**Influence of instream habitat and water chemistry on amphibians in channelized
agricultural headwater streams**

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ABSTRACT

The widespread use of stream channelization and subsurface tile drainage for removing water from agricultural fields has led to the development of numerous channelized agricultural headwater streams within agricultural watersheds of the Midwestern United States, Canada, and Europe. Channelized agricultural headwater streams have been documented to serve as habitat for amphibians, but information on amphibian habitat relationships within these streams is lacking and needed for developing effective conservation strategies. We quantified instream habitat, water chemistry, and sampled amphibians from seven sites in three channelized streams in Cedar Creek, Indiana in 2008 and 2009 and five sites in five channelized streams in Upper Big Walnut Creek, Ohio in 2009. We conducted an indirect gradient analysis involving the use of principal component analysis and generalized linear mixed effect model analysis to determine which variables had the greatest influence on amphibian community and population structure. Overall, amphibian community and population structure was most strongly correlated with water chemistry rather than instream habitat within channelized agricultural headwater streams in Indiana and Ohio. Eleven of 12 amphibian response variables were most strongly correlated with either a water chemistry gradient of nitrate+nitrite and acetochlor, a gradient of total nitrogen and ammonia, or a gradient of simazine and total phosphorus. Only one amphibian response variable was most strongly correlated with an instream habitat gradient of dissolved oxygen and water depth. Our results suggest that conservation strategies that target reductions of nutrients and herbicides will provide the greatest benefits for amphibians within channelized agricultural headwater streams.

Keywords: hydrology; nutrients; herbicides; anurans

1. Introduction

Throughout regions dominated by agricultural land use, headwater streams are often channelized or created to maximize the removal of excess water from crop fields with poorly drained soils. The watersheds of these streams are often further modified through the installation of subsurface tile drains. It is estimated that 25% of land within the Midwestern United States would not be available for cultivation without stream channelization and subsurface tile drainage (Skaggs *et al.*, 1994) and it is estimated that these practices impact 80% of the landscape of the region (Blann *et al.*, 2009). Stream channelization alters instream habitat through increased hydrological dynamics, sediment accumulation, and the simplification of habitat structure (Blann *et al.*, 2009; King *et al.*, 2014). Additionally, water quality within channelized streams is influenced by the application of nutrients and pesticides within the adjacent agricultural fields to promote crop productivity. Surface and subsurface runoff leads to the input of these chemicals within channelized streams at rapid rates, especially following storm events after nutrient and pesticide application (Kladivko *et al.*, 2001; Gaynor *et al.*, 2002; Brown and Van Beinum, 2009; Smiley *et al.*, 2014; Smith *et al.*, 2015; Williams *et al.*, 2015). Channelized streams are also subjected to periodic removal of riparian vegetation and dredging of accumulated sediment to ensure hydraulic capacity is maintained. The management of channelized headwater streams is primarily oriented toward maximizing drainage for agricultural productivity without regard to the potential of these streams to serve as habitat for plants and animals (Herzon and Helenius, 2008; Smiley and Gillespie, 2010). Thus, an enhanced understanding of the relationships of the riparian and aquatic biota with the physical and chemical habitat conditions within channelized headwater streams is needed to develop conservation strategies capable of mitigating the effects of agriculture.

Fish and aquatic macroinvertebrate communities have been the subject of most of the ecological research in channelized agricultural headwater streams (Sullivan *et al.*, 2004; Lau *et al.*, 2006; Morris *et al.*, 2006; Smiley and Gillespie, 2010; Leslie *et al.*, 2012; D'Ambrosio *et al.*, 2014). Amphibians are another group that use these degraded streams as habitat but they have received little study (Twisk *et al.*, 2000; Piha *et al.*, 2007b; Maes *et al.*, 2008; Hartel *et al.*, 2011; Smiley *et al.*, 2011). The lack of amphibian research within channelized agricultural headwater streams is likely due in part to a focus on the wetlands and ponds that tend to be preferred habitats for amphibian species found within agricultural landscapes (Kolozsvar and Swihart, 1999; Knutson *et al.*, 2004). Additionally, the presence of predatory fish within channelized agricultural headwater streams may lead to amphibians having a greater preference for isolated wetlands and ponds than channelized agricultural headwater streams. Channelized agricultural headwater streams can serve as dispersal corridors (Reh and Seitz, 1990; Mazerolle, 2004) and may provide much needed refugia for amphibians in agricultural landscapes (Maisonneuve and Rioux, 2001; Chester and Robson, 2013) where preferred habitats, in the form of standing surface water, are actively removed and fragmented by drainage practices. The Midwestern United States is characterized by intensified agricultural land use and wetland loss, which makes it highly probable that channelized agricultural headwater streams are serving as amphibian refugia. However, basic information on the occurrence of amphibian species and their habitat relationships within these small degraded streams in this region has not been explored.

Water chemistry may play a prominent role in determining amphibian community and population structure within channelized agricultural headwater streams. The application of agricultural chemicals during the growing season coincides with breeding and larval development in temperate latitudes. Amphibians may be particularly susceptible to the uptake of

environmental toxicants due to the high permeability of their skin (Quaranta *et al.*, 2009; Brühl *et al.*, 2011) and the effects of agricultural runoff on the survival, maturation, reproduction, and immunity of amphibians has been widely documented in ecotoxicological studies (Hayes *et al.*, 2003; Relyea, 2005; Rohr *et al.*, 2006; Mann *et al.*, 2009; Smalling *et al.*, 2015). At the landscape level, population declines have been associated with proximity to agricultural land use (Bishop *et al.*, 1999; Houlihan and Findlay, 2003; Davidson, 2004; Piha *et al.*, 2007a). Nutrient and pesticide levels in wetlands have been shown to be negatively correlated with the diversity and abundance of amphibians (Bishop *et al.*, 1999; Houlihan and Findlay, 2003), but whether these relationships also occur in channelized and unchannelized streams in agricultural watersheds in the Midwestern United States remains obscure. Physical habitat variables (i.e., those variables that describe the physico-chemical conditions, the hydrological characteristics, and the substrate and cover types) within streams are also likely to have an important influence on amphibian community and population structure (Twisk *et al.*, 2000; Ficetola *et al.*, 2011). To our knowledge no studies have evaluated the relative influence of water chemistry and instream habitat variables across streams in a homogenous agricultural landscape. Understanding the relative influence of these two habitat factors is important for conservation of amphibians in agricultural watersheds because most conservation efforts in the Midwestern United States only focus on reducing nutrient, pesticide, and sediment inputs into agricultural watersheds. If amphibians are more strongly correlated with water chemistry then these conservation efforts are likely to benefit amphibians. Conversely, if amphibians are more strongly correlated with instream habitat then conservation efforts focused solely on water chemistry improvements may be ineffective for them.

The objective of this paper is to examine the community and population structure of amphibians, and their relationship to instream habitat and water chemistry in channelized agricultural headwater streams in northeastern Indiana and central Ohio. Following previous field and laboratory research in wetland amphibians, we expected that water chemistry would influence amphibian community and population structure through elevated nutrients and pesticide concentrations. However, water chemistry has been suggested to play a minor role relative to habitat in structuring fish and aquatic macroinvertebrate communities that occupy headwater streams in agricultural regions (Dyer *et al.*, 1997; Fitzpatrick *et al.*, 2001; D'Ambrosio *et al.*, 2009; Leslie *et al.*, 2012). Similar results with fish and aquatic macroinvertebrate communities have also been documented within the watersheds of our study sites (Smiley *et al.*, 2008, 2009; McKinney, 2012; Sanders, 2012), which will enable us to make direct comparisons regarding the observed habitat relationships of amphibians with those of fishes and aquatic macroinvertebrates. Our analysis will provide novel information on the structure of amphibian communities and populations within channelized agricultural headwater streams and will assist with development of conservation strategies for aquatic biota in these streams (Herzon and Helenius, 2008; Smiley and Gillespie, 2010).

2. Materials and methods

2.1. Study area and sampling sites

Cedar Creek (CC) is the largest tributary of the St. Joseph River located in northeast Indiana (latitudes 41°53'78" – 41°19'23", longitudes 85°31'88" – 84°91'50") and is part of the Maumee River Basin that drains into Lake Erie. The lower 22 km of CC is part of the Indiana State Natural, Scenic, and Recreational River system and this designation provides this section of

CC with protection from construction, dam, and drainage projects. This has led to the upper reaches of CC being designated as an Environmental Quality Incentives Program priority area (St. Joseph River Watershed Initiative, 2005). Dominant land use in CC is cropland consisting of soybean and corn and the majority of streams within the CC watershed have been channelized for drainage of agricultural fields. Additionally, increased loadings of nutrients and pesticides from agricultural fields and bacteria from failed septic tanks are nonpoint source pollutants of concern within the watershed (St. Joseph River Watershed Initiative, 2005). Upper Big Walnut Creek (UBWC) is located in central Ohio (latitudes 40°60''–40°32'30'', longitudes 82°56'00''–82°42'00'') and is part of the Scioto River watershed, which is one of the most biologically diverse watersheds in Ohio that drains into the Ohio River basin (Sanders, 2001). Dominant land use in the UBWC watershed is cropland consisting of soybean and corn. Headwater streams in the watershed are impaired by nutrient enrichment, pathogens, and habitat degradation stemming from current agricultural management practices (Ohio EPA, 2005).

 Instream habitat measurements, water samples for nutrients and herbicide measurements, and amphibians were collected from seven sites in three channelized headwater streams within CC in 2008 and 2009 and five sites in five channelized headwater streams in UBWC in 2009. The watershed size of channelized streams in CC ranged from 13 to 43 km² while those in UBWC ranged from 1 to 10 km². Each site was 125 m long and near automated water samplers or locations where grab sample were collected for the measurement of nutrients and herbicides. These sites possessed riparian habitats consisting mostly of herbaceous riparian vegetation and exhibited the straightened, over-enlarged, trapezoidal channel shape typical of channelized agricultural headwater streams in the Midwestern United States (Smiley *et al.*, 2011).

2.2. *Instream habitat measurements*

Hydrologic (water depth, velocity, wet width), substrate/cover type, and physicochemical measurements (dissolved oxygen, temperature, pH, and conductivity) were recorded from each site in May and July 2008 and 2009 in CC and May and July 2009 in UBWC. Four measurements of water depth and velocity and one measurement of wet width were obtained from six transects located 25 m apart in each site. One additional transect was established for the calculation of instantaneous discharge and 10 equidistant measurements of water depth and velocity were made along this transect. Water depths were measured with a meter stick or wading rod, water velocity was measured with an electromagnetic velocity meter, and wet widths were measured with a tape measure. The dominant substrate type and cover type was identified at four points along each of the six transects within each site used for the measurement of hydrologic variables. Substrate and cover type categories included clay (particle size < 0.004 mm), silt (particle size 0.004-0.06 mm), sand (particle size (0.06 – 2.0 mm), gravel (particle size 2.0 mm to 64 mm), cobble (particle size 64-256 mm), and boulder (particle size > 256 mm), terrestrial vegetation (herbaceous and woody terrestrial plants found within the wetted portion of the stream), aquatic plant, small instream wood, large instream wood, leaf litter, and algae.

We calculated the mean and standard deviation of water depth, water velocity and wet width and instantaneous discharge from each site during each month of each year. We also calculated five variables (dominant substrate size, substrate type richness, cover type richness, substrate+cover type richness, percent cover types) that described the composition and diversity of substrate and cover types present within each site during each month. Dominant substrate size was calculated by first determining the most frequently occurring substrate type within the site and then assigning a substrate size value. The substrate size values assigned to each substrate

type are: 1) clay – 0.002 mm; 2) silt – 0.032 mm; 3) sand – 1.03 mm; 4) gravel – 33.0 mm; 5) cobble – 160 mm; and 6) boulder – 260 mm. In the case of ties the average substrate size value of the two most commonly occurring substrate types was used. Substrate richness is the number of substrate types found in a site and cover type richness is the number of cover types found. Substrate+cover type richness is the number of all substrate and cover types present in a site. Percent cover types is the number of points having at least one cover type divided by the total number of points (24) in a site. *In situ* measurements of dissolved oxygen, temperature, pH, and conductivity were collected from a consistent location (typically a pool or run) within each site during daylight hours (8:00 am EST to 6:00 pm EST) with a multiparameter meter concurrently with hydrologic and substrate/cover types measurements.

2.3. Nutrient and herbicide measurements

Water samples, collected on a weekly basis in CC with automated water samplers for the measurement of nutrients and herbicides, were analyzed by the USDA-ARS National Soil Erosion Research Laboratory. Weekly water samples from the UBWC for the measurement of nutrient and herbicides were analyzed by the USDA-ARS Soil Drainage Research Unit. Specifically, concentrations of six nutrients (ammonia, nitrate+nitrite, total nitrogen, dissolved reactive phosphorus, total phosphorus, dissolved organic carbon) and four herbicides (acetochlor, atrazine, metolachlor, simazine) were measured from each water sample.

Concentrations of nitrate+nitrite, ammonia, and dissolved reactive phosphorus were determined colorimetrically. Ammonia and nitrate+nitrite were determined by application of the copperized-cadmium or hydrazine-sulfate reduction method and dissolved reactive phosphorus was determined by the ascorbic acid reduction method (Parsons *et al.*, 1984). Total phosphorus

analyses were performed on unfiltered samples following alkaline persulfate oxidation (Koroleff, 1983) with subsequent determination of nitrate+nitrite and dissolved reactive phosphorus. Concentrations of total nitrogen were analyzed with alkaline persulfate oxidation (Koroleff, 1983), while concentrations of total nitrogen from CC samples were calculated by as the sum of total Kjeldahl nitrogen and nitrate+nitrite concentrations. Total Kjeldahl nitrogen from CC water samples was measured using U.S. EPA method 351.2 (U.S. EPA, 1983). Dissolved organic carbon was determined by heated-persulfate oxidation using a total organic carbon analyzer with in-line sample acidification and sparging (Menzel and Vaccaro, 1964).

Concentrations of acetochlor, atrazine, metolachlor, and simazine were determined using gas chromatography following standard protocols for pesticide analyses (U.S. EPA, 1995). We selected these herbicides because they are more frequently detected and often occur at greater concentrations than insecticides within agricultural watersheds in the United States (Gilliom, 2007). We calculated the median concentration value of each nutrient and herbicide from water samples collected during a three week period that encompassed the week before amphibian sampling, the week of amphibian sampling, and the week after amphibian sampling. We used a three week time period for our calculation of median concentrations to attain a more representative measure of amphibian exposure to nutrients and herbicides than what would be indicated by a single water sample collected on the day of amphibian sampling.

2.4. Amphibian sampling

Amphibians were collected with unbaited gee minnow traps (43 cm length x 23 cm width, two 2.54 cm openings on each end, 0.32 cm mesh size) in May and July of 2008 and 2009 within CC and in June and August 2009 within UBWC. Fourteen minnow traps were set in each

site and were spaced 9 m apart with traps alternating in position from the right bank to the left bank throughout the site. Traps were set for two nights in each month and resulted in 28 trap nights for each site for each month and 56 trap nights in each site per year. At the end of each 48 hour period the traps were examined and all captured animals were identified, enumerated, and released. Our sampling design resulted in an equal sampling effort among all sites in each watershed and among all years.

Sixteen amphibian response variables were calculated that described amphibian community and population structure within each site during each month of each year. Community response variables included abundance (number of all amphibians captured), tadpole abundance (number of tadpoles captured), post-metamorph abundance (number of post-metamorph frogs captured), species richness (number of species captured), tadpole species richness (number of species calculated from only tadpole captures), post-metamorph species richness (number of species calculated from only post-metamorph captures) Shannon diversity index (Shannon diversity index calculated based on all amphibians captured; Magurran, 1988), tadpole Shannon diversity index (Shannon diversity index calculated based only on tadpoles captured), post-metamorph Shannon diversity index (Shannon diversity index calculated based only on post-metamorphs captured). We also calculated seven population response variables describing the abundance of green frog (*Lithobates clamitans*) tadpoles, bullfrog (*Lithobates catesbianus*) tadpoles, wood frog (*Lithobates sylvaticus*) tadpoles, green frog post-metamorphs, bullfrog post-metamorphs, northern leopard frog (*Lithobates pipiens*) adults, and wood frog adults. We originally planned on using all 16 amphibian community and population response variables in our generalized linear mixed effect model analyses. However, we eliminated four response variables (bullfrog post-metamorph abundance, wood frog tadpole abundance, wood

frog abundance, and northern leopard frog abundance) because these species-life stages had too few occurrences within our dataset (i.e., occurred within < 5 sites). Additionally, all three Shannon diversity index response variables were rounded to the nearest whole number to meet the requirements of the generalized linear mixed effect model analyses.

2.5. Statistical analyses

We conducted an indirect gradient analysis involving the combined use of ordination and generalized linear mixed effect model analysis to examine the relationships of instream habitat and water chemistry with the 12 amphibian community and population response variables within channelized agricultural headwater streams. We first conducted two separate principal component analysis (PCA) using PC-ORD (McCune and Mefford, 2011) on the 16 instream habitat variables to obtain the site scores from PCA axis of instream habitat and on 10 water chemistry variables to obtain the site scores from PCA axes of water chemistry. Each PCA axis represents the underlying gradient in combinations of instream habitat and water chemistry variables that occurs among our study sites. We retained the first three PCA axes for use as independent variables within our regression analyses. Our use of PCA also enabled us to objectively reduce the number of independent variables in our generalized linear mixed effect model.

We then performed generalized linear mixed effect model analysis to obtain the standardized coefficients and determine the relationships that occurred between the amphibian community and population response variables with instream habitat and water chemistry. Generalized linear mixed effect models are an extension of the linear modeling process that allows regression models with both fixed and random factors to be fit to data that do not exhibit

normal probability distributions (i.e., Poisson, binomial, multinomial) (Zuur *et al.*, 2009). Our initial inspection of our community and population response variables noted the frequent occurrence of zero values and that the distributions appeared follow the negative binomial distribution. Subsequent Akaike's Information Criteria (AIC) analyses confirmed that generalized linear mixed effect model analyses with either the poisson or negative binomial distribution would provide greater predictive accuracy than the use of a linear mixed effect model analysis intended for use with normally distributed data. Specifically, we used the site scores from the PCA axes of instream habitat and water chemistry as independent variables and the amphibian response variables as dependent variables in our generalized linear mixed effect model analyses. In all analyses we used the random factors of watersheds and sites nested within watersheds to address the issue of pseudoreplication that results from repeatedly sampling the same sites through time (Zuur *et al.*, 2009). We also used AIC scores to select whether to use the poisson or the negative binomial distribution within our analysis of each amphibian response variable. Generalized linear mixed effect model analyses with either the poisson (species richness, tadpole species richness, post-metamorph species richness, abundance, Shannon diversity index, tadpole Shannon diversity index, post-metamorph Shannon diversity index, bullfrog tadpole abundance) or negative binomial distribution (tadpole abundance, post-metamorph abundance, green frog tadpole abundance, green frog post-metamorph abundance) and watersheds and sites as nested random factors were conducted with R (R Core Team, 2014) and the lme4 package [glmer function (Bates et al., 2015)].

3. Results

3.1. Instream habitat summaries and ordination results

Sites within CC exhibited greater water velocity and discharge than sites within UBWC (Table 1). Most sites in CC were dominated by sand substrate, while UBWC sites were dominated by either clay or gravel substrate (Table 1). Mean physicochemical conditions were typically within acceptable levels for aquatic life (Table 1). Sites in CC and UBWC exhibited increases in water temperatures, decreases in dissolved oxygen, and increases in conductivity that would be expected to occur with decreases in water depth, water velocity, wet width, and discharge between the May and July sampling periods (Table 1).

The observed mean and range of nutrient and herbicide concentrations indicated that our sites exhibited wide variations in concentrations expected within agricultural streams (Table 2, Smiley *et al.*, 2014). Mean and maximum total nitrogen and total phosphorus concentrations exceeded levels found to be capable impacting stream fish, macroinvertebrate, and algal community structure (Table 2, Justus *et al.*, 2010). All minimum total phosphorus concentrations and the minimum total nitrogen concentrations in May within CC and in July within UBWC also exceeded levels found to be capable impacting stream fish, aquatic macroinvertebrate, and algal communities (Table 2, Justus *et al.*, 2010). Additionally, mean and maximum nitrate concentrations did not exceed the minimum level documented to cause amphibian mortality (Table 2, Mann *et al.*, 2009). Mean and maximum concentrations of acetochlor, atrazine, and metolachlor were less than chronic and acute toxicity levels for fish and aquatic macroinvertebrates (Table 2, U.S. EPA, 2015). Mean and maximum concentrations of simazine were less than acute toxicity levels for fish and aquatic macroinvertebrates (Table 2, U.S. EPA, 2015).

The first two PCA axes of instream habitat possessed eigenvalues greater than the broken stick eigenvalues (Jackson, 1993) and were used as independent variables in the generalized

linear mixed effect model analyses. We also chose to use the third PCA axis of instream habitat as an independent variable in the generalized linear mixed effect model analyses because it represented an interpretable axis that represented a different habitat gradient than the first two PCA axes of instream habitat. These three PCA axes explained 55.8% of the cumulative percent of the variance in the instream habitat data (Table 3). The PCA of instream habitat identified a hydrology gradient (axis 1), a substrate-cover type and pH gradient (axis 2), and a water depth and dissolved oxygen (axis 3) (Table 3). Sites with increasing site scores along the first PCA axis of instream habitat exhibited decreasing discharge, mean velocity, and mean water depth values. Sites with increasing site scores along the second PCA axis of instream habitat had increasing pH values and decreasing diversity of substrate and cover types on the stream bottom. Sites with increasing site scores along the third PCA axis of instream habitat exhibited increasing dissolved oxygen concentrations and decreasing mean water depths.

The first three PCA axes of water chemistry also possessed eigenvalues greater than the broken stick eigenvalues (Jackson, 1993) and were used as independent variables in the generalized linear model analyses. These three PCA axes explained 70.5% of the cumulative percent of the variance in the water chemistry data (Table 3). The PCA of water chemistry identified one nutrient gradient (axis 1) and two nutrient and herbicide gradients (axes 2 and 3) (Table 3). Sites with increasing site scores along the first PCA axis of water chemistry exhibited increasing concentrations of total nitrogen and ammonia. Sites with increasing site scores along the second PCA axis of water chemistry exhibited increasing concentrations of simazine and decreasing total phosphorus concentrations. Sites with increasing site scores along the third PCA axis of water chemistry exhibited increasing nitrate+nitrite concentrations and decreasing acetochlor concentrations.

3.2. Summary of amphibian captures and generalized linear mixed effect model results

We documented four frog species from 153 captures of tadpoles, post-metamorphs, and adult animals during our study (Table 4). No salamanders were captured. Our captures consisted mostly of tadpoles and post-metamorphs (97 %), but we also captured northern leopard frog and wood frog adults (Table 4). The three most frequently captured species-life stages captured in both watersheds combined were: 1) green frog tadpoles; 2) bullfrog tadpoles; and 3) green frog post-metamorphs (Table 2). Green frog tadpoles and post-metamorphs, bullfrog tadpoles and post-metamorphs, and wood frog tadpoles were captured in both watersheds, while adult wood frogs and northern leopard frogs were only captured in CC (Table 4).

Overall, the generalized linear mixed effect models indicated that water chemistry influenced amphibian community and population structure more than instream habitat as the greatest standardized coefficient values for all amphibian response variables occurred within the three PCA axes of water chemistry more often than it did for the three PCA axes of instream habitat (Table 5).

Five (species richness, tadpole species richness, tadpole abundance, tadpole Shannon diversity index, bullfrog tadpole abundance) of 12 amphibian response variables were most strongly correlated with PCA axis 3 of water chemistry (i.e., nitrate+nitrite and acetochlor gradient) (Table 5). Four (Shannon diversity index, post-metamorph species richness, post-metamorph abundance, post-metamorph Shannon diversity index) of 12 amphibian response variables were most strongly correlated with PCA axis 1 of water chemistry (i.e., total nitrogen and ammonia gradient) (Table 5). Only abundance was most strongly correlated with PCA axis 2 of water chemistry (i.e., simazine and total phosphorus gradient) (Table 5).

The generalized linear mixed effect model analysis for green frog tadpole abundance did not converge when all six independent variables were included in the analysis. We reanalyzed this response variable with a reduced generalized linear mixed effect model containing only one instream habitat variable and one water chemistry variable. Specifically, for the reduced model we selected the instream PCA axis (i.e., PCA axis 1) and the water chemistry PCA axis (i.e., PCA axis 3) that exhibited the greatest standardized coefficients in the non-converged full model. Green frog post-metamorph abundance was most strongly correlated with PCA axis 3 of water chemistry (i.e., nitrate+nitrite and acetochlor gradient) (Table 5).

Generalized linear mixed effect model analyses also indicated that species richness, tadpole species richness, tadpole abundance, tadpole Shannon diversity index, green frog tadpole abundance, and bullfrog tadpole abundance were positively correlated with PCA axis 3 of water chemistry, which indicates these response variables increased with increasing nitrate+nitrite concentrations and decreasing acetochlor concentrations (Table 5, Figure 1). Shannon diversity index, post-metamorph species richness, post-metamorph abundance, and post-metamorph Shannon diversity index were negatively correlated with PCA axis 1 of water chemistry and decreased with increasing total nitrogen and ammonia concentrations (Table 5, Figure 2). Abundance was positively correlated with PCA axis 2 of water chemistry and increased with increasing simazine concentrations and decreasing total phosphorus concentrations (Table 5, Figure 3). Green frog post-metamorph abundance was negatively correlated with PCA axis 3 of instream habitat and decreased with increasing dissolved oxygen concentrations and decreasing mean water depths (Table 5, Figure 3).

4. Discussion

4.1 Community and Population Level Effects of Water Chemistry

Our results suggest that water chemistry has a greater role in shaping amphibian communities and populations than instream habitat in channelized agricultural headwater streams. Eleven of 12 amphibian response variables exhibited stronger relationships with water chemistry gradients than to those derived from instream habitat variables. In particular, community response variables calculated for all amphibians and tadpoles either increased along a gradient of increasing nitrate+nitrite and decreasing acetochlor concentrations, decreased along a gradient of increasing total nitrogen and ammonia concentrations, or increased along a gradient of increasing simazine and decreasing total phosphorus concentrations. Post-metamorph community response variables decreased along a gradient of increasing total nitrogen and ammonia. At the population level, the abundance of green frog and bullfrog tadpoles increased along a gradient of increasing nitrate+nitrite and decreasing acetochlor concentrations, while the abundance of green frog post-metamorphs decreased as streams became shallower and more oxygenated.

The literature on amphibian habitat relationships within stream ecosystems is sparse and few studies provide information on relationships of amphibians with nutrient and herbicide concentrations in the field. The studies that have been done suggest a minimal role for the effect of water chemistry. In a polder landscape in the Netherlands, it was found that the frequency of dredging for maintenance of hydraulic capacity within channelized streams was correlated with the presence of amphibian larvae, but the amount of nutrients applied on adjacent fields was not (Twisk *et al.*, 2000). Analysis of amphibian communities in streams within a predominantly agricultural landscape in Italy indicated a potential role for nutrient concentrations, but this

relationship was likely an indirect effect of adjacent land use (Ficetola *et al.*, 2011). It should be noted that the previous two studies (Twisk *et al.*, 2000; Ficetola *et al.* 2011) did not quantify herbicide concentrations and both were focused on amphibian occurrence patterns at a landscape scale.

The relationship between water quality and amphibian communities in agricultural wetlands has received more attention (Hecnar and M'Closkey, 1996; Bishop *et al.*, 1999; Brodman *et al.*, 2003; Houlahan and Findlay, 2003; Knutson *et al.*, 2004; Jeliazkov *et al.*, 2014). An index of water quality that included nutrient and physical parameters was positively correlated with species richness in ponds in northern France (Jeliazkov *et al.*, 2014). Nitrogen pollution (total nitrogen, total Kjeldahl nitrogen, and ammonia) has been found to be associated with reduced species richness in wetlands in Wisconsin, USA, (Knutson *et al.*, 2004) and Ontario, Canada (Bishop *et al.*, 1999; Houlahan and Findlay, 2003; but see Hecnar and M'Closkey, 1996 for contrasting results). While there is evidence of generally sub-lethal effects of herbicides on individual species (reviewed in Mann *et al.*, 2009), documented effects on community structure are either weak or lacking in natural systems and this has been attributed to low chronic concentrations typically present within agricultural wetlands and other aquatic ecosystems (Knutson *et al.*, 2004). When relationships between water chemistry and community structure have been documented, these relationships occurred as a result of comparing amphibian community structure among wetlands with contrasting land use (Bishop *et al.*, 1999; Houlahan and Findlay, 2003; Knutson *et al.*, 2004), which suggests that land use may be the underlying factor in amphibian response to water quality (Houlahan and Findlay, 2003; Ficetola *et al.*, 2011).

A growing literature on fish and aquatic macroinvertebrate communities in channelized agricultural headwater streams allows the comparison of our results to the habitat relationships exhibited by other taxa found within this stream type. In CC and UBWC, it has been found that instream habitat, specifically hydrology and substrate type, is more important than riparian habitat and water chemistry in explaining variation in the structure of fish (Smiley *et al.*, 2008; Sanders, 2012) and aquatic macroinvertebrate communities (McKinney, 2012). Other studies on fish in channelized headwater streams in Ohio (Dyer *et al.*, 1997; Miltner and Rankin, 1998; D'Ambrosio *et al.*, 2009) and Wisconsin (Fitzpatrick *et al.*, 2001) have also suggested that water chemistry has less influence relative to instream habitat, geomorphology, riparian habitat, and adjacent land use. Stream size and water velocity has been documented to explain greater variation in aquatic macroinvertebrate community metrics than water quality parameters within channelized agricultural headwater streams in Maryland (Leslie *et al.*, 2012). While water chemistry is considered to be a relatively important habitat factor for fish and aquatic macroinvertebrates in other stream types in North America (Sawyer *et al.*, 2004; Johnson and Ringler, 2014) and Europe (Hering *et al.*, 2006; Leps *et al.*, 2015), these studies were conducted at much larger spatial scales than our study and encompassed greater number of stream types differing in channel modification and stream size.

The relationships of amphibian community and population structure with water chemistry gradients that we have observed are novel. In contrast to much of the literature related to the effects of water chemistry on aquatic biota, a potentially important difference is that our study occurred at a sub-regional scale in two separate watersheds having similar land uses. Although fish and aquatic macroinvertebrates within channelized agricultural headwater streams in CC and UBWC appear to respond to the variation in instream habitat that occurs within and among these

sites (Smiley *et al.*, 2008; McKinney, 2012; Sanders, 2012), perhaps the variation in instream habitat is not great enough for amphibians to perceive these streams as complex habitats containing multiple microhabitat types. If so, this would explain why amphibian community structure was more strongly correlated with water chemistry than physical habitat. More research on amphibian use of different microhabitats within channelized agricultural headwater streams is needed to confirm this observation.

While community interactions are complex in water containing nutrient and pesticide mixtures (Boone and James, 2003; Boone *et al.*, 2007; Mann *et al.*, 2009; Relyea, 2009), the specific relationships that we observed are intriguing. Species richness, tadpole species richness, tadpole abundance, and tadpole diversity increased in response to the combined effect of increasing nitrite+nitrate concentrations and decreasing acetochlor concentrations. The nitrite+nitrate concentrations within the streams during amphibian sampling was below 10 mg/L and the lethal effect concentrations compiled by Mann *et al.* (2009) (>20 mg/L). Such sub-lethal concentrations may benefit tadpoles by increasing productivity which can provide food through increased periphyton biomass (de Wijer *et al.*, 2003; Smith *et al.*, 2006; Boone *et al.*, 2007). Acetochlor effects on amphibians are less well studied, but there is evidence that it can accelerate metamorphosis in northern leopard frogs (Cheek *et al.*, 1999), alter periphyton and phytoplankton dynamics within aquatic food webs, and potentially lead to decreases in pH and dissolved oxygen (Relyea, 2009). We also observed that increasing concentrations of ammonia among sites was correlated with reduced species richness, abundance, and diversity of post-metamorphic amphibians. Ammonia is well known to be highly toxic to tadpoles (Rouse *et al.*, 1999; Mann *et al.*, 2009) and their sensitivity to ammonia can be greater than fish (Diamond *et al.*, 1993). The maximum ammonia concentrations documented in the streams during amphibian

sampling exceeded the concentrations capable of causing significant levels of embryo mortality and reductions in embryo and tadpole growth and development in green frogs (0.6 mg/L) and northern leopard frogs (1.5 mg/L) (Jofre and Karasov, 1999) suggesting that such toxicity may be reducing the numbers of individuals that proceed to and complete metamorphosis from channelized agricultural headwater streams. Additionally, we observed increasing amphibian abundance with increasing simazine concentrations and decreasing total phosphorus concentrations. The influence of simazine on amphibians has not been examined (Smalling *et al.*, 2013), but simazine concentrations in the streams during amphibian sampling were less than acute toxicity levels for fish and aquatic macroinvertebrates (Table 2, U.S. EPA, 2015). In contrast, increasing eutrophication caused by increasing phosphorus concentrations has been linked to increased occurrence of malformations (i.e., missing, extra, or misshapen limbs) in amphibians in North America as a result of increased parasitic infections (Mann *et al.*, 2009). Increasing phosphorus concentrations may also reduce the types and abundance of macroinvertebrate prey preferred by amphibians (Ficetola *et al.*, 2011). Clearly future research needs to quantify the influence of nutrient and pesticide mixtures on amphibians within agricultural streams.

4.2 Channelized Agricultural Headwater Streams as Amphibian Habitat

Only a handful of studies have documented amphibian use of channelized agricultural headwater streams (Twisk *et al.*, 2000; Piha *et al.*, 2007b; Maes *et al.*, 2008; McDaniel *et al.*, 2008; Hartel *et al.*, 2011), and ours is among the first to do so for the Midwestern United States (Smiley *et al.*, 2011). Our results are consistent with these previous studies in that we documented ranid frogs (Family Ranidae) within channelized agricultural headwater streams in

our watersheds. In contrast, previous studies from Europe (Twisk *et al.*, 2000; Maes *et al.*, 2008; Hartel *et al.*, 2011) documented toads (Family Bufonidae, Family Pelobatidae), tree frogs (Family Hylidae), and salamanders (Order Caudata) within channelized agricultural headwater streams in addition to ranid frogs. Additionally, a previous study (McDaniel *et al.*, 2008) in Canada documented toads (Family Bufonidae) in channelized agricultural headwater streams in as well as ranid frogs. Among aquatic breeding amphibian that occur in the counties of our study sites, we only captured 4 of 19 species in Indiana (Minton, 2001) and 3 of 24 species in Ohio (Pfingsten, 1998). One of these species (wood frog) is considered to be sensitive to disturbance and prefers forested habitats (Micacchion, 2011), and a second (northern leopard frog) has experienced population declines and is listed as Species of Concern in Indiana (Minton, 2001). The reduced amphibian diversity and dominance by tolerant, habitat generalists within our study may be a function of its small spatial scale, the high degree of agricultural landuse adjacent to our study areas, and/or the timing of our sampling. Future research in the United States needs to be conducted over the full range of the amphibian breeding season at larger spatial scales to more fully document use of channelized agricultural headwater streams by amphibians.

Green frogs and bullfrogs were the most abundant species within our study sites. Both species favor permanent, open canopy wetlands in forested landscapes but are known to be habitat generalists that will use streams and will readily colonize anthropomorphic bodies of water (Dodd, 2013), and are able to persist in remnant habitats within agricultural landscapes (Kolozsvarly and Swihart, 1999). Green frogs and bullfrogs are also tolerant of the presence of fish, a major contributor to tadpole community structure in wetlands (Knutson *et al.*, 2004; Boone *et al.*, 2007; Werner *et al.*, 2007) that has been documented to decrease amphibian diversity and abundance. Bullfrogs are well known to persist in the larval stage for two to three

seasons and green frogs in the northern United States and southern Canada have also been documented to remain in larval stage for more than one season (Dodd, 2013). Tadpoles of these two species are exposed to aquatic conditions for a relatively extended period of time, which could increase their susceptibility to agricultural contaminants. While comparative information for bullfrogs is not available, green frogs appear to be more tolerant of reduced water quality than other amphibian species (Hecnar and M'Closkey, 1996; McDaniel *et al.*, 2008).

Among the species we observed, green frogs have the closest association with stream habitats (Martof, 1953a; Dodd, 2013). In Michigan, green frogs living in streams will migrate to nearby ponds and wetlands to breed (Martof, 1953b), with females spending only one week away from their home stream. Martof (1953a) observed stream-dwelling residents to remain in place to overwinter and it has also been found that green frogs will migrate from ponds and wetlands to overwinter within streams (Lamoureux and Madison, 1999). It is assumed that stream overwintering sites provide higher oxygen availability due to flowing water. While green frogs and bullfrogs may prefer to breed in open canopy ponds and wetlands with long hydroperiods, our captures of tadpoles in channelized agricultural headwater streams confirms that breeding can occur in this stream type. Further study will be required to determine if the use of streams for reproduction is linked to the lack of proximate ponds and wetlands or if there is polymorphism in breeding site choice within populations.

Our results indicate that channelized agricultural headwater streams provide habitat for anurans in anthropogenically disturbed landscapes, but habitat quality within these streams is highly dependent on the use of management practices that maximize their ecological value (Herzon and Helenius, 2008; Chester and Robson, 2013). Our results provide empirical support for previous amphibian management guidelines (BC WLAP, 2004; Kingsbury and Gibson, 2012)

for agricultural landscapes in the United States and Canada that recommend reductions in the use of agricultural fertilizers and pesticides for amphibian conservation. Channelized streams with subsurface drainage in the watersheds present a special challenge for managing water quality because subsurface tile drains enable agricultural contaminants to bypass grass filter strips and other types of riparian buffers (Smiley *et al.*, 2011). Thus, effective conservation practices will be those that reduce nutrient and herbicide concentrations within subsurface runoff. Routing subsurface drainage into treatment wetlands created to capture nutrients and pesticides has been promoted to reduce nutrient and pesticide inputs into streams (Smiley *et al.*, 2011). Creation of treatment wetlands within riparian habitats of channelized agricultural headwater streams would also benefit amphibians by providing additional habitat and increasing riparian habitat diversity. Additionally, conservation practices that dissipate subsurface runoff through riparian habitats [i.e., saturated buffers (Jaynes and Isenhardt, 2014)] or practices that filter subsurface runoff [i.e., blind inlets (Smith and Livingston, 2013)] represent additional options for reducing nutrient and pesticide concentrations in channelized agricultural headwater streams. Although we recommend the use of conservation practices targeting water quality improvement at the local scale within channelized agricultural headwater streams as part of amphibian conservation efforts within agricultural landscapes, **it is our opinion** that amphibian conservation efforts should involve the management of both local and landscape-scale factors. Specifically, management of terrestrial factors **(e.g., land use characteristics, riparian corridor width, regional scale density of wetlands, etc.)** known to influence amphibian diversity and abundance in agricultural wetlands (Kolozsvary and Swihart, 1999; Houlahan and Findlay, 2003; Semlitsch and Bodie, 2003; Jeliazkov *et al.*, 2014) is needed but this has rarely been explored for streams (Maisonneuve and Rioux, 2001; Ficetola *et al.*, 2011).

5. Conclusions

Our results indicate that anuran community and population structure was most strongly correlated with water chemistry (i.e., nutrient and herbicide gradients) rather than instream habitat within channelized agricultural headwater streams in Indiana and Ohio. Our results also highlighted the relationships of anuran community and population structure with agricultural contaminants commonly found within agricultural streams. Within these small degraded streams we documented that anuran community and population response variables either increased along a gradient of increasing nitrate+nitrite and decreasing acetochlor concentrations, decreased along a gradient of increasing total nitrogen and ammonia concentrations, or increased along a gradient of increasing simazine and decreasing total phosphorus concentrations. Our results suggest local scale practices that reduce nutrients and herbicides within channelized agricultural headwater streams will provide greater benefits for amphibians than practices address physical habitat degradation. Amphibian conservation strategies within agricultural landscapes have focused mostly on wetland conservation. Future research needs to more fully explore the habitat value of channelized agricultural headwater streams and how these streams can be managed to contribute to amphibian conservation efforts.

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Table 1

Mean (standard deviation) of selected instream habitat variables within channelized agricultural headwater streams from May and July 2008 and 2009 within Cedar Creek, Indiana (CC) and May and July 2009 within the Upper Big Walnut Creek, Ohio (UBWC).

	CC		UBWC	
	May	July	May	July
Water depth (m)	0.25 (0.09)	0.19 (0.08)	0.21 (0.09)	0.14 (0.06)
Water velocity (m/sec)	0.16 (0.16)	0.06 (0.07)	0.04 (0.03)	0.00 (0.01)
Wet width (m)	2.69 (0.86)	2.44 (0.95)	2.63 (0.77)	2.27 (0.78)
Discharge (m ³)	0.12 (0.20)	0.02 (0.04)	0.02 (0.01)	0.00 (0.00)
Dominant substrate	sand	sand	clay	gravel
Substrate+cover type richness	5.36 (1.45)	5.36 (1.15)	7.00 (0.71)	6.40 (0.89)
Percent cover types	61.67 (26.14)	67.86 (28.23)	46.67 (24.01)	87.39 (5.90)
Water temperature (°C)	13.47 (2.70)	19.33 (3.52)	14.19 (1.37)	20.68 (1.86)
Dissolved oxygen (mg/L)	9.60 (3.89)	7.33 (2.66)	10.41 (1.47)	5.38 (2.54)
Conductivity (microsiemens/cm)	579.35 (72.70)	672.62 (52.52)	457.60 (212.56)	758.40 (234.85)
pH	8.31 (0.81)	8.55 (0.83)	7.89 (0.15)	7.74 (0.47)

Table 2

Mean (range) of water chemistry variables within channelized agricultural headwater streams from May and July 2008 and 2009 within Cedar Creek, Indiana (CC) and May and July 2009 within Upper Big Walnut Creek, Ohio (UBWC).

	CC		UBWC	
	May	July	May	July
Dissolved organic carbon (mg/L)	13.00 (5.78-26.75)	13.62 (4.41-39.49)	13.62 (4.41-39.49)	7.15 (5.13-11.58)
Ammonia (mg/L)	0.21 (0.00-0.67)	0.43 (0.00-7.05)	0.43 (0.00-7.05)	0.05 (0.00-0.25)
Nitrate+nitrite (mg/L)	2.78 (0.09-7.72)	0.50 (0.00-3.07)	0.50 (0.00-3.07)	0.94 (0.01-3.45)
Total nitrogen (mg/L)	5.53 (1.85-13.79)	3.36 (0.07-53.42)	3.36 (0.07-53.42)	1.65 (0.51-4.59)
Dissolved reactive phosphorus (mg/L)	0.02 (0.00-0.29)	0.03 (0.00-0.23)	0.03 (0.00-0.23)	0.07 (0.01-0.25)
Total phosphorus (mg/L)	0.46 (0.02-3.22)	1.14 (0.03-20.28)	1.14 (0.03-20.28)	0.12 (0.02-0.32)
Acetochlor (µg/L)	0.60 (0.00-18.35)	0.04 (0.00-0.50)	0.04 (0.00-0.50)	0.00 (0.00-0.00)
Atrazine (µg/L)	2.28 (0.00-30.59)	0.12 (0.00-1.53)	0.12 (0.00-1.53)	0.20 (0.00-0.34)
Metolachlor (µg/L)	0.99 (0.00-10.90)	0.04 (0.00-0.33)	0.04 (0.00-0.33)	0.14 (0.07-0.65)
Simazine (µg/L)	1.20 (0.00-16.71)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.10 (0.00-0.23)

Table 3

Loadings for instream habitat variables and percent variance explained for the first three principal component analysis (PCA) axes of instream habitat and for nutrient and herbicide variables on the first three PCA axes of water chemistry from channelized agricultural headwater streams in Cedar Creek, Indiana, and Upper Big Walnut Creek, Ohio, 2008 to 2009. Underlined loadings are those that were statistically significant (i.e., > 0.3 or < -0.3) (Hair *et al.*, 1997) and bolded loadings are those that best characterized the underlying habitat gradients.

	Axis 1	Axis 2	Axis 3
<i>Instream Habitat</i>			
Mean depth	<u>-0.368</u>	-0.055	<u>- 0.441</u>
SD depth	-0.156	-0.246	<u>-0.329</u>
Mean velocity	<u>-0.387</u>	0.278	0.118
SD velocity	<u>-0.349</u>	0.263	0.205
Mean wet width	<u>-0.343</u>	-0.062	<u>-0.314</u>
SD wet width	-0.065	-0.196	-0.043
Discharge	<u>-0.388</u>	0.187	-0.077
Dominant substrate score	-0.036	-0.216	0.260
Substrate richness	-0.163	<u>-0.370</u>	0.097
Cover type richness	-0.178	-0.295	<u>0.312</u>
Substrate+cover type richness	-0.223	<u>-0.433</u>	0.267
Percent cover types	-0.178	-0.295	<u>0.312</u>
water temperature	0.213	-0.043	0.267
Dissolved oxygen	-0.230	0.086	<u>0.377</u>
Conductivity	0.199	-0.013	-0.083
pH	0.119	<u>0.402</u>	-0.008
% variance explained by axis	<u>25.7</u>	<u>19.2</u>	<u>10.9</u>
<i>Water Chemistry</i>			
Dissolved organic carbon	0.293	-0.047	<u>-0.356</u>
Ammonia	<u>0.436</u>	<u>-0.392</u>	0.045
Nitrate+nitrite	0.122	<u>0.404</u>	<u>0.503</u>
Total nitrogen	<u>0.460</u>	-0.130	0.290
Dissolved reactive phosphorus	0.147	-0.000	<u>0.320</u>
Total phosphorus	<u>0.396</u>	<u>-0.437</u>	0.089
Acetochlor	0.269	0.185	<u>-0.546</u>
Atrazine	<u>0.345</u>	<u>0.341</u>	-0.291
Metalachlor	0.250	<u>0.331</u>	0.204
Simazine	0.256	<u>0.462</u>	0.015
% variance explained by axis	<u>32.5</u>	<u>20.9</u>	<u>17.1</u>

Table 4

Number of captures (#) and relative abundance (%) of frogs captured in channelized agricultural headwater streams within Cedar Creek, Indiana (CC) and Upper Big Walnut Creek, Ohio (UBWC), 2008 to 2009. Percentages within the table are calculated based on the number of captures from within each watershed.

	CC		UBWC	
	#	%	#	%
<i>All amphibians</i>				
tadpoles	37	53.6	72	85.7
post-metamorphs	28	40.6	12	14.3
adults	4	5.8	0	0
<i>Green Frog (Lithobates clamitans)</i>				
tadpole	22	31.9	37	44.0
post-metamorph	23	33.3	9	10.7
<i>Bullfrog (Lithobates catesbiana)</i>				
tadpole	13	18.8	33	39.3
post-metamorph	5	7.2	3	3.6
<i>Wood Frog (Lithobates sylvaticus)</i>				
tadpole	2	2.9	2	2.4
adult	2	2.9	0	0.0
<i>Northern Leopard Frog (Lithobates pipiens)</i>				
adult	2	2.9	0	0.0

Table 5

Standardized regression coefficients from generalized linear mixed effect model analyses depicting the relative influence of instream habitat (IH) and water chemistry (WC) on amphibians within channelized agricultural headwater streams in Cedar Creek, Indiana, and Upper Big Walnut Creek, Ohio, 2008 to 2009. PCA – principal component analysis axis. Bolded standardized coefficients identify the environmental factors with the greatest influence on amphibian community and population response variables. Underlined standardized coefficients are those independent variables that were documented to have significant effect ($P < 0.05$) within the regression model.

	IH PCA 1	IH PCA 2	IH PCA 3	WC PCA 1	WC PCA 2	WC PCA 3
<i>Community</i>						
Species richness	0.054	-0.028	-0.132	-0.486	0.194	0.530
Tadpole species richness	0.018	-0.140	-0.208	-0.360	0.253	0.579
Post-metamorph species richness	<u>0.219</u>	-0.107	0.057	-0.666	0.530	0.376
Abundance	-0.236	-1.159	1.024	-1.006	1.259	-0.098
Tadpole abundance	<u>1.324</u>	-0.583	-2.212	-2.841	2.028	10.541
Post-metamorph abundance	0.350	-0.221	-0.586	-0.609	0.221	0.150
Shannon diversity index	0.048	-0.043	-0.080	-0.344	0.172	0.332
Tadpole Shannon diversity index	0.031	-0.121	-0.221	-0.303	0.219	0.460
Post-metamorph Shannon diversity index	<u>0.217</u>	-0.125	0.092	-0.601	0.517	0.290
<i>Population</i>						
Green Frog tadpole abundance*	‡	0.896	‡	‡	‡	6.026
Bullfrog tadpole abundance	-0.337	-0.020	-4.047	-6.477	-5.115	12.216
Green Frog post-metamorph abundance	0.186	-0.287	-0.592	-0.530	0.259	-0.132

* The model for this response variable did not converge and required reanalysis with a reduced number of independent variables. The results of the reduced model are presented above.

Figure 1

Predicted relationship of amphibian species richness and tadpole abundance with site scores of the third Principal Component Axis (PCA) axis of water chemistry (WC) within channelized agricultural headwater streams in Cedar Creek, Indiana and Upper Big Walnut Creek, Ohio, 2008 to 2009. Predicted values were calculated with multiple regression equations using a range of values for the third PCA of WC and the values of the other independent variables held constant.

Figure 2

Predicted relationship of post-metamorph (pmet.) species richness and pmet. abundance with site scores of the first Principal Component Axis (PCA) axis of water chemistry (WC) within channelized agricultural headwater streams in Cedar Creek, Indiana and Upper Big Walnut Creek, Ohio, 2008 to 2009. Predicted values were calculated with multiple regression equations using a range of values for the first PCA of WC and the values of the other independent variables held constant.

Figure 3

Predicted relationship of amphibian abundance with site scores of the second Principal Component Axis (PCA) axis of water chemistry (WC) and predicted relationship of green frog post-metamorph (gf pmet.) abundance with third PCA axis of instream habitat (IH) within channelized agricultural headwater streams in Cedar Creek, Indiana and Upper Big Walnut Creek, Ohio, 2008 to 2009. Predicted values were calculated with multiple regression equations using a range of values for the second PCA of WC (amphibian abundance) or third PCA of IH (gf pmet. abundance) and values of the other independent variables held constant.

Figure 1

Figure 2

Figure 3