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Potential interactions among disease, pesticides, water quality and adjacent land cover in amphibian habitats in the United States

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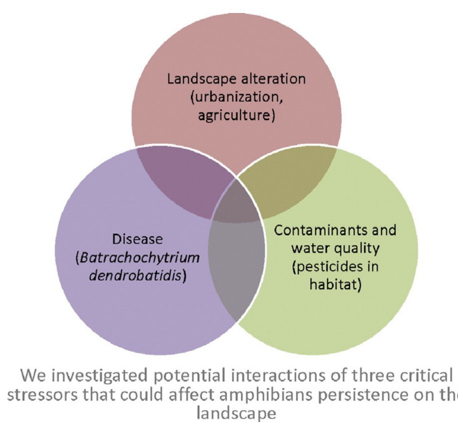
HIGHLIGHTS

- Disease, pesticides, and landscape alteration can cause amphibian decline.
- We assessed Bd and pesticides in frog tissue and their aquatic habitats.
- Pesticide concentrations in tissue were correlated to land cover around the sites.
- Bd correlated positively with DOC, total nitrogen and total phosphorous in water.
- Bd in water was not associated with differences in land use around sites.
- Fungicides occurred more frequently than expected in frog habitats and tissue.

GRAPHICAL ABSTRACT



We investigated potential interactions of three critical stressors that could affect amphibians persistence on the landscape.

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ABSTRACT

To investigate interactions among disease, pesticides, water quality, and adjacent land cover, we collected samples of water, sediment, and frog tissue from 21 sites in 7 States in the United States (US) representing a variety of amphibian habitats. All samples were analyzed for >90 pesticides and pesticide degradates, and water and frogs were screened for the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) using molecular methods. Pesticides and pesticide degradates were detected frequently in frog breeding habitats (water and sediment) as well as in frog tissue. Fungicides occurred more frequently in water, sediment, and tissue than was expected based upon their limited use relative to herbicides or insecticides. Pesticide occurrence in water or sediment was not a strong predictor of occurrence in tissue, but pesticide concentrations in tissue were correlated positively to agricultural and urban land, and negatively to forested land in 2-km buffers around the sites. Bd was detected in water at 45% of sites, and on 34% of swabbed frogs. Bd detections in water were not associated with differences in land use around sites, but sites with detections had colder water. Frogs that tested positive for Bd were associated with sites that had higher total fungicide concentrations in water and sediment, but lower insecticide concentrations in sediments relative to frogs that were Bd negative. Bd concentrations on frog swabs were positively correlated to dissolved organic carbon, and total nitrogen and phosphorus, and negatively correlated to pH and water temperature.

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Data were collected from a range of locations and amphibian habitats and represent some of the first field-collected information aimed at understanding the interactions between pesticides, land use, and amphibian disease. These interactions are of particular interest to conservation efforts as many amphibians live in altered habitats and may depend on wetlands embedded in these landscapes to survive.

1. Introduction

For a variety of reasons, many of the world's amphibian species face extinction or decreasing populations (Adams et al., 2013; Lannoo et al., 2011; Sodhi et al., 2008). Numerous studies have demonstrated that exposure to agricultural chemicals can have adverse effects on amphibians (Bruhl et al., 2013; Hayes et al., 2006; Mann et al., 2009; Relyea and Diecks, 2008; Rohr et al., 2003; Sparling and Fellers, 2009). For example, fungicides occur in water and sediment in agricultural, urban, and remote locations (Battaglin et al., 2011; Reilly et al., 2012; Smalling et al., 2012, 2013a) and recent studies have indicated that fungicides may be of particular concern to amphibians, freshwater fish, and invertebrates (Belden et al., 2010; Hooser et al., 2012; Junges et al., 2012; Li et al., 2016; McMahon et al., 2011; Morrison et al., 2013). In amphibians, the presence of fungicides has been linked to alteration of predator–prey interactions (Junges et al., 2012; Teplitsky et al., 2005), endocrine disruption (McMahon et al., 2011; Poulsen et al., 2015), and mortality (Belden et al., 2010; Hooser et al., 2012). Glyphosate-based herbicides also are of concern as they occur widely in the environment (Battaglin et al., 2014; Coupe et al., 2012) and have been shown to cause poor survivorship and mortality of larval and adult amphibians (Moore et al., 2012; Relyea, 2005a, 2005b; Wagner et al., 2013).

Pesticides found in frogs and their habitats can have effects beyond the scale of individual animals or populations. Similar to results reported for metamorphosing insects and the movement of contaminants from aquatic to terrestrial systems (Kraus et al., 2014), amphibians accumulate pesticides (Smalling et al., 2015) and may potentially concentrate some of them during metamorphosis. This transfer of contaminants across ecological boundaries (i.e., water and sediment to plants and animals) is relevant to subsequent offspring (contaminants transferred to eggs) (Metts et al., 2013) and to the animals that prey on juveniles or adults (Wu et al., 2009). Amphibians can represent a substantial source of biomass in a pond (Gibbons et al., 2006) such that the implications of this transfer may be important.

Despite a large body of literature on contaminants in the laboratory (Mann et al., 2009; Sparling and Fellers, 2009), there are few field studies that assess the effects of pesticides, or the potential interactions between disease and pesticides, on individual amphibians or populations of amphibians persisting in contaminated environments (Egea-Serrano et al., 2012; Smalling et al., 2015). A focus on amphibians and the effects of pesticides is warranted because, as a taxonomic group, they are disappearing at a rate far greater than the expected background rate of extinction (McCallum, 2007), representing a likely mass extinction event (Wake and Vredenburg, 2008).

Land-use change resulting in habitat destruction or alteration remains a leading cause of amphibian decline (Lannoo et al., 2011; Sodhi et al., 2008). Since the mid-1700s, humans have transformed landscapes across the US from forests, grasslands, and wetlands to farmland, rangeland, and urban land (U.S. Department of Agriculture, 2013). A notable result of the increase in developed land was the loss of natural wetland landscapes, an estimated 53% of which were removed between 1780s and 1980s (Dahl, 1990). More recently, various conservation programs have started to reverse this trend by encouraging the construction of new wetlands and restoration of natural wetlands (Dahl, 2011; Sucik and Marks, 2014). Some amphibians persist in altered habitats and some may benefit from practices associated with agriculture (e.g., farm ponds; Knutson et al., 2004). There is also evidence that amphibians may develop some tolerance to pesticide exposure in agricultural settings (Cothran et al., 2013; Hua et al., 2013, 2015). In contrast, some such situations may represent environmental traps or "sinks" (Pulliam, 1988) where amphibians may survive but are unable to successfully reproduce. Such scenarios may represent an opportunity to manage wetlands for the benefit of amphibians. However, a better understanding of the biotic and abiotic interactions occurring within these systems is needed before management actions can be formulated.

Disease is another contributor to amphibian decline that may exacerbate the effects of other stressors (Puglis and Boone, 2007; Hayes et al., 2010). Chytridiomycosis, caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd; Longcore et al., 1999; Van Rooij et al., 2015) is one such disease. Amphibian responses to Bd vary widely by species and individuals (Retallick and Miera, 2007). Chytridiomycosis caused by Bd exposure is implicated in local declines and extinctions of amphibians worldwide (Bosch et al., 2001; Lips et al., 2006), including North America (Muths et al., 2008; Vredenburg et al., 2010). Despite considerable attention to the effects of chytridiomycosis on individuals and populations, the effect of stressors such as pesticides on Bd occurrence is unknown, and the effect of Bd on amphibians when they are already challenged by pesticide exposure is equivocal. Exposure to pesticides can exacerbate the effect of Bd on host animals (Hayes et al., 2010), decrease negative effects (Brown et al., 2013; Gahl et al., 2011; Hanlon and Parris, 2012; Rumschlag et al., 2014), or have no effect (Edge et al., 2013).

Three stressors - landscape alteration, contaminants, and disease - are critical factors in the persistence of amphibians on the landscape. Efforts to address multiple stressors simultaneously can be fraught with difficulty, especially in observational studies from the field. However, these real-world situations and resulting data are vital to provide context for results from controlled laboratory and mesocosm efforts. Specifically, few field studies have measured current-use pesticides in key habitat components (water or sediment) in conjunction with their occurrence in tissue or blood of wildlife (Grant et al., 2013; Masia et al., 2013; Nilsen et al., 2015; Smalling et al., 2013b, 2015). Amphibians, especially those in altered habitats, offer a system with the potential to provide a better understanding of the interactions among pesticides, disease and the movement of contaminants from aquatic to terrestrial landscapes (Reeves et al., 2016). We combined analyses of pesticide concentrations in sediment, water, and frog tissue with concurrent analysis of Bd in water and on frogs at sites representing a range of amphibian habitats surrounded by a variety of land uses. We hypothesize that the area of agricultural and urban land surrounding a site is positively correlated with the pesticide content of the amphibians' food source or potential for direct dermal exposure in cropped fields or other pesticide treated landscapes. Our objectives were to (1) characterize pesticide occurrence and concentrations in water, sediment, and frog tissue and evaluate potential site differences; (2) identify interactions between pesticide concentrations in water, sediment, frog tissue and the surrounding land use; and (3) identify interactions between pesticide concentrations in water, sediment, frog tissue, land use, and the occurrence of Bd.