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Pesticide concentrations in frog tissue and wetland habitats in a landscape dominated by agriculture

Kelly L. Smalling [a,*], Rebecca Reeves [b], Erin Muths [c], Mark Vandever [c], William A. Battaglin [d], Michelle L. Hladik [e], Clay L. Pierce [f]

[a] US Geological Survey New Jersey Water Science Center, Lawrenceville, NJ, USA
[b] Department of Natural Resource Ecology and Management, Iowa State University, Ames, IA, USA
[c] US Geological Survey, Fort Collins Science Center, Fort Collins, CO, USA
[d] US Geological Survey, Colorado Water Science Center, Lakewood, CO, USA
[e] US Geological Survey, California Water Science Center, Sacramento, CA, USA
[f] US Geological Survey, Iowa Cooperative Fish and Wildlife Research Unit, Ames, IA, USA

HIGHLIGHTS

- Habitat quality was similar between restored and reference wetlands in Iowa.
- Complex mixtures of pesticides are detected in frog tissues (liver and whole body).
- The number of fungicides (up to 8) in frog tissues is largest reported to date.
- Life history has the potential to impact pesticide bioaccumulation in frogs.

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ABSTRACT

Habitat loss and exposure to pesticides are likely primary factors contributing to amphibian decline in agricultural landscapes. Conservation efforts have attempted to restore wetlands lost through landscape modifications to reduce contaminant loads in surface waters and providing quality habitat to wildlife. The benefits of this increased wetland area, perhaps especially for amphibians, may be negated if habitat quality is insufficient to support persistent populations. We examined the presence of pesticides and nutrients in water and sediment as indicators of habitat quality and assessed the bioaccumulation of pesticides in the tissue of two native amphibian species *Pseudacris maculata* (chorus frogs) and *Lithobates pipiens* (leopard frogs) at six wetlands (3 restored and 3 reference) in Iowa, USA. Restored wetlands are positioned on the landscape to receive subsurface tile drainage water while reference wetlands receive water from overland run-off and shallow groundwater sources. Concentrations of the pesticides frequently detected in water and sediment samples were not different between wetland types. The median concentration of atrazine in surface water was 0.2 μg/L. Reproductive abnormalities in leopard frogs have been observed in other studies at these concentrations. Nutrient concentrations were higher in the restored wetlands but lower than concentrations thought lethal to frogs. Complex mixtures of pesticides including up to 8 fungicides, some previously unreported in tissue, were detected with concentrations ranging from 0.08 to 1500 μg/kg wet weight. No significant differences in pesticide concentrations were observed between species, although concentrations tended to be higher in leopard frogs compared to chorus frogs, possibly because of differences in life histories. Our results provide information on habitat quality in restored wetlands that will assist state and federal agencies, landowners, and resource managers in identifying and implementing conservation and management actions for these and similar wetlands in agriculturally dominated landscapes.

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1. Introduction

Degradation and loss of habitat are among the primary reasons amphibian populations have declined worldwide (Collins and Storfer, 2003). Between 1850 and 1950 the amount of farmland in the United States increased from less than 300 million to more than 1.1 billion

* Corresponding author at: 3450 Princeton Pike, Suite 110, Lawrenceville, NJ 08648, USA. Tel.: +1 609 331 4850; fax: +1 609 771 3915.
E-mail address: ksmall@usgs.gov (K.L. Smalling).

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acres (U.S. Department of Agriculture, 2013). However, in the last 5 decades the amount of total cropland in the United States has decreased as the amounts of urban, residential and special-use lands (mostly parks and wildlife areas) are increasing (U.S. Department of Agriculture, 2007). In agriculturally dominated states like Iowa, 75% of the acreage is cropland (U.S. Department of Agriculture, 2007). These land-use changes may not always destroy habitat but usually include alterations, such as the application of chemicals that can threaten amphibian survival. Amphibian declines and abnormalities have been attributed to contaminants, often with a focus on water quality, specifically water at breeding sites. In a recent survey 30–60% of shallow groundwater and 60–95% of streams across different land-use categories in the United States were contaminated with at least one pesticide (Gilliom, 2007).

Contaminants have the potential to cause lethal effects in amphibians, such as reduced survival or sub-lethal effects such as immunosuppression, malformations, compromised reproduction and reduction in growth and development (Hecnar, 1995; Taylor et al., 2005; Johnson et al., 2007; Gahl et al., 2011; Groner and Relyea, 2011). For example, the herbicide atrazine has the potential to cause immunosuppression in adult northern leopard frogs (Brodkin et al., 2007) and impair sexual development of male frogs (Hayes et al., 2003). Glyphosate formulations (Howe et al., 2004; Relyea, 2005) as well as some fungicide formulations (Belden et al., 2010; Bruhl et al., 2003) are toxic to amphibians at environmentally relevant concentrations. Decreases in growth and development have also been observed after exposure to fungicide formulations containing pyraclostrobin (Hartman et al., 2014). Laboratory studies designed to identify acute and chronic effects frequently focus on a single compound or a specific class of compounds that are often conducted in simplified mesocosm settings (Relyea and Mills, 2001; Relyea, 2005; Boone et al., 2005; Boone, 2008). However, pesticides in the environment exist as mixtures and efforts in the field to elucidate some of these relationships in non-laboratory settings have been limited.

Exposure to pesticides can lead to suppression of the immune system, preventing amphibians from developing a normal response against pathogens (Mann et al., 2009). Christin et al. (2004) exposed frogs (*Xenopus laevis* and *Rana pipiens*) to a mixture of herbicides and insecticides and found that at environmentally relevant concentrations, combinations of these pesticides altered aspects of the immune system. However, Davidson et al. (2007) found no correlation between a common insecticide, carbaryl, and amphibians' susceptibility to the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, Bd). Increased eutrophication due to nitrogen based fertilizers, coupled with pesticide application, may cause trophic cascades resulting in increased rates of parasitism in wetlands and has been linked to immunosuppression in amphibians (Brodkin et al., 2007; Johnson et al., 2007). Although a direct link has been made between pesticide exposure and infection by trematodes (Rohr et al., 2008; Kiesecker, 2002), a general understanding of these interactions in the field is lacking because results vary by species and land-use (King et al., 2007).

Despite landscape alterations and the suite of potentially negative effects related to such alterations, there are examples of amphibians persisting in modified habitats. For example, in Eastern Europe amphibians breed successfully in man-made drainage ditches (Hartel et al., 2001), in the Midwest, certain species persist despite agrochemical inputs and habitat modifications (Kolozsvary and Swinhart, 1999; Gilliland et al., 2001) and in Florida not all species of anurans appeared to be adversely affected by development as long as permanent habitat was available for breeding (Delis et al., 1996). In areas of California where habitats have been altered by human activities many of the amphibian species (with the exception of *Ambystoma californiense*) have significantly declined (Davidson et al., 2002). Although animals can persist in altered landscapes, careful assessments of long-term persistence and population health are warranted. The presence of amphibians, or the appearance of population persistence can mask a host of problems that may manifest in the long-term such as increased susceptibility to disease, reduced probability of survival and recruitment or other genetic issues (i.e. breeding) related to lack of habitat connectivity.

Much of Iowa illustrates the changes made to the land for crop production over the last two centuries; greater than 90% of the wetlands have been drained and replaced with row crop agriculture, primarily corn and soybeans (Whitney, 1994). Despite the dramatic changes, there is still a rich herpetofauna represented in Iowa and much of the Midwest. Although some amphibians persist, approximately 45% of the amphibian and reptile species in Iowa are imperiled because of habitat fragmentation and anthropogenic activities (Green, 2005; IDNR, 2006). Thus, we chose the Des Moines Lobe of central Iowa to assess the presence of pesticides and nutrients in water and sediment as indicators of habitat quality and to assess the bioaccumulation of pesticides in the tissue of two amphibian species (chorus frogs (*Pseudacris triseriata*) and leopard frogs (*Lithobates pipiens*)). Furthermore, in 2001, a major initiative between the state of Iowa and United States Department of Agriculture (USDA) Farm Service Agency enacted the Conservation Reserve Enhancement Program (CREP) to help identify and restore wetlands lost through landscape modifications as a means to reduce nitrogen concentrations and loads in surface waters. The Des Moines Lobe hosts 72 of these CREP sites in 29 counties. The resulting wetlands appear to provide additional ecosystem services such as habitat for migrating waterfowl (O'Neal et al., 2008), however, for many organisms, such as amphibians the related costs of living within a matrix of highly modified habitat have not been determined. For example, the value of increased habitat for species with low vagility (e.g., amphibians) is assumed to be high, but benefits may be negated if the quality of the habitat is insufficient to support amphibian populations' overtime.

Our objective was to determine if restored wetlands in an agricultural landscape provide similar quality habitat for amphibians as adjacent reference wetlands as determined by the occurrence and distribution of 1) pesticides and nutrients in water, 2) pesticides in bed sediment and 3) pesticides in tissues of leopard and chorus frogs, two amphibians found commonly in this area. Understanding the occurrence and distribution of contaminants provides information on habitat quality in restored wetlands that can assist state and federal agencies, landowners, and resource managers in identifying and implementing conservation and management actions for these and similar wetlands and their associated amphibian fauna. Our data also provides useful covariates (i.e. pesticide and nutrient concentrations) for assessing population demographics and the long-term trajectory of populations faced with the challenges of living in an altered landscape.

2. Materials and methods

2.1. Site information

Six wetlands in the Des Moines Lobe landform of central Iowa (Fig. 1) were sampled in 2012 and 2013 (3 restored and 3 reference). The restored wetlands were developed through the CREP and were positioned on the landscape to receive substantial amounts of tile drainage water to reduce nitrate concentrations to surrounding surface water bodies. Approximately, 80% of the flow into the restored wetlands is from tile drains and all wetlands sampled had several tile lines and ditches leading directly into them. Two tile drains were observed at Marshall and Story while four large ditches with at least one outflow drain were observed at Greene. All drains were considered laterals and were about 20–25 cm in diameter. The reference wetlands are likely remnant wetlands but were restored from past agricultural use by landowners and are not typically positioned in the landscape to accept a significant amount of tile drainage from active agricultural fields and are not part of the CREP. Reference wetlands receive the majority of their water from overland flow (i.e. run-off) and, to a lesser extent, from shallow groundwater sources and tile drain outlets. Bjorkboda