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A meta-analysis of the effects of pesticides and fertilizers on survival and growth of amphibians

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HIGHLIGHTS

- ► We used meta-analytic techniques to examine agro-chemical impacts on amphibians.
- ► We looked at survival and growth metrics for available amphibian data.
- ► Pesticides and fertilizers negatively impacted amphibian survival.
- ► Pesticides and fertilizers negatively impacted amphibian growth.

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ABSTRACT

The input of agrochemicals has contributed to alteration of community composition in managed and associated natural systems, including amphibian biodiversity. Pesticides and fertilizers negatively affect many amphibian species and can cause mortality and sublethal effects, such as reduced growth and increased susceptibility to disease. However, the effect of pesticides and fertilizers varies among amphibian species. We used meta-analytic techniques to quantify the lethal and sublethal effects of pesticides and fertilizers on amphibians in an effort to review the published work to date and produce generalized conclusions. We found that pesticides and fertilizers had a negative effect on survival of −0.9027 and growth of −0.0737 across all reported amphibian species. We also observed differences between chemical classes in their impact on amphibians: inorganic fertilizers, organophosphates, chloropyridinyl, phosphonoglycines, carbamates, and triazines negatively affected amphibian survival, while organophosphates and phosphonoglycines negatively affected amphibian growth. Our results suggest that pesticides and fertilizers are an important stressor for amphibians in agriculturally dominated systems. Furthermore, certain chemical classes are more likely to harm amphibians. Best management practices in agroecosystems should incorporate amphibian species-specific response to agrochemicals as well as life stage dependent susceptibility to best conserve amphibian biodiversity in these landscapes.

1. Introduction

Anthropogenic impacts on natural systems are of growing concern as human populations expand and global biological diversity declines (Benton, 2007; Donald and Evans, 2006). Among the many stressors attributed to humans, chemical contaminants are anthropogenically created, used, and distributed, and may pose significant risk to a variety of taxa and ecosystems (Relyea, 2005b). Agricultural practices often occur near freshwater ecosystems, which put these freshwater systems at a high risk for chemical exposure. Direct and indirect pathways exist for exposure of freshwater systems, such as intentional application for pest control, accidental overspray, runoff, leaching, and sediment deposition (Boone et al., 2005; Relyea, 2005a, 2005b, 2005c).

Several groups of non-target organisms have been found to be highly sensitive to pesticide exposure, including amphibians, crustaceans, bivalves, nematodes, annelid worms, and non-target insects (Kerby et al., 2010). Although amphibian and fish species tend to be less susceptible to pesticide and fertilizer exposure than invertebrate species, there is evidence of significant negative effects on survival and growth (Davidson et al., 2002; Kerby et al., 2010; Relyea, 2005a; Shelley et al., 2009). Impacts on amphibians are of particular interest because amphibian population declines are occurring worldwide (Alford and Richards, 1999; Blaustein et al., 1994; Mendelson et al., 2006; Stuart et al., 2004). Additionally, many amphibian species are data deficient, meaning we cannot accurately assess their conservation status (Stuart

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et al., 2004). Investigating stressors such as pesticides and fertilizers may help fill in knowledge gaps and potentially contribute to amphibian conservation efforts worldwide.

The effects of pesticides and fertilizers on amphibians include increased mortality, reduced growth, developmental abnormalities, and increased susceptibility to disease (e.g., Boone and Bridges, 2003; Mills, 2004; Relyea, 2005a). The effect of these chemicals can vary among chemical classes and species. For example, survival of the green frog (*Rana clamitans*) decreased when exposed to Abate®, an organophosphate pesticide, whereas Release, a chloropyridinyl pesticide, did not result in decreased survival in the same species (Sparling et al., 1997; Wojtaszek et al., 2005). In addition, carbaryl, a carbamate pesticide, negatively impacted survival of the spotted salamander (*Ambystoma maculatum*) but did not impact the survival of the southern leopard frog (*Rana sphenocephala*) (Boone and James, 2003; Boone et al., 2004).

Sublethal impacts can include longer larval periods, smaller size at metamorphosis, and increased susceptibility to predation due to decreased swim speed and endurance (Boone and Bridges, 2006; Mills, 2004). Additionally, indirect impacts on growth can be attributed to food web disruptions initiated by these chemicals. Herbicides may also decrease primary production, resulting in increased competition and reduced growth rates (Boone and Bridges, 2003; Relyea, 2006; Relyea and Diecks, 2008).

We posit that a comprehensive look at the effects of pesticides and fertilizers on amphibians would better direct management and conservation decisions worldwide. Most studies focus on single chemicals or species, and quantify only the LC50 (lowest concentration needed to kill 50% of the test subjects; Relyea, 2004). With hundreds of pesticides and dozens of fertilizers in use (Gail and Leonard, 2000), a comprehensive approach is needed to quantify effects of these chemicals on amphibians.

Here, we used a meta-analytic technique to synthesize published studies on lethal and sublethal impacts of pesticides and fertilizers on amphibian species worldwide. Meta-analytic techniques are the most statistically rigorous method for summarizing independent data (Bancroft et al., 2008; Gurevitch et al., 1992) and hence are ideal for reviewing lethal and sublethal impacts of chemical contaminants on amphibians. We quantified the overall effect of 16 classes of chemicals, representing both pesticides and fertilizers, on survival and growth of amphibians. Chemicals were analyzed as groups based on parent chemical classes. Chemical classes were defined as groups of chemicals that have similar structures and activity (Kegley et al., 2008) and this allowed for a more generalized representation of the chemicals used in previous studies (Table 1). We hypothesized that pesticides and fertilizers would have an overall negative effect on growth and survival in amphibians and that chemical classes would differ in their effects on both survival and growth.

2. Methods

2.1. Data selection

We used five databases to identify studies for analysis (Aquatic Sciences and Fisheries Abstract, BIOSIS, Environmental Sciences and Pollution Management, Web of Science, and Wildlife and Ecology Studies Worldwide). To find primary literature on the effects of agricultural chemicals on amphibians within these databases, we searched for all combinations of five search terms: pesticide or fertilizer, survival, growth, mortality, and amphibian. We limited our search to experimental manipulations of pesticides and fertilizers. To avoid potential biases in the selection of studies, we established a priori criteria for the inclusion of studies in the meta-analysis: 1) each study must give the mean survival or growth data for both an experimental group (chemical exposed) and an appropriate control group (no chemical exposure), 2) each study must give the sample size for both the experimental group and control group, and 3) chemical concentrations must be ecologically relevant, which means that they must be within a range of possible concentrations that one would expect to see in the environment after a spray event (Table 1). Any data points within an article that met these criteria were considered for inclusion.

Table 1
Summary of pesticides and fertilizers used in the meta-analysis organized by chemical classes, chemicals, and expected environmental concentrations (EEC) represented as mg/L.

Chemical class	Chemical	EEC	References
Carbamate	Carbaryl	5	Boone and Bridges (2003)
Chloro-nicotinyl	Imidaclorpid	42	EPA (1992)
Chlorophenoxy acid	2,4-D	0.12	Relyea (2005c)
Chloropyridinyl	Release	5.77	Wojtaszek et al. (2005)
Dithiocarbamate	Mancozeb	0.008	Harris et al. (2000)
Inorganic fertilizers	Calcium	15	Hammer et al. (2004)
	Phosphate	50	WHO (2007)
	Nitrate	50	WHO (2007)
Organochloride	Endosulfan	10	Harris et al. (2000)
Organophosphorus	Malathion	1.8	Relyea and Dieckс (2008)
	Abate	0.05	EPA (1998)
	Chlorpyrifos	0.0037	Wood and Stark (2002)
	Diazinon	0.082	EPA (2005)
Organotin	Triphenyltin	0.002	Fioramonti et al. (1997)
Phenol	Octylphenol	0.05	Rohr et al. (2003)
Polyalkyloxy compound	POEA	0.6	Howe et al. (2004)
Pyrethroid	Permethrin	0.05	Johansson et al. (2006)
	Alpha-cypermethrin	0.006	Greulich and Pflugmacher (2003)
Triazine	Atrazine	0.002	Boone and Bridges (2006)
	Cyanazine	0.9	Johansson et al. (2006)
Urea	Urea	154	Schuytema and Nebeker (1999)
	Diuron	10	Schuytema and Nebeker (1998)
Other	Methoprene	0.05	Chu et al. (1997)
	Azadiracthin	0.5	Punzo (1997)

Several studies included more than one species, chemical, dose, or sampling period. All species and chemicals from a given study were included in our analyses if the overall inclusion criteria were met. Although including all species or chemicals from one study might decrease the independence among some data points, the inclusion of all available species and chemicals allowed us to more fully explore the effects of pesticides and fertilizers in these systems (Bancroft et al., 2007). However, if more than one dose of the same chemical was used in the original article, we then randomly selected only one dose level for inclusion in our analysis. If the study reported survival or growth over a time series, we selected the final measurement for analysis. When studies quantified growth using several response variables (i.e., length and mass), we randomly selected one variable for inclusion. All data were obtained from primary research articles and, when necessary, data were extracted from published figures using TechDig V.2.0 software.

2.2. Effect sizes

To calculate an overall measure of pesticide and fertilizer effect on survival and growth in amphibians, including magnitude and direction (positive or negative), we used a log response ratio (lnR) as our metric of standardized effect size (Hedges et al., 1999). We defined the control group as the group not exposed to any pesticides or fertilizers; therefore, a negative value in our response ratio indicates a negative effect of pesticides and fertilizers on survival or growth. MetaWin Version 2.0 (Rosenberg et al., 2000) was used to generate