Endocrine disrupting compounds that affect gonadal development and effectively alter sex ratios (e.g., Hogan et al., 2008) in breeding amphibian populations could also reduce fecundity (Kloas and Lutz, 2006), especially in species for which explosive breeding episodes require parity in operational sex ratio and breeding frequency (Alho et al., 2008). Disparities in maturation time can also contribute to male-biased sex ratios associated with declining amphibian populations (Swannack and Forstner, 2007), although female-biased sex ratios have also been associated with vulnerable populations of amphibians (Di Minin and Griffiths, 2011). In response to observed effects of endocrine-disrupting compounds on clutch size, sexual differentiation, and gonadal development (Orton and Tyler, 2015), we also simulate reduction in reproductive potential and the population-level impacts.

For larval amphibians developing in an ephemeral habitat, growth and survival are density dependent (Wilbur, 1977; Vonesh and De la Cruz, 2002; Schiesari et al., 2007; Rogers and Chalcraft, 2008), and ecological and demographic factors unique to different species can influence long-term effects of stressors (Lips et al., 2003). As multiple stressors contribute to amphibian population declines (Grant et al., 2016), ecologically relevant risk assessment methods are necessary to present variable long-term effects of habitat availability and chronic pesticide exposure (Bruhl et al., 2011; Weir et al., 2016; Willson et al., 2012). One objective of this study is to demonstrate the influence of delayed tadpole development on reduced survival. We also compare the resulting mortality and reduced fecundity among species of amphibians with different life histories to further explore population-level vulnerabilities. We expand on a simple, stage-based comparative matrix model approach advocated elsewhere (Biek et al., 2002; Weir et al., 2016) for initial evaluation of population sensitivity and offer perspectives on differential species sensitivity for consideration in population-level ERA.

2. Methods

2.1. Larval period estimation

The time to metamorphosis ranges were used to develop cumulative distribution functions (CDF) of metamorphosis during a standard larval period for six amphibian species (western toad, *Anaxyrus boreas;* southern toad, *A. terrestris;* southern leopard frog, *Lithobates sphenocephalus;* common frog, *Rana temporaria;* northern red-legged frog, *R. aurora;* California red-legged frog, *R. draytonii*). These species were selected because they are frequently studied and adequate demographic data existed in the literature for subsequent population modeling (discussed below). Their life cycles include terrestrial juvenile and adult stages, unlike aquatic model species. In addition to these six species, we also developed CDF for one aquatic species commonly used in toxicity testing (African clawed frog, *Xenopus laevis*). The range of time to metamorphosis data used to develop the CDFs were obtained from AmphibiaWeb (AmphibiaWeb, 2017), Encyclopedia of Life (Encyclopedia of Life, 2017), or published literature (Beck and Congdon, 1999). Estimates for *X. laevis* were based on laboratory observations (unpublished data) which were similar to standard protocol guidelines (OECD, 2015). For each CDF, the inflection point (e) represents the approximate median age at which metamorphosis occurs and the slope (b) represents the rate at which larvae for each species transition. Steeper slopes represent species with smaller estimated time ranges during which they undergo metamorphosis. The slope values were determined iteratively using a log-logistic function so that 5–95% metamorphosis fell within the published ranges:

$$S_l = S_{max}(\exp\ (-\exp\ (b(\log\ (x) - \log\ (e)))))$$

2.2. Delayed development as larval mortality

To simulate delays in development, we shifted the inflection point of the curve, or the point at which 50% of the population reaches metamorphosis, to the right by 1–15 days while keeping the slope constant. Exposure to some pesticides can delay metamorphosis by weeks or cause cessation of development (Howe et al., 2004). Most pesticide-induced developmental delays tend to be more modest, on the order of a few to several days (Sullivan and Spence, 2003; Yahnke et al.,