AN ABSTRACT OF THE THESIS OF

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Title: Agricultural Impacts on Amphibian Survival, Growth, and Distributions.

Abstract approved:

Tiffany Sacra Garcia

The potential impact of chemical contaminants and conservation practices on amphibians in agricultural landscapes is a key research topic globally. Amphibians represent a common group in many freshwater systems and are currently experiencing worldwide population declines. Global amphibian declines may be attributed to a number of causes, including habitat loss, introduced species, global climate change, disease, and chemical contaminants; most species declines are not a function of only one factor, but a result of interacting factors and synergistic impacts.

I analyzed the impact of field conservation efforts employed in the Calapooia watershed, located in the central Willamette Valley in Oregon, on amphibian species diversity using Simpson's Diversity Index. In the Calapooia watershed the value of this index increased when conservation efforts, such as retaining crop residue and riparian buffers, were present. This suggests that species diversity increased with increased conservation effort at the field level.

In addition, I found species-specific habitat associations in the Calapooia watershed. Long-toed salamanders (*Ambystoma macrodactylum*) were associated with stream channel cover. Rough-skinned newts (*Taricha granulosa*) and Red-legged

frogs (*Rana aurora*) showed similar relationships to pH, bank width, depth, and riparian habitat where as Pacific treefrogs (*Pseudacris regilla*) showed strong relationships to increased structural heterogeneity, increased distance to nearest agricultural field, and increased human disturbance. These results indicate that conservation efforts can impact amphibian biodiversity, and that there are species-specific habitat associations in the Calapooia watershed.

My third chapter looked at pesticides and fertilizers, which have been shown to negatively affect many species of amphibians. I used meta-analytic techniques to quantify the lethal and sub-lethal effects of pesticides and fertilizers on amphibians in an effort to review the published work to date. I found that, in general, pesticides and fertilizers negatively affect amphibians by reducing both survival and growth. Pesticide and fertilizer chemical classes showed differences in their impacts on amphibians: inorganic fertilizers, organophosphates, phosphonoglycines, and triazines negatively affected amphibian survival, while, organophosphates and triazines negatively affected amphibian growth.

Agricultural Impacts on Amphibian Survival, Growth, and Distributions

by

Nick J. Baker

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APPROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Nick J. Baker, Author

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I have a special connection to the Zoology department since that is where I got my science start. The Blaustein lab in particular has always been a core group of friends and support network for all of my undergraduate and graduate work. I have made a lot of friends in that department and I’m thankful I was introduced to them as they are some of the most amazing people I’ve ever met.

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CONTRIBUTION OF AUTHORS

Betsy A. Bancroft assisted with many aspects of this thesis. She helped analyze data and provided statistical advice for Chapter 3. Betsy also provided editorial assistance with reviews and comments during the formation of Chapters 2 and 3.

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DEDICATION

To my friends and my family, thank you so much for everything!

Agricultural Impacts on Amphibian Survival, Growth, and Distributions

CHAPTER 1: GENERAL INTRODUCTION

Agricultural impacts on global biodiversity are of growing concern as agricultural land use continues to increase. Currently agriculture takes up approximately 38% of the planet's available land and the amount of land in agriculture is expected to continue to increase (Tilman 2001, Donald 2006). Agricultural land expansion is expected to occur in areas of high wild biodiversity and conservation value, such as wetlands and riparian zones (Tilman 2002, Mattison 2005, Donald 2006) Wetlands and riparian areas cleared for new planting can fragment entire landscapes, cutting off migratory routes, and eliminating habitat (Mattison 2005). Wetlands represent a mosaic of ephemeral and permanent water bodies that provide a multitude of potential habitats for a variety of wildlife. Heavy modification of agricultural lands eliminates a portion of these wetland habitats, often infilling temporary wetlands, channelizing streams to reduce flooding, and providing irrigation (Gergel 2005). Relative to aquatic biota in particular, successfully integrating priorities of environmental health with agricultural production will be important in order to prevent global biodiversity declines.

Some efforts have been made to minimize the impacts of fragmentation and habitat loss via agricultural environmental schemes. These schemes include conservation programs to promote sustainable land use and management on private lands (Benton 2007). The purpose of the Conservation Efforts and Assessment Project (CEAP) is to establish how the application of conservation practices would have the largest ecological and economic benefit. The CEAP program began in 2003 as a multi-agency effort to quantify the environmental benefit of conservation practices, and is currently monitoring 37 watersheds in the United States (Mausbach 2004). In the Calapooia watershed, located in the Willamette Valley in Western Oregon, the CEAP workgroup investigated the use of biodiversity as an indicator of water quality and conservation effort. The use of biodiversity as an indicator of environmental quality is unique in the CEAP project and currently the Calapooia watershed is the only workgroup focusing on this approach (Figure 2.1). My research focused on amphibian taxa, how this species group is impacted by conservation efforts and how they are distributed across the Calapooia watershed.

Of the approximately 6,000 species of amphibians, an estimated 43% are experiencing population declines, while 32% are listed by the IUCN as threatened (Blaustein 1994, Alford 1999, Stuart 2004, Mendelson *et al.* 2006). There are many hypotheses for amphibian population declines, but two of these hypotheses include: habitat loss and chemical exposure. Amphibians often are used as indicator species for degraded environments due to their unique physiological characteristics, such as thin, permeable skin, unshelled eggs, and complex life cycle (Alford 1999, Blaustein 2002). Because of their unique physiology, amphibians could potentially be used to gauge environmental health for both terrestrial and aquatic systems.

I explored the benefit of field level conservation practices, consisting of various tillage and ground management techniques, on amphibian species diversity in the Calapooia watershed. I found a positive relationship between field-level conservation practices and amphibian species diversity suggesting that field conservation practices may provide some benefit. In addition to monitoring conservation impacts on amphibians I looked at amphibian distributions in the Calapooia watershed to establish habitat associations. Four native amphibian species were identified in the Calapooia watershed, including: *Ambystoma macrodactylum*, *Taricha granulosa*, *Pseudacris regilla*, and *Rana aurora*. Habitat associations were the same for *Taricha granulosa* and *Rana aurora*, while *Ambystoma macrodactylum* and *Pseudacris regilla* differed from the other two species.

Lastly, I analyzed data on chemical contaminants common in agriculturally dominated systems worldwide. There is a growing body of literature on the impacts of pesticides and fertilizers on amphibians, but the literature lacks synthesis there is a (Relyea 2005*b*). I employed meta-analytical techniques (e.g., Gurevitch 1992, Gurevitch & Hedges 1999, Bancroft *et al.* 2007, Bancroft *et al.* 2008) to synthesize published reports on survival and growth of amphibians when exposed to pesticides and fertilizers. Pesticides and fertilizers have an overall negative effect on amphibian survival and growth. Additionally I found that there was variation in the effect of chemical classes on amphibian survival and growth. Organophosphate, triazine, phosphonoglycine, with the chemical surfactant POEA, and inorganic fertilizers impacted amphibian survival, while organophosphates and triazines significantly reduced growth.

Chapter 2

Conservation matters: A Multi-Scaled Approach to Quantifying Amphibian Diversity in Agricultural Landscapes

Nick J. Baker and Tiffany S. Garcia

Introduction

Anthropogenic impacts on natural systems have become an increasing problem as human population densities continue to increase (Donald 2006). Impacts such as habitat loss and degradation, climate change, introduction of non-native species, and increased contaminant exposure are all contributing to a global biodiversity crisis (Root et al. 2003, Worm et al. 2006, Whitfield et al. 2007). In particular, human agricultural production requirements have altered many existing natural habitats and reducing wild biodiversity. In 2000, approximately 5.01 x 10^9 ha of land around the world had been converted to agriculture; this number is predicted to reach approximately 6.0 x 10^9 ha by the year 2050. Converting this additional 10,000,000,000 ha of land to agricultural needs would represent a worldwide land conversion larger than the area of the United States (Tilman 2001). Thus, successfully integrating priorities of environmental health with agricultural production is a necessary challenge, as agricultural ecosystems are both economically and environmentally important.

Agricultural products provide billions of dollars in revenue to the United States annually, while the farming industry provides jobs and stability to small, rural communities (Miles 1994). Environmentally, agricultural systems often overlap with areas of high conservation value, such as wetland habitats and riparian zones (Tilman et al. 2002, Mattison 2005). Additionally, the establishment of these human-dominated systems often fragments existing habitats, decreases species biodiversity and population densities, and negatively impacts dispersal and migratory patterns

(Bowers 1999). It is important to investigate the mechanisms by which these habitat modifications have altered the ecology of these systems to better understand how to manage and protect regions of conservation concern.

There have been efforts to minimize the impacts of fragmentation and habitat loss via government-sanctioned incentive programs. These conservation-oriented programs exist at the federal, state, and local levels and offer a variety of options to promote sustainable management on privately owned land (Donald 2006, Benton 2007, House Bill 6124). A variety of state and federal programs, like the 2008 Farm Bill, provide farmers and landowners with cost-share incentives, tax incentives and grants in exchange for voluntarily taking farmland out of production and/or the conservation of wetland and riparian habitats (Donald 2006, Benton 2007, House Bill 6124). For example, agricultural field conservation efforts such as low/no till practices or leaving vegetative cover on fields in the form of native grasses are represented in the USDA-sponsored program Environmental Benefits Index (EBI) (Khanna 2009). The EBI program has been able to increase the cost effectiveness of implementing conservation practices and has generated increased enrollment from sites with highly erodible areas (Khanna 2009). Possible downsides to programs like the EBI and other field-level conservation efforts are clear links to environmental quality improvements (Khanna 2009). These programs often depend on multiple-year investments to fully mature and many areas can take over a decade before noticeable environmental benefits are achieved. Unfortunately, farmer participation is not consistent because participation turnover rates are quite high (Khanna 2009).

A challenge of these programs is trying to tailor management strategies to restore and maintain these complex ecosystems. Aquatic habitats add specific elements of complexity to these landscapes. Agricultural wetland habitats are generally flooded during part of the year, creating a mosaic of ephemeral and permanent water bodies (Keddy 2000). Natural hydrological regimes and wetlands have been heavily modified to either prevent flooding, which eliminates a portion of the wetland habitat, or for irrigation purposes (Gergel 2005). In addition to hydrological modification, there has been heavy riparian corridor loss throughout many agricultural systems to maximize crop area (Mensing 1998). These habitat modifications have dramatically altered ecosystem processes, biological communities, and have created new challenges for the management of wild biodiversity (Mensing 1998, Gergel 2005, Donald 2006).

To gain a better understanding of how habitat modifications have impacted these communities, research often focuses on environmentally sensitive species, as these populations can be indicators of environmental health. Amphibians are proposed to be an excellent indicator species in both managed and natural environments (e.g., Blaustein and Kiesecker 2002). Amphibians typically have a complex life cycle, which exposes individuals to both terrestrial and aquatic stressors, and possess unique physiological characteristics, such as permeable skin and unshelled eggs, that can result in increased exposure to environmental stressors. Moreover, amphibians are of serious conservation concern on a global scale. A recent study found that 7% of the 5,948 total amphibian species on the planet are listed as critically endangered, and 22% of these species are too poorly known to assess population status (Stuart et al. 2004). In addition, the extinction of nine amphibian species since 1980 has been documented and another 122 species can no longer be found (Stuart et al. 2004). Because amphibians face immediate conservation threats and possess unique physiological characteristics, they are an ideal indicator species in agricultural systems (Blaustein et al 1994). Using amphibians as a gauge, researchers can assess overall environmental health of both terrestrial and aquatic systems associated with agricultural landscapes.

Many potential amphibian-breeding, aquatic habitats occur in agriculturally dominated systems. These breeding habitats are often heavily fragmented and manipulated, which can lead to extirpation of amphibians from historic breeding sites and hibernation sites and can expose them to enhanced physiological stressors (e.g. temperature, fluctuating hydroperiods, ultraviolet radiation, toxins; Blaustein & Kisecker 2002, Johannson 2005, Relyea 2005). Agricultural systems also introduce chemical stressors, such as pesticides and fertilizers, which can have severe impacts on growth and survival of amphibian embryos and larvae (Boone 2002, Kats and Ferrer 2003, Hayes et al. 2006). Conservation practices can be implemented to reduce impacts of habitat loss, fragmentation, and physiological stressors, such as managing for riparian corridors, restoration of wetlands, and practicing field-level conservation techniques. Conservation techniques can include reduced or no-till practices, leaving crop residue (i.e. residue management) or planting native plant species over the winter season, and reducing the use of chemical additives. In addition, understanding where amphibians breed, hibernate, and reside in managed habitats will increase our ability to implement conservation practices in the future.

The Calapooia River, located in the central Willamette Valley, Oregon, USA, is a large tributary of the Willamette River. The 90,806 ha watershed encompasses 115.9 km of river and dissects a variety of land use practices (Runyon 2004; Figure 2.1). Land in the Calapooia watershed includes U.S. Forest land in the headwaters, industrial forests and mixed agriculture in the central region, and predominantly grass seed farming in the lower section (Floyd 2004, Runyon 2004). This makes the Calapooia watershed an ideal location to assess agricultural impacts on amphibian biodiversity.

In a collaborative effort with the USDA National Forage Seed Production Center, Oregon State University's Departments of Fisheries and Wildlife and Agricultural Economics, and local farmers, a large-scale biodiversity project has been initiated to quantify the economic trade-offs of implementing various conservation efforts throughout the Calapooia watershed. This USDA/Cooperative Research Education and Extension Service (CREES) sponsored project, (the Conservation Efforts Assessment Project [CEAP]) will establish how the application of conservation practices will have the largest ecological effect and least economic impact. The amphibian component of this multi-taxa study was designed to quantify amphibian species composition and population distributions across the Calapooia watershed and to quantify the potential economic impacts of applying conservation practices, such as reduced tillage, crop residue management, and enhanced riparian buffer zones.

I hypothesized that areas of high conservation effort would be positively correlated with diverse and species-rich amphibian communities associated with streams and ponds. In addition, I predicted that amphibian species presence and relative abundance would be correlated with local habitat variables at pond and stream sites in the Calapooia watershed.

Methods

Site Selection

Remote sensing classification was conducted on the Calapooia watershed in 2005, 2006, and 2007 by the USDA National Forage Seed Production Center (NFSPC) (Mueller-Warrant in press *a*). Using the information provided by NFSPC, the Calapooia watershed was divided into 124 sub-basins based on hydrological independence using ArcGIS 9.2 (Figure 2.2). Only sub-basins classified as independent hydrological drainages were considered for amphibian survey site selection (i.e., independent drainages only receive runoff from fields within the sub-basin instead of multiple sub-basins). Using these independent sub-basins, percent conservation (Table 2.1) was calculated using the area of the sub-basin in a conservation practice divided by the total area of land in the sub-basin (Figure 2.3). Conservation practices were defined as a list of 8 possible residue management practices such as leaving vegetation residue on the fields over winter, no-till practices after the growing season, and planting native vegetation during the off season (Table 2.2; Mueller-Warrant in press *a*).

The percent conservation established from these practices ranged from 0.0% to 100.0% conservation across all sub-basins. This gradient of percent conservation was incorporated into a raster file by the USDA NFSPC and was used to establish a list of potential sampling sites (Table 2.1; Mueller-Warrant in press *b*). Sub-basins were ranked by percent conservation and landowners within each of the sub-basins were contacted to secure access to potential sampling sites. Sites were selected to represent the gradient of possible conservation practices; these ranged from 22.7% to 78.9% for the sites used in this study (Table 2.2). For the amphibian component of this project, we obtained access to 10 pond sites and 15 stream sites, representing 19 out of 124 sub-basins throughout the Calapooia watershed (Table 2.1; Figure 2.3).

We quantified amphibian assemblage composition (i.e. species occurrence and relative abundance) and distribution by sampling ponds and streams within selected sub-basins of the Calapooia watershed (Table 2.1). Surveys were conducted for seven months from December 2007 through June 2008, and each site was sampled three times. Multiple sample times were necessary for these sites due to the different breeding and development rates for our 5 endemic amphibian species. In the Willamette Valley, Oregon, Long-toed salamanders (*Ambystoma macrodactylum*) are the first species to emerge (December), followed by Rough-skinned newts (*Taricha granulosa),* Pacific treefrogs (*Pseudacris regilla*), Red-legged frogs (*Rana aurora*), and Northwestern salamanders (*Ambystoma gracile*) (January through March, respectively) (Gordon 1939, Henry 1940, Stebbins 1951, Storm 1960, Leonard 1993, Twedt 1993, Corkran 1996).

At each site particle-board cover boards, approximately 1-meter squared in size, were implemented to serve as refuge habitat for adult amphibians. At pond sites four boards were evenly spaced around the perimeter of the ponds, while at stream sites boards were randomly placed at four of the eleven transects for the duration of the field season. Along with cover boards, minnow traps baited with salmon eggs were also placed at pond and stream sites 24 hours before the next sampling period to attempt to catch any free-swimming adults.

Pond Protocols for Amphibian Surveys

Ponds were divided into three sampling zones: shore, shallow water, and deep water (Figure 2.4). Shore sampling took place within a 3-meter belt along the perimeter of the pond focusing entirely on terrestrial habitats. Shallow water surveys were conducted in a 1-meter belt around the perimeter of the pond and deep water surveys took place from the edge of the shallow water survey to approximately three meters out from the shoreline. Two team members moving in opposite directions surveyed the perimeter of each zone. Surveyors stopped every two meters, for two minutes, and visually scanned for amphibian adults, larvae and egg masses, and used a dipnet to manually sweep for larvae and adults. In addition, calling adults were also documented with auditory surveys. These methods were repeated in each of the three pond sampling zones. In addition to the above protocols, surveyors turned over rocks, logs, and debris on the shore to locate adult and juvenile amphibians. These methods were adapted from previously established protocols (Olson *et al.* 1997).

Stream Protocols for Amphibian Surveys

Amphibian surveys were conducted on selected streams using a Random X survey technique (Bury *et al.* 2000). At each stream site eleven 10-meter belts were selected for surveying, representing a 110-meter stretch of the stream (Figure 2.5). Streams were split into two zones for surveys: stream bank and stream channel. For stream bank and stream channel surveys, two team members, moving upstream on opposite banks, visually scanned for amphibian adults, larvae, and egg masses at each belt for two minutes. Any rocks and debris found along the stream bank were moved to search for adults. Dipnetting was performed in the channel zone in addition to visual surveys. For both zones, calling adults also were documented.

Habitat protocols

Physical habitat data were obtained following the Environmental Protection Agency protocols for quantifying physical habitat in wadeable streams for 33 different categories of micro- and macrohabitat classification (Table 2.3). The habitat variables collected were condensed to twelve variables (Table 2.4), following protocols outlined in Kaufmann 1999, to combine similar habitat metrics. Habitat surveys were conducted in November 2007 and December 2007 at each site prior to the start of the sampling season. Additionally, water samples were taken at each site three times during the sampling season. Water samples were analyzed by the USDA National Forage Seed Production Center for pH, dissolved organic carbon, total nitrogen, ammonium, and nitrate.

Diversity Index

Species diversity at each site was calculated using Simpson's Index (*SI)*:

$$SI = 1 - \frac{\sum(n(n-1))}{N(N-1)}$$

where n = total number of organisms of a particular species and N = total number of organisms of all species. *SI* ranges in value between 0 and 1, with 1 representing infinite diversity and 0 representing no diversity. The value of *SI* represents the probability that two individuals randomly selected from a sample will belong to different species (Simpson 1949).

Statistical Analysis

A linear regression was used to examine the association between relative species diversity (*SI*) and percent conservation for all sites. Pond and stream sites were analyzed separately (SigmaPlot version 11.0 Systat Software, Inc.).

Nonmetric Multidimensional Scaling (NMS) was used to examine relationships between the relative abundance, presence of amphibians species, and 12 habitat and water quality variables per site (Table 2.4; PC-ORD version 6.0 McCune 2002). NMS is an ordination method suited to ecological data, which tends to be non-normal (McCune 1994). Pond and stream data were split and analyzed separately. We used the Sorenson (Bray-Curtis) distance measurement to calculate distance metrics for the ordination space.

For the stream ordination, we used presence/absence data to generate probability distributions for amphibian community composition in ordination space. We coded individual habitat associations in the matrix as presence/absence in dummy variables and then performed a Beals smoothing transformation for each site (row).

Beals smoothing transforms categorical presence/absence data into continuous probabilities based on common occurrences in each category. For example, if Red-legged frog presence co-occurs with other species in our data set, the Beals smoothing takes that into consideration and calculates the probability of Red-legged frog presence based on the presence or absence of the other species. This procedure transforms sparse data sets, containing many zeros, into a data set that contains species presence probabilities. Ordination techniques are then able to extract patterns from the new probability data sets (McCune 1994).

Results

During the 2007-2008 sampling season 2,002 amphibians were collected: 1,201 from ponds and 796 from streams (Table 2.5). These samples included four species of native amphibians: *Ambystoma macrodactylum* (Long-toed salamander), *Rana aurora* (Red-legged frog), *Taricha granulosa* (Rough-skinned newt), and *Pseudacris regilla* (Pacific treefrog). The invasive American Bullfrog (*Rana catesbeiana*) was found at one pond and one stream site, and the native Northwestern salamander (*Ambystoma gracile*) was never detected.

We found a positive relationship ($p = 0.018$) between amphibian species diversity (*SI*- species index) and percent land in conservation in each of the 19 surveyed sub-basins (Figure 2.6). A similar relationship was found when stream sites were analyzed separately ($p = 0.045$; Figure 2.7). For the pond sites, there was no relationship between *SI* and percent land in conservation ($p = 0.848$; Figure 2.8).

NMS Ordination on Ponds

The pond ordination used relative species abundance at each site to establish amphibian community composition as a function of habitat. Amphibian communities based on relative abundance differed between sites in pond habitats (Figure 2.9). American Bullfrogs were originally included in this analysis but their presence increased model stress, preventing a solution, so they were dropped from the analysis. Long-toed salamander abundance was not correlated with any habitat variables measured in the pond data set (Table 5). Rough-skinned newt and Red-legged frog abundance were both positively correlated with increases in pH, understory cover, depth, and bank width (Table 2.6). Pacific treefrog abundance was positively correlated with increases in depth, channel cover, bank width, understory cover, ground cover, and distance to nearest agricultural field (Table 2.6).

NMS Ordination on Streams

In streams, the probability of Long-toed salamander presence was positively correlated with decreased channel cover in stream habitats (Table 2.7; Figure 2.10). American Bullfrogs were originally included in this analysis but their presence increased model stress, preventing a solution, so they were dropped from the analysis. The probability of Rough-skinned newt and Red-legged frog presence was positively correlated with increases in canopy density, canopy cover, understory cover, ground cover, pH, distance to nearest agricultural field, and depth (Table 2.7). The probability of Pacific treefrog presence was positively correlated with increases in depth, and human disturbance (Table 2.7).

Discussion

Amphibian community composition and distribution in an agricultural landscape were analyzed at both the landscape (sub-basin conservation) and local (habitat characteristics) levels. Species diversity in the Calapooia watershed was positively correlated with field conservation efforts (Figure 2.6). Additionally, amphibian species abundance showed positive correlations with many local habitat variables in both stream and pond sites located within the Calapooia watershed (Table 2.3; Table 2.4). These results reveal that amphibian assemblage composition and diversity are related to many habitat characteristics specific to the aquatic environment and amphibians showed a positive association with residue management practices in the Calapooia watershed.

Differences in habitat type were observed between ponds and streams in the Calapooia watershed. Pond sites had well-established vegetation and shoreline habitat and were typically further away from agricultural fields. Stream sites had considerable variation in available riparian habitat, ranging from well-established riparian corridors with complex channels to simple ditches in the middle of an agricultural field. This variation in available habitat altered amphibian communities as a function of species specific life history traits. Some amphibians such as Pacific treefrogs and Long-toed salamanders were found across a variety of habitats in the Calapooia watershed. Rough-skinned newts and Red-legged frogs, however, are typically found in established riparian or densely vegetated areas and are often associated with ponds and wetland habitats (Twitty 1942, Hayes 2002).

Landscape Scale: Conservation efforts implemented by farmers

Amphibian species diversity was calculated using Simpson's Index and then compared to a landscape level conservation gradient that was created by the CEAP work group. The results showed a positive linear relationship between the field level conservation practices and the Simpsons Index calculated for each site (Figure 2.6; $p = 0.018$). However, when analyzed separately, no significant linear relationship between SI and percent conservation at pond sites was detected (Figure 2.8; $p = 0.848$), whereas a positive linear relationship was detected at stream sites (Figure 2.7; $p = 0.045$).

Pond site amphibian species diversity was high across all sites, which could explain why there was no relationship between conservation practices and species diversity. Only one pond site, RRA (Table 2.1), had less than 4 species present: this site was missing Red-legged frogs. Pond sites were also typically further away from agricultural fields (average distance = 20.56 meters) relative to streams (average distance = 8.65 meters) and thus may not be impacted by conservation practices at the field level. Stream sites, however, were typically much closer to agricultural practices and may be influenced by field level conservation practices due to their proximity. Vegetative cover may provide habitat for amphibians as well as buffer them from other natural stressors, such as ultraviolet radiation, predation, and desiccation. Full field tillage, which was represented as bare/disturbed ground, would have negative impacts on any burrowed amphibians near the channels on the field edges, and would eliminate potential cover and habitat by removing all grasses and vegetation in the area.

Local Scale: Habitat Relationships

Overall patterns in amphibian community composition in local pond and stream habitats conformed to common habitat associations of the local amphibian species. Long-toed salamanders and Pacific treefrogs were associated with habitats that differed from the types of habitats that Rough-skinned newts and Red-legged frogs were associated with. Rough-skinned newts and Red-legged frogs had strong correlations to heavily vegetated habitats suggesting that they may be associated with relatively undisturbed areas, usually further away from agricultural practices (Figure 2.9; Figure 2.10).

Long-toed salamanders

Long-toed salamanders did not show a strong relationship with any of the local pond habitat variables, and therefore we were unable to indentify habitat characteristics related to their abundance. In stream sites, Long-toed salamander presence was positively correlated only with an increase in channel cover. Increased channel cover may provide habitat and egg deposition sites for Long-toed salamander (Hamilton 1998). It is likely that Long-toed salamanders have high enough densities that their patterns of presence and abundance were not clear from the data we collected. In total, we sampled over 600 long-toed salamanders across 10 pond sites relative to 122 Red-legged frog individuals, 210 Pacific treefrog individuals, and 267 Rough-skinned newt individuals. Similarly, Long-toed salamanders were present at

14 out of 15 stream sites. This abundance suggests that Long-toed salamanders are capable of successfully occupying a variety of habitats.

Long-toed salamander abundance may be explained in the context of the species life history. They are opportunistic breeders and gape limited carnivores, and have been known to inhabit a variety of habitats ranging from heavily disturbed areas to pristine systems (Hamilton 1998, Petranka 1998). Long-toed salamanders are also the first Willamette Valley amphibian species to breed, migrate to sites, and lay eggs (Leonard 1993). Early breeding may allow Long-toed salamanders to attain a large enough body size to forage on the other amphibian species larvae and embryos (Walls 1993), in addition to micro invertebrates and crustaceans. Emerging from aquatic habitats early in the spring may also allow Long-toed salamanders to avoid additional agricultural stressors, such as spring pesticide spraying and field harvesting.

Rough-skinned newts & Red-legged frogs

Rough-skinned newt and Red-legged frog abundance at pond sites were positively correlated with increases in pH and bank width. Similarities between these species are not surprising as they share many of the same life history characteristics. Both Rough-skinned newts and Red-legged frogs prefer to breed in lentic habitats or slow moving streams and wetlands. Both species lay eggs on submerged vegetation, and their larvae both utilize submerged rocks and dense vegetation for cover. As both species are closely tied to water, correlations to pH may be an important factor for determining potential habitat suitability and restoration (Twitty 1942, Hayes 2002).

Rough-skinned newt and Red-legged frog habitat associations in local streams showed different patterns from the pond habitat associations. At stream sites predicted species presence for both newts and frogs were positively correlated to increased water depth, canopy density, canopy cover, understory cover, ground cover, pH, and distance to nearest agricultural field. These results suggest that both species are associated with habitats that have established riparian corridors that are not adjacent to agricultural fields. Our data reflects the habitat associations of adults from both species, which prefer to remain in riparian areas year round, which often act as a buffer between streams and agricultural fields (Hayes 2003).

Red-legged frogs and Rough-skinned newts seemed to associate with similar habitat type in both ponds and streams. The ponds in the Calapooia watershed were typically heavily vegetated and usually far away from agricultural practices. Stream sites were much more variable in available riparian and channel habitat and often times occurred in the middle of an agricultural field. Because of this range in available local habitats, we were able to see associations for established riparian zones emerge for Rough-skinned newts and Red-legged frogs. Red-legged frogs, listed by the IUCN as near threatened, are a species of concern in Oregon and our data supporting their association with established riparian habitats may enable appropriate conservation efforts to be implemented (Oregon Department of Fish and Wildlife 2005).

Pacific treefrogs

Pacific treefrog abundance in local pond habitats was positively correlated with increased depth, channel cover, bank width, understory cover, ground cover, and distance to nearest agricultural field. Pacific treefrogs were negatively correlated with canopy density and canopy cover and total nitrogen. This suggests that Pacific treefrogs are associated with well-established channel, understory and ground cover and were associated with pond habitats that were further away from agricultural practices with lower nitrogen concentrations. Previous studies have shown that Pacific treefrog larvae are highly sensitive to nitrates and nitrites, which could explain their negative correlation to increased nitrogen concentrations in pond sites (Marco 1999, Schuytema 1999). Pacific treefrog abundance being positively correlated with understory and ground cover but not canopy cover and density shows associations for increased vegetation complexity near the ground. High vegetation complexity at the ground level could provide increased refuge accessibility and foraging opportunities.

Interestingly, Pacific treefrog predicted presence at stream sites was negatively correlated with channel cover and positively correlated with increased human disturbance and depth. Pacific treefrog positive correlation with increased human disturbance could indicate their ability to adapt and survive as local habitats change. Amphibian species that are associated with established riparian habitat, such as Red-legged frogs and Rough-skinned newts, may not be capable of surviving in sites occupied by Pacific treefrogs. This could be an indication of a reduction in the home range of other local amphibian species, as sites with lower channel complexity and increased human disturbance may result in increased competition for food and other resources.

Conclusions

The conservation efforts in this study represent a small fraction of the available options for farmers and ranchers. Many potential conservation options are available to land owners; programs such as CEAP were designed to assess the benefits and costs associated with implementing these conservation efforts. The CEAP workgroup collected water quality information, habitat information and detailed species information from a variety of taxa, including birds, fish, amphibians, and invertebrates. This information was used to establish broad landscape level changes that conservation practices may have on an agricultural system. Additionally, the CEAP program looked to provide farmers with an economic and ecological model to predict the costs and benefits of implementing conservation efforts on their land (Mueller-Warrant in press. *b*).

Understanding patterns of amphibian species presence and community composition at multiple spatial scales is critical to the design and implementation of conservation programs. If scientists can attempt to give economic value to conservation efforts and can demonstrate economic viability for the landowner to implement conservation strategies, then these programs will likely succeed. Successfully implementing conservation practices on agricultural lands is an important step in conserving biodiversity worldwide. Much of the agricultural land in the United States is privately owned, leaving conservation efforts up to the farmer. Establishing that conservation efforts can be cost effective and ecologically effective is needed to insure successful implementation on private lands. More research is needed to monitor the effectiveness of these conservation programs. Very little information is available on the hydrological effects, either positive or negative, of field level remediation. Additionally, more information is needed at the local habitat and species level for these systems so we can better design and implement cost effective and ecologically effective conservation practices (Khanna 2009).

Table 2.1: Stream and pond sites ranked by percent conservation in each sub-basin. Percent conservation was defined by total land area in the sub-basin in a conservation practice divided by the total land area of the sub-basin. Conservation consisted of residue management practices as defined by table 2.2

Stream Sites	Percent Conservation	Pond Sites	Percent Conservation
67	65.57	Calapooia Acres North	78.98
49	65.41	Calapooia Acres South	78.98
85	63.16	McLagan	64.33
108 C	59.23	Ash Swale	60.24
111	57.93	83 F	60.24
92	56.13	13 T	56.22
14 A	55.78	RRC	49.68
110	55.04	RRB	49.68
98 A	54.94	RRA	49.68
83	54.49	107 T	42.14
107	42.51		
108 B	39.83		
3	35.45		
98 B	30.56		
11	22.67		

Table 2.2: Field level conservation practices as defined by Mueller-Warrant (in press (b)).

Conservation Practices	Non-conservation Practices
Full straw Italian ryegrass	Bare / Disturbed ground – Other crops
Spring planting new grass seed	Bare / Disturbed ground – Italian ryegrass
Established perennial ryegrass	Bare / Disturbed ground – New perennial ryegrass
Established orchardgrass	Bare / Disturbed ground – Tall fescue
Established tall fescue	Bare / Disturbed ground – New clover
Mixed grass pasture	Wheat or other cereals
Established clover	Meadowfoam
Established Mint	
Mixed grass haycrop	

Table 2.3: Physical habitat variables measured at each site following protocols outlined by Kaufmann 1999.

Category	Habitat Variable
General	Depth
	Substrate
	Embeded %
	Bank Angle
	Wetted Width
	Bankfull width
	Bankfull height
	Incised height
Macrophytes	Macrophytes
	Filamentous algae cover
Channel Cover	Woody debris greater than 0.3 m
	Brush / woody debris less than 0.3m
	Live trees or roots
	Overhanging vegetation
	Undercut banks
	Boulders
	Artificial structures
Canopy Cover	Canopy vegetation type
	Big trees greater than 0.3 m
	Small trees less than 0.3 m
Understory Cover	Understory vegetation type
	Woody shrubs and saplings
	Non-woody herbs, grasses, forbs
	Woody shrubs and saplings
Ground Cover	Non-woody herbs, grasses, forbs
	Barren bare dirt, duff
Human Disturbance	Wall / dike / revetment / dam / riprap
	Buildings
	Pavement / cleared lot
	Road / railroad
	Pipes: inlet / outlet
	Landfill / trash
	Park / lawn

Table 2.4: Micro- and macro-habitat variables condensed from the list of measured variables presented in table 2.3.

Category	Habitat Variable
Channel Habitat	Depth
	Macrophytes
	Channel cover
	Bank width
Vegetated Habitat	Canopy density
	Canopy cover
	Understory cover
	Ground cover
Anthropogenic	Human disturbance
	Distance to Ag. field
Water Quality	pH
	Total nitrogen

Table 2.5: Total amphibian numbers for ponds and streams

Species	Number of Individuals Pond Sites	Stream Sites
Ambystoma macrodactylum (Long-toed salamander)	602	335
Rana aurora (Red-legged frog)	122	17
Taricha granulosa (Rough-skinned newt)	267	149
Pseudacris regilla (Pacific treefrog)	210	274
Rana catesbeiana (American Bullfrog)	5	21

Table 2.6: Pond Nonmetric Multidimensional Scaling Ordination results showing r (direction and magnitude) between amphibian species relative abundance and habitat variables.

Category	Ordination Axis Class	Axis 1 r	Axis 2 r
Relative Abundance	Long-toed Salamander	-0.902	0.104
	Rough-skinned Newt	0.898	-0.239
	Red-legged Frog	0.750	-0.551
	Pacific Treefrog	0.127	-0.929
Channel Habitat	Depth	0.396	-0.345
	Macrophytes	0.073	0.354
	Channel cover	-0.205	-0.480
	Bank width	0.626	-0.301
Riparian Habitat	Canopy density	-0.350	0.688
	Canopy cover	-0.358	0.511
	Understory cover	0.435	-0.537
	Ground cover	-0.076	-0.847
Anthropogenic	Human disturbance	-0.268	0.191
	Distance to Ag. field	0.498	-0.716
Water Quality	pH	0.502	-0.182
	Total nitrogen	0.405	0.504

Table 2.7: Stream Nonmetric Multidimensional Scaling Ordination results showing r (direction and magnitude) between amphibian predicted species presence and habitat variables.

Category	Ordination Axis Class	Axis 1 r	Axis 2 r
Relative Abundance	Long-toed Salamander	-0.813	0.168
	Rough-skinned Newt	0.498	0.980
	Red-legged Frog	0.223	0.958
	Pacific Treefrog	0.951	0.345
Channel Habitat	Depth	0.392	0.429
	Macrophytes	0.093	-0.258
	Channel cover	-0.253	-0.163
	Bank width	0.081	0.166
Riparian Habitat	Canopy density	0.017	0.588
	Canopy cover	0.036	0.594
	Understory cover	0.052	0.535
	Ground cover	0.028	0.439
Anthropogenic	Human disturbance	0.365	-0.203
	Distance to Ag. field	-0.020	0.455
Water Quality	pH	0.053	0.340
	Total nitrogen	-0.098	0.291

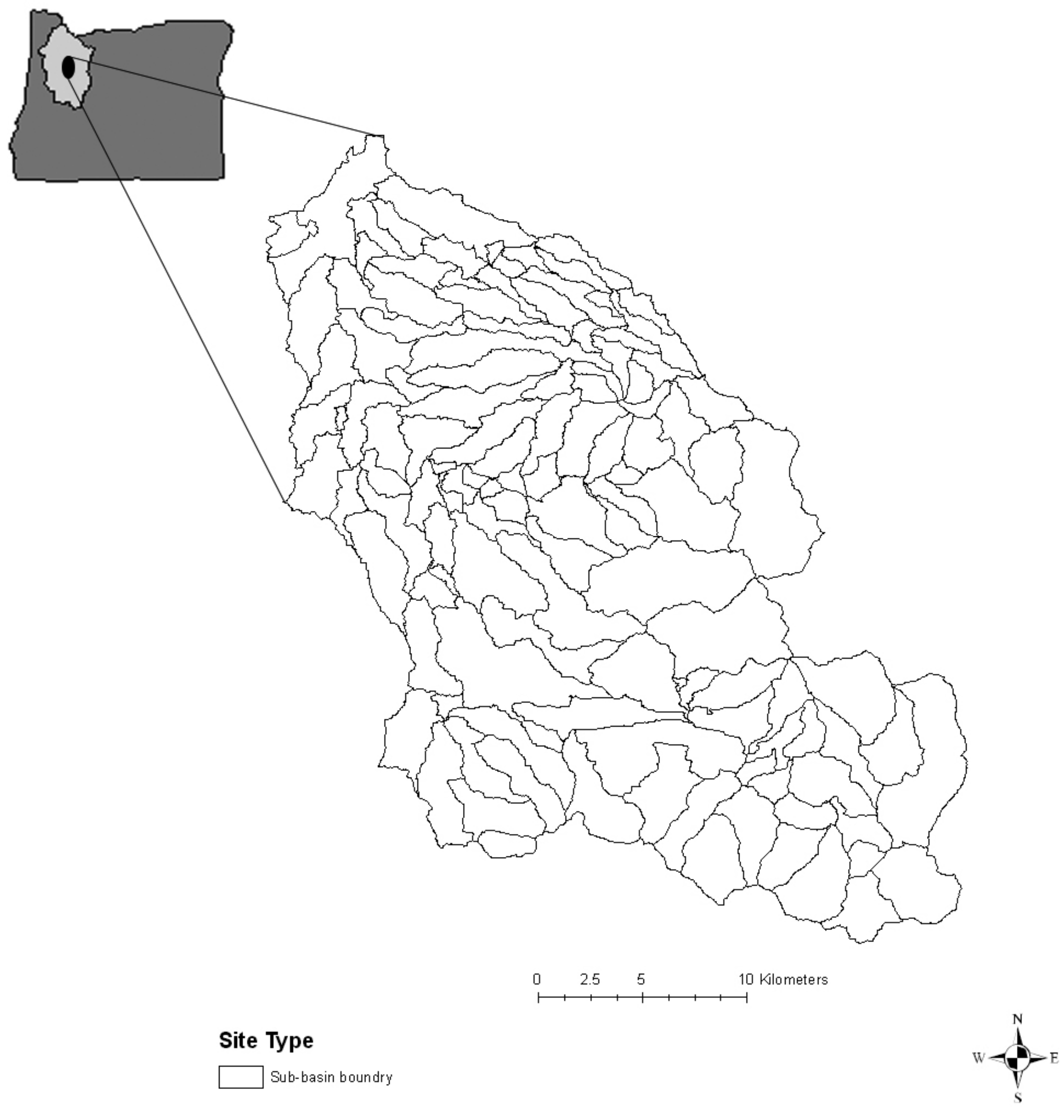

Figure 2.1. Calapooia watershed, located in Oregon's Willamette Valley. Sub-basins showed here were created by George Muller-Warrant (National Forage Seed Production Center) using DEM's. Resolution for these sub-basins approximate HUC6 sub-basins.

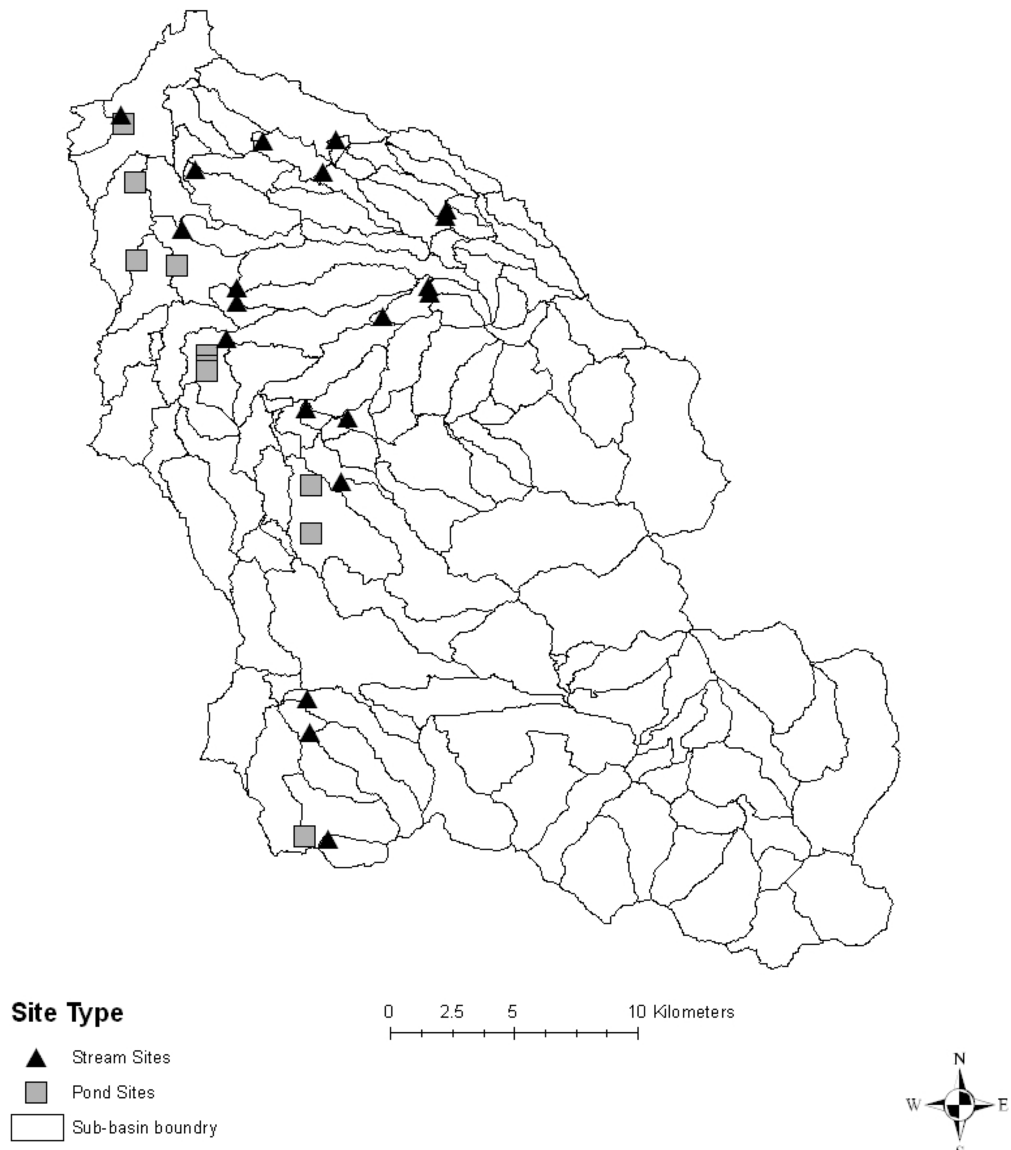

Figure 2.2. Calapooia watershed map created using ArcGIS version 9.2. Map shows location of pond (squares) and stream (triangle) sites inside the watershed. Boundary lines represent sub-basin divisions in the watershed. Sub-basin divisions were created created by George Muller-Warrant (National Forage Seed Production Center) using DEM's and are at a scale approximating HUC-6.

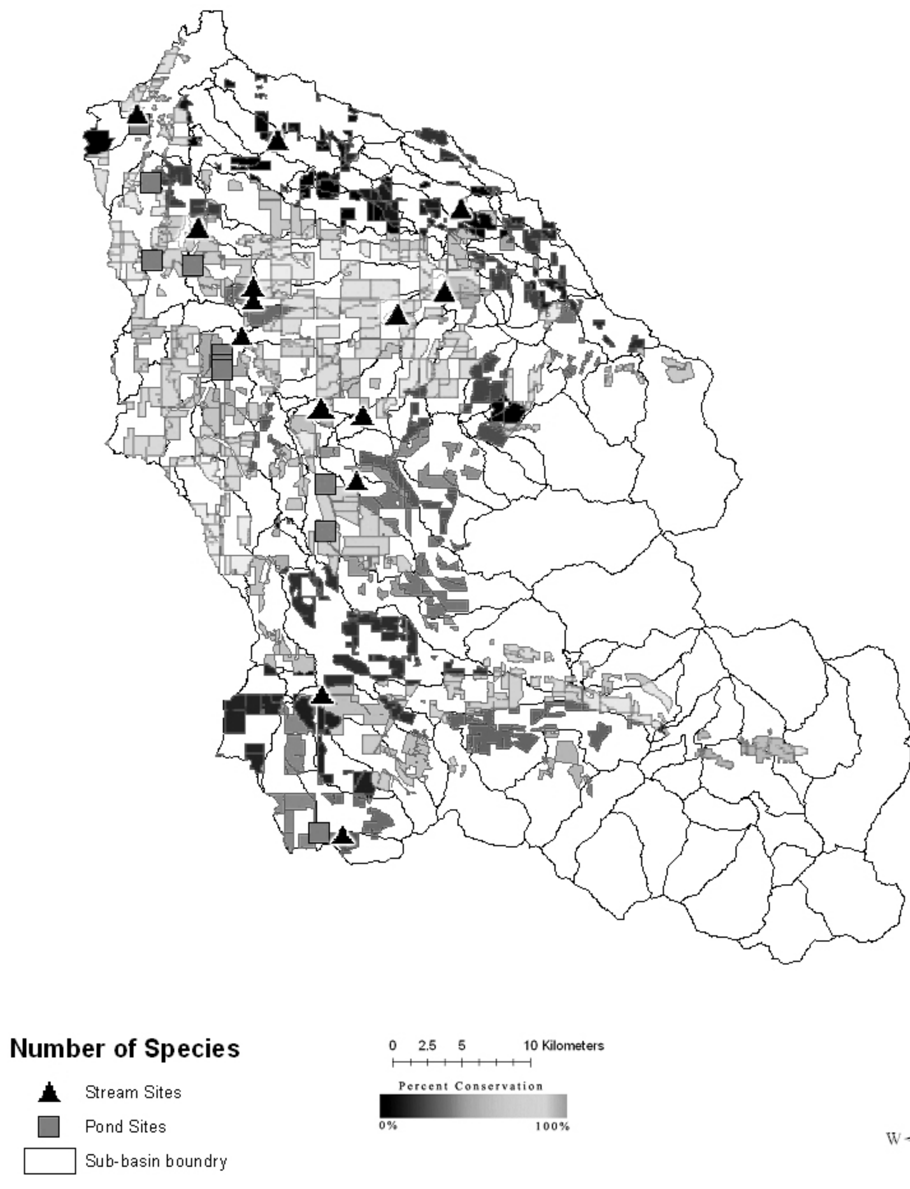

Figure 2.3. Calapooia watershed map created using ArcGIS version 9.2. Map shows location of pond (squares) and stream (triangle) sites and conservation gradient inside the watershed, darker areas represent areas of lower conservation. Boundary lines represent sub-basin divisions in the watershed. Sub-basin divisions were created created by George Muller-Warrant (National Forage Seed Production Center) using DEM's provided by the USDA and are at a scale approximating HUC-6.

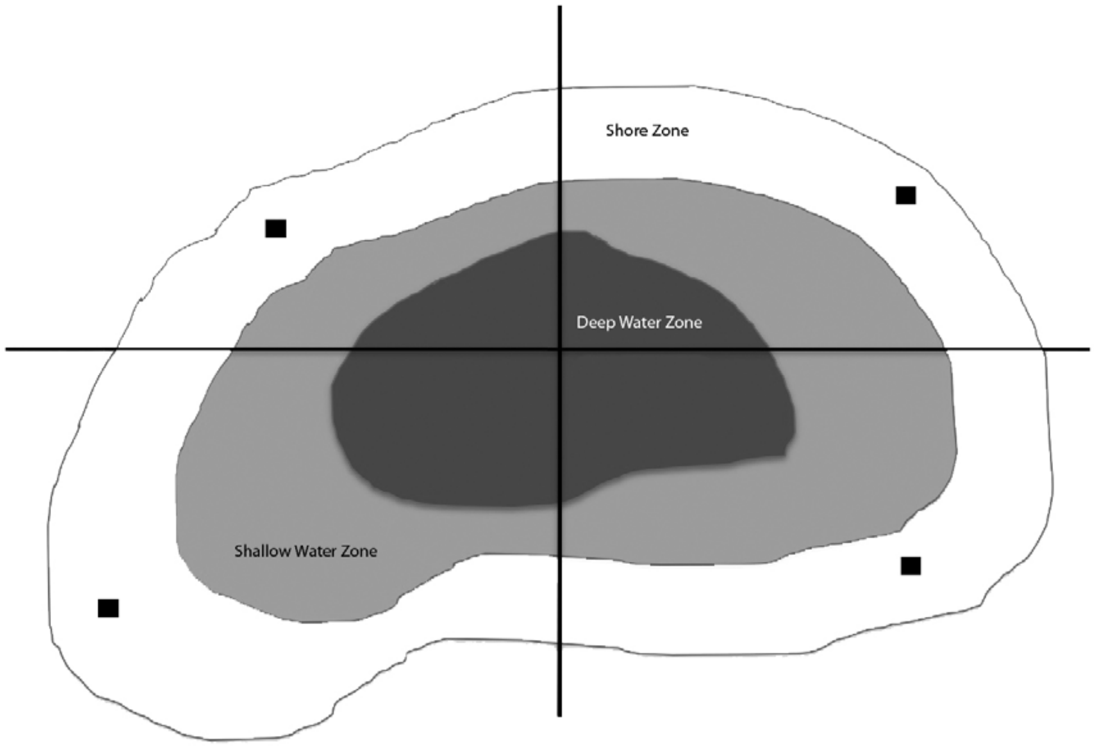

Figure 2.4. Pond sampling diagram. Three sample zones were used, shore zone, shallow water zone, and deep water zone. The pond was divided into quadrants for sampling purposes. The black squares represent coverboards that were placed at each of the quadrants to serve as potential refuge for adult amphibians.

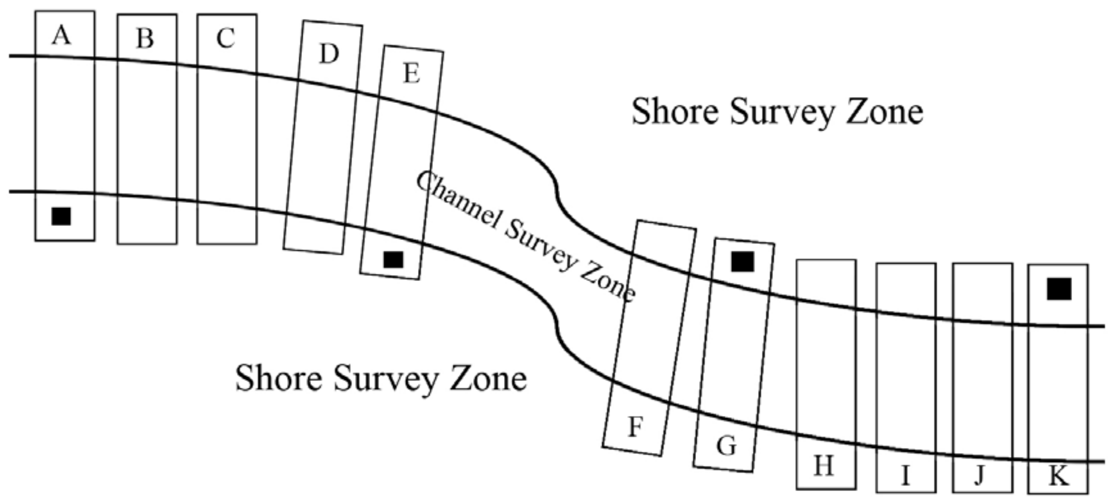

Figure 2.5. Stream sampling diagram. Stream sites had 11 (A-K) 10-meter transects representing 110 meters along the stream. Two sampling zones were surveyed including Shore survey zones and channel survey zones. Black squares represent coverboards placed randomly at four of the 11 transects.

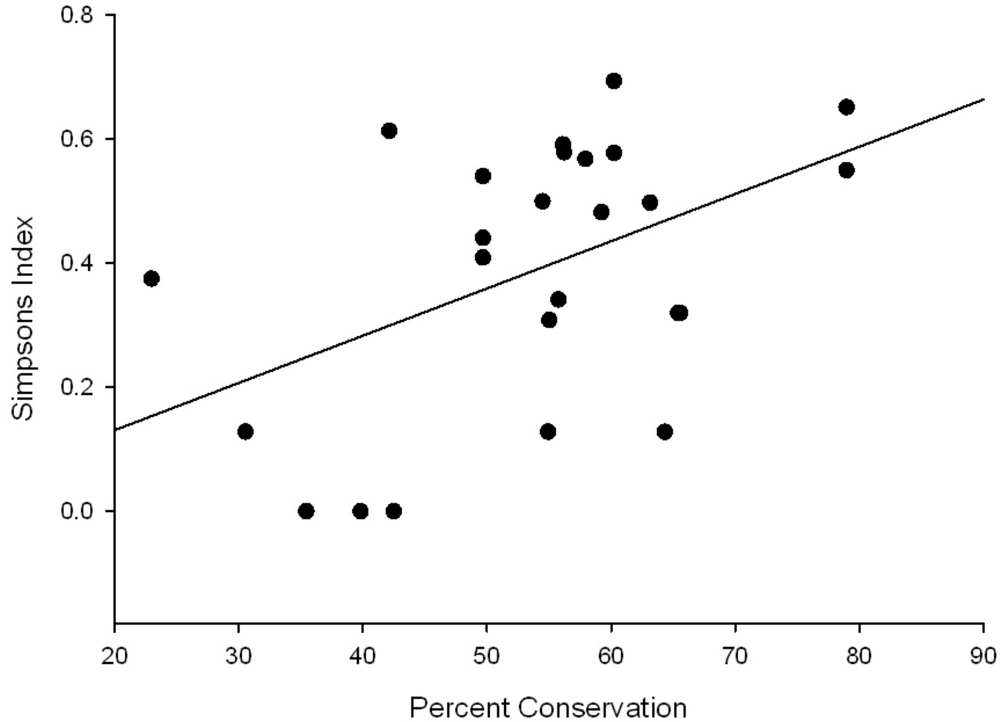

Figure 2.6. Linear regression of Simpsons species diversity index versus percent conservation for all sites ($p = 0.018$). Amphibian data was collected during the 2007-2008 field season and the Simpsons Index was calculated using relative abundance from the survey data. Percent conservation was defined as total area in the sub-basin under a field level conservation practice divided by the total area of the sub-basin.

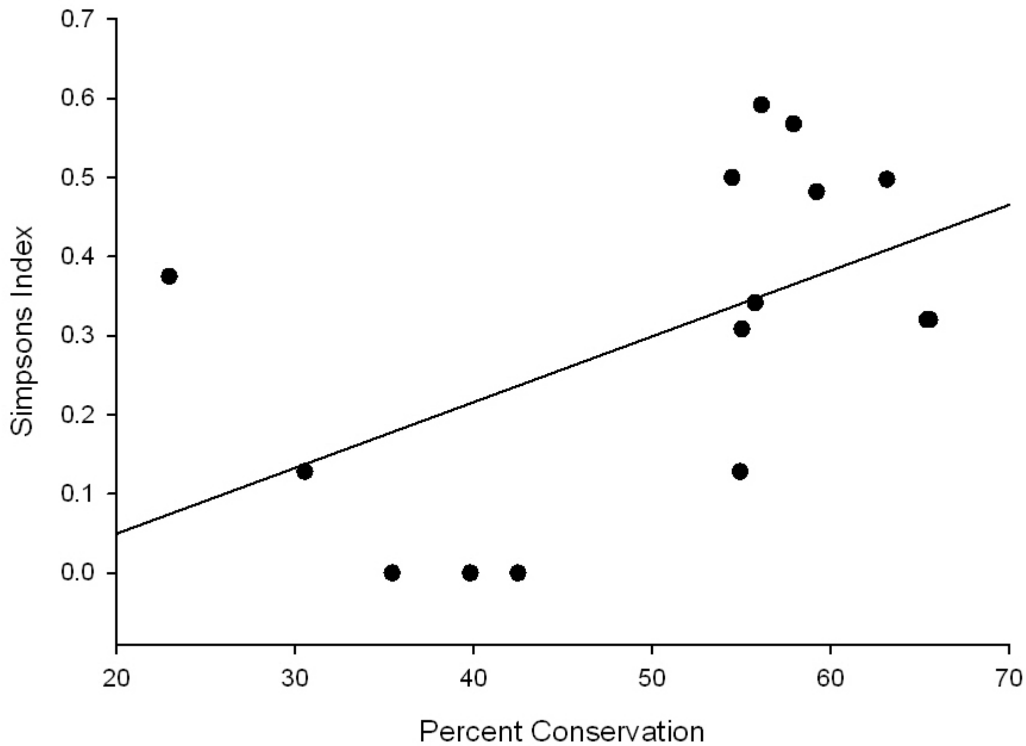

Figure 2.7. Linear regression of stream sites Simpsons species diversity index versus percent conservation, (p = 0.045). Amphibian data was collected during the 2007-2008 field season and the Simpsons Index was calculated using relative abundance from the stream sites. Percent conservation was defined as total area in the sub-basin under a field level conservation practice divided by the total area of the sub-basin.

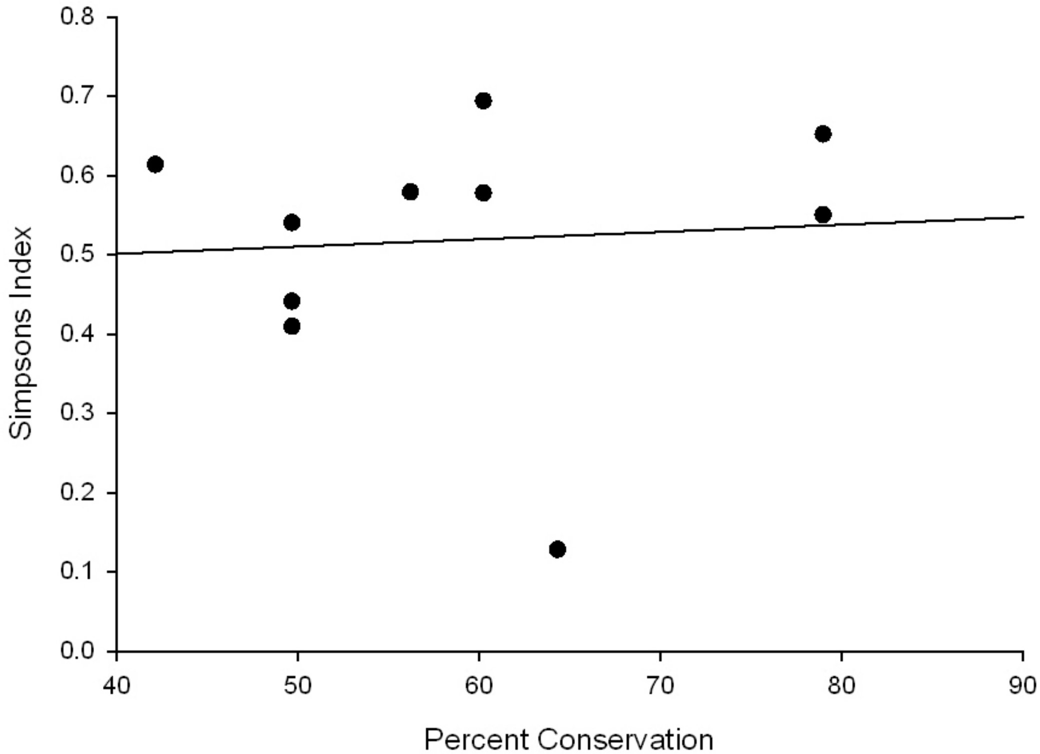

Figure 2.8. Linear regression of pond sites Simpsons species diversity index versus percent conservation (p = 0.848). Amphibian data was collected during the 2007-2008 field season and the Simpsons Index was calculated using relative abundance from the pond data. Percent conservation was defined as total area in the sub-basin under a field level conservation practice divided by the total area of the sub-basin.

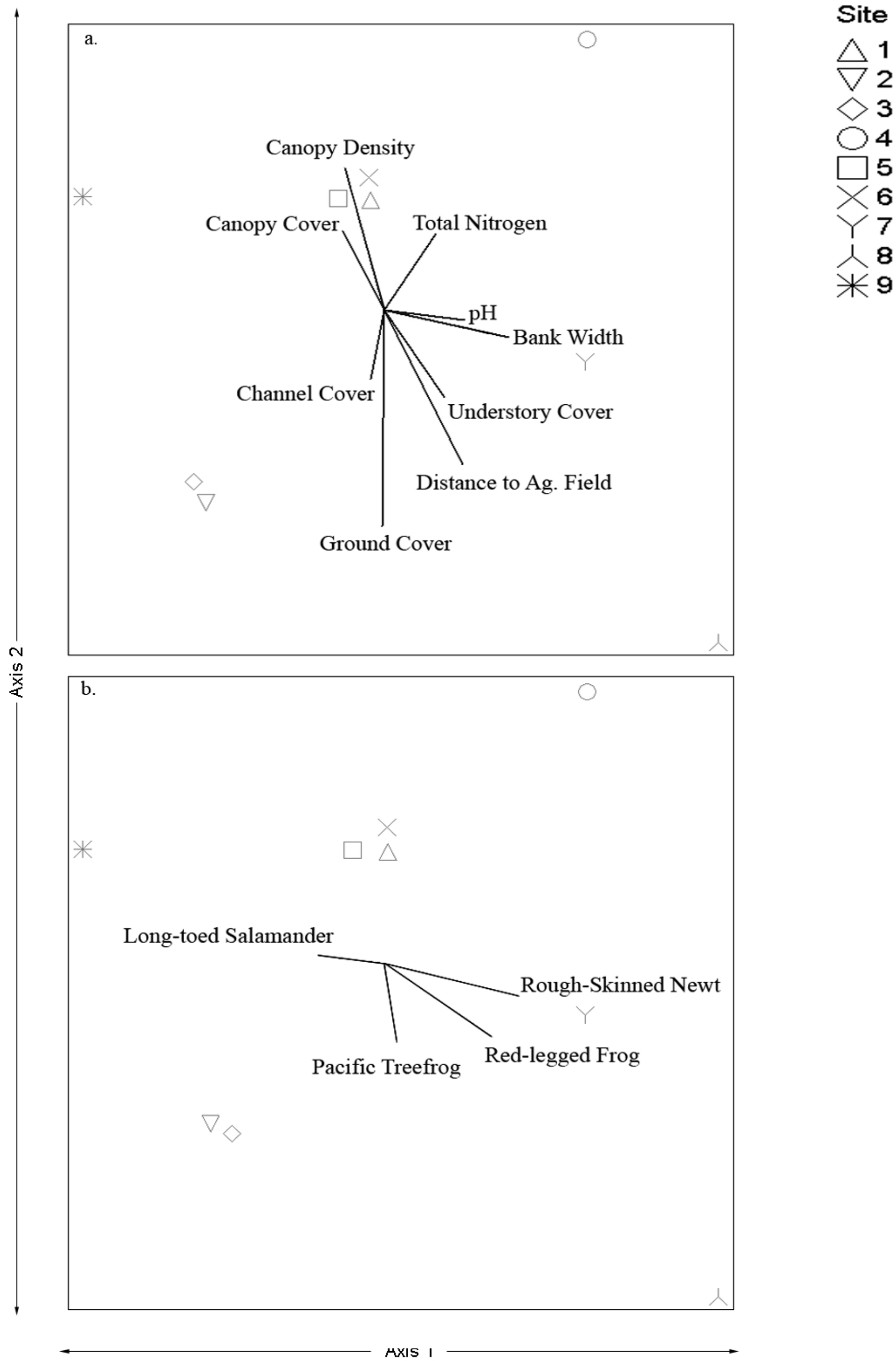

Figure 2.9. Nonmetric Multidimensional Scaling ordination of 10 pond sites in the Calapooia watershed in Oregon's Willamette Valley. (a) Vectors indicate the strength and direction of correlations between environmental variables and amphibian assemblage composition. (b) Species vectors and correlations with site assemblage. Symbols represent amphibian assemblage based on relative abundance collected at pond sites during the 2007-2008 sampling season.

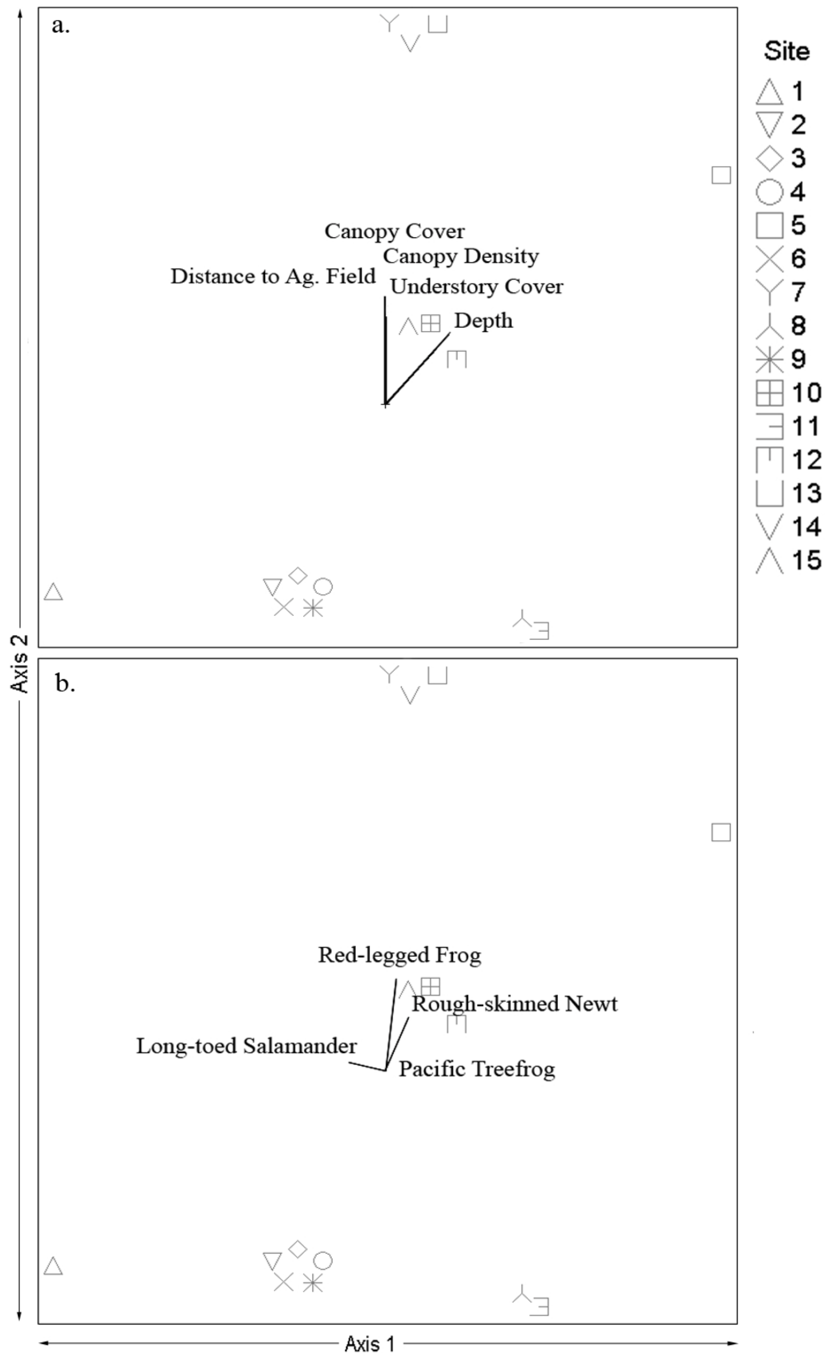

Figure 2.10. Nonmetric Multidimensional Scaling ordination of 10 pond sites in the Calapooia watershed in Oregon's Willamette Valley. (a) Vectors indicate the strength and direction of correlations between environmental variables and amphibian assemblage composition. (b) Species vectors and correlations with site assemblages. Symbols represent amphibian assemblages based on relative abundance collected at pond sites during the 2007-2008 sampling season.

Chapter 3

Pesticide and Fertilizer Impacts on Amphibians: A Synthesis Though Meta-analysis

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Introduction

Anthropogenic impacts on natural systems are of growing concern as human populations expand and global biological diversity declines (Donald 2006, Benton 2007). 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 2005*b*). Pesticides and fertilizers are common additions to many managed systems given their general availability, economic value, and benefit to agricultural yield (Relyea *et al.* 2005, Boone 2008, USDA 2006). At least 600 million pounds of pesticides and 45 billion pounds of fertilizers are used every year in the United States alone (Gail 2000, USDA 2006, EPA 2008.)

Presence of pesticides and fertilizers has been detected in a variety of habitats worldwide. For example, two pesticides, endosulfan and lindane, have been detected in Arctic ice core samples (Carvalho 2009). These chemicals have the potential to impact the ecology of natural systems, including community composition, trophic cascades, and many other biological interactions (Boone 2005, Relyea *et al.* 2005). Changes at the community and ecosystem level can trickle down to the species or individual level, resulting in changes in reproductive ability, behavior, growth and susceptibility to disease (Shelley 2009). Potential cumulative changes pose unique challenges for ecologists when looking at the impacts of these contaminants on natural ecosystems and biological communities.

Freshwater ecosystems have a high risk of chemical exposure from local, regional, and potentially global agricultural practices. Multiple indirect pathways exist for exposure of freshwater systems, such as runoff, leaching, and sediment deposition (Boone 2005, Relyea *et al.* 2005). In addition, direct application of pesticides to freshwater systems is a common practice to control for various aquatic pests (e.g., mosquitoes) and can have negative impacts on many non-target organisms. Additionally, unintentional run-off after rain events are also mechanisms by which freshwater systems can experience high pulses in pesticide exposure rates. Contamination in freshwater habitats has been implicated in a number of species declines, including wild salmon stocks via changes in fish immune systems (Shelly 2009) and amphibians (Davidson 2001, Relyea 2005).

Impacts on amphibians are of particular interest because amphibian population declines have been reported worldwide (Blaustein 1994, Alford 1999, Stuart *et al.* 2004, Mendelson *et al.* 2006). Recent estimates suggest that approximately 43% of amphibian species are currently experiencing population declines, and approximately 32% of amphibian species are considered threatened (Stuart *et al*. 2004). Additionally many amphibian species are considered data deficient, meaning we can’t accurately assess their conservation status because we lack information (Stuart *et al.* 2004). Investigating stressors such as pesticides and fertilizers may help fill in the gaps in the data and could 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 2003, Mills 2004, Relyea 2005). 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, where as Release®, a chloropyridinyl pesticide, did not result in decreased survival in the same species (Sparling 1997, Wojtaszek 2005). In addition carbaryl, a carbamate pesticide, negatively impacted survival of the spotted salamander (*Ambystoma maculatum*) but did not impact the survival of the leopard frog (*Rana sphenocephala*) (Boone 2003*b*, Boone 2004).

Sublethal impacts can include longer larval periods, smaller mass at metamorphosis, and increased susceptibility to predation by decreased swim speed and endurance (Boone 2006, Mills 2004). Additionally, impacts on growth can be attributed to food web impacts from these chemicals. Herbicides may decrease primary production levels causing increased competition which can lead to lowered growth rates (Boone 2003, Relyea 2006, Relyea 2008).

We are in need of a more comprehensive look at the effects of pesticides and fertilizers on amphibians. Most studies focus on one chemical or one 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 worldwide, (Gail 2000) a comprehensive approach is needed to quantify the effects of these chemicals on amphibians.

Here, we have used a meta-analysis technique to synthesize published studies on lethal and sublethal impacts of pesticides and fertilizers on amphibian species worldwide. Meta-analysis techniques are designed to aid in the summary and interpretation of the findings from a collection of primary data (Gurevitch & Hedges

1999). These techniques have been used to explore the effects of UVB radiation and other environmental stressors on aquatic organisms, including amphibians (e.g. Bancroft *et al.* 2007, Bancroft *et al.* 2008). Meta-analytic techniques are the most statistically rigorous method for summarizing independent data (Gurevitch 1992, Bancroft *et al.* 2008) 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. Survival was selected as a response variable because the majority of studies have quantified amphibian mortality rate to various levels of pesticide and fertilizer exposure. Growth was chosen because it generally has been used to quantify sublethal effects and has been often measured in conjunction with survival. Other sublethal response variables were also quantified in these types of studies (i.e., reproduction and life stage duration) but growth is commonly measured for many species across multiple life stages (i.e., embryonic, larval, juvenile; Bancroft et al. 2007). 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 2008) and this allowed for a more generalized representation of the chemicals used in previous studies (Table 3.1). 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.

Methods

Data selection

We used five databases to identify studies for analysis (Aquatic Sciences & Fisheries Abstract, BIOSIS, Environmental Sciences and Pollution Management, Web of Science, and Wildlife & 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 and selected survival and growth as response variables to explore both lethal and sublethal effects. 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 a measure of error (i.e., standard deviation, standard error, or 95% confidence intervals) and sample size for both the experimental group and control group, and 3) chemical dosage levels must be ecologically relevant (Table 3.1). Any data points within an article that met these criteria were considered for inclusion.

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 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.

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 & Gurevitch 1999). The response ratio is an estimate of the ratio of the population means defined by the experimental group divided by the control group. Taking the natural log of this ratio makes the metric more linear and helps to normalize skewed data (Hedges & Gurevitch 1999). Also, while the ratio is affected more acutely by changes in the denominator, the log ratio is affected equally by changes in either the numerator or denominator (Hedges & Gurevitch 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 for all statistical procedures. Normality assumptions were checked via normal QQ plots using MetaWin Version 2.0.

Full Models

Response variables were selected with the intention of quantifying both lethal and sublethal effects. To accomplish this, we ran two separate analyses quantifying survival and growth effects. Random effects models were then used to calculate the grand mean effect size for each analysis. We chose not to use a fixed effects model, which is more typical in meta-analyses, because we expected the true effect size to vary among studies due to the variety of chemicals used (Gurevitch & Hedges 1999). Random effects models use the pooled standard deviation to estimate the distribution of effect sizes within the population. Therefore, using a random effects model allows effect size estimates to vary not only due to sampling error, but due to real biological or environmental differences between organisms and studies (Bancroft 2007). The output of each statistical test consisted of the grand mean effect size for the analysis with an accompanying biased-corrected bootstrapped 95% confidence interval (CI) (Adams *et al.* 1997). The mean effect is significantly different from zero if the CIs do not overlap with zero.

Chemical Class Analysis

In addition to examining effect size on survival and growth, we examined whether or not effect size changed among specific classes of chemicals. Chemical classes represent groups of chemicals that share similar structure and behave both environmentally and physiologically in similar ways (Kegley 2008). The mode of action of each chemical varies by the type of chemical class. For example, carbamates and organophosphates are cholinesterase inhibitors, pyrethrins are ion channel manipulators, and organochlorines are endocrine and chloride channel disrupters (Kegley 2008). We compared the mean effect size between chemical classes across both survival and growth studies. A mean effect size and bias-corrected bootstrapped 95% CI were calculated for each group in the exploratory analyses and groups with fewer than 4 comparisons were removed from the analyses.

Publication Bias

The potential effect of publication bias, commonly referred to as the File Drawer Problem (Rosenthal 1979), was tested using Rosenberg's failsafe number (Rosenberg 2005). The failsafe number is a quantitative representation of the importance of publication bias to the outcome of our analyses. Rosenberg's failsafe number is the number of studies with a non-significant effect size necessary to change the results of an analysis from significant to non-significant. A robust failsafe number is considered anything above (5* n +10) where n equals the number of studies (Rosenberg 2005). Total number of studies in the survival and growth analyses was 66 and 45 respectively. Rosenberg's failsafe number was large for both the survival (20,941) and growth (401) analyses, which indicates publication bias is not influencing the outcome of our analyses.

Results

Pesticides and fertilizers were predicted to have an overall negative impact on survival in amphibians. We found that exposure to pesticides and nitrogen-based fertilizers resulted in an 18.8% (95% CI: 11.6% to 27.2%) decrease in survival compared with controls (Figure 3.1). Our results indicate that there are four chemical classes with significant negative effects on survival. Exposure to chemicals in the phosphonoglycine (with POEA surfactant) class decreased survival by 61.3% (95% CI: 20.5% to 124.5 %), while exposure to organophosphate, triazine, and inorganic fertilizers reduced survival by 17.9% (95% CI: 5% to 35.6%), 18.1% (95% CI: 2.4% to 47.9%), and 23.7% (95% CI: 10.2% to 41.8%), respectively (Figure 3.2).

We found an overall 7.7% (95% CI: 3.6% to 11.9%) decrease in amphibian growth compared to controls (Figure 3.1). In particular, triazines reduced growth by 5.2 (95% CI: .3% to 10.7%) while organophosphorus reduced growth by 19.0% (95% CI: 8.6% to 28.7%) (Figure 3.3).

Differences were observed between amphibian families in both the survival and growth analyses. For survival we found that pesticides and fertilizers had no effect on the Myobatrachidae and Pipdae families, while a negative effect was observed in the: Ambystomatidae, Bufonidae, Hylidae, and Ranidae families (Figure 3.4). The effect of pesticides and fertilizers on growth was negative for the Ambystomatidae, Bufonidae, and Ranidae families, while the Hylidae familiy showed no effect on growth (Figure 3.5).

Sensitivity Analysis

We conducted a sensitivity analysis to test whether extreme values (i.e., outliers) were significantly impacting the results of our study. We removed all observations that were more than two standard deviations from the mean and ran the analyses again. The results for both survival and growth were qualitatively the same without these studies included in the analysis, suggesting that a few extreme studies are not driving the results we observed.

Over one-third (34%) of the comparisons in the survival analysis and 23% of the comparisons in the growth analysis were from the work of Rick Relyea and colleagues. To explore the possibility of bias, we removed all comparisons generated by these researchers and reran the analyses. Our model for survival was qualitatively the same without studies conducted by this group. Without the Relyea laboratory comparisons in the analysis, pesticides and fertilizers reduced survival by 18.5% (95% confidence interval 10.3% to 28.4%), compared to 18.8% (95% CI 11.6% to 27.2%) with all studies included in the analysis. However, removing the Relyea-generated data from the growth analysis produced results that differed from the full model. Without the Relyea-generated data, growth was reduced by 3% (95% CI -.01% to 7%), compared to 7.7% (95% confidence interval 3.6% to 11.9%) with all studies included in the analysis. As the confidence intervals overlapped with zero, there was no significant effect of pesticides and fertilizers on growth without the Relyea-generated data is indicated. However, the exclusion of the Relyea laboratory data removed 30% of the observations from the carbamate chemical class, 50% of the observations from the organophosphate chemical class, and 86% of the observations from the phosphonoglycine class. The removal of more than 50% of the data points from two chemical classes is likely confounding the results of this analysis. While it is possible that the Relyea-generated data is influencing the original analysis, the loss of these data points equates to a substantial loss of the total data available, thus an analysis run without those observations should not be compared to the full model.

Discussion

Amphibians are negatively impacted by a host of chemical stressors and can suffer both lethal and sublethal effects. These effects, however, are not consistent across all chemicals or chemical classes. As such, a detailed and statistically rigorous synthesis of pesticide and fertilizer effect studies is necessary to provide an overall framework in which to interpret individual results. In our meta-analysis, we collected and synthesized the results from 66 survival studies and 45 growth studies (Appendix A, Appendix B) and found an overall negative effect of the chemicals examined on both the survival and growth of amphibians.

Exposure to pesticides and fertilizers resulted in an 18.8% decrease in survival of amphibians across all chemical classes. When breaking down these findings by chemical class, only four classes of chemicals (inorganic fertilizers, organophosphates, phosphonoglycines, and triazines) significantly reduced amphibian survival. The studies using pesticides and fertilizers from these four chemical classes represent approximately 55% of the total observations in this analysis. These four chemical classes rank amongst the most prevalent in the United States and can reach a combined total of over 180 million pounds of active ingredient used each year in the United States (Gail 2000). In addition, more than 45 billion tons of inorganic fertilizers are used each year, and this number is predicted to increase (Lanyon 1996, USDA 2006). Compounding their prevalence, these chemicals often are applied multiple times during the spring agricultural growing season; amphibians typically breed in the spring, thereby increasing the chance of exposure during the sensitive larval and embryonic life stages (Cox 2006, Ortiz 2007, Relyea 2008).

We predict these effects will have negative impacts on the biological community as a whole. A significant reduction in amphibian survival due to pesticides and fertilizer exposure will eventually lead to population and community level changes in these environments. Because amphibians represent a unique position in trophic food webs, their presence or absence in a system can lead to dramatic changes in community function (Colón-Guad 2009). In neotropical streams, grazing tadpoles can influence basal resources by reducing the amount of food available to primary consumers (Whiles *et al.* 2006). In temperate forests, amphibians represent a significant portion of the vertebrate biomass, potentially exceeding the total biomass of all other non-amphibian vertebrates (Rodenhouse 2009).

The complex life cycle of amphibians represents an energetic link between aquatic and terrestrial systems (Colón-Guad 2009). Many amphibian larvae develop in freshwater habitats and then move to terrestrial habitats, transferring the energy and nutrients gained from one system to the other (Colón-Guad 2009). Adult amphibians return to the freshwater habitats to deposit energy-rich eggs back into the aquatic food webs creating a cycle of energy transfer between these systems (Colón-Guad 2009). In addition to basic food web contributions, amphibians also play a role in both intraguild and classic predation. Intraguild predation is when a potentially competing species occupying the same trophic level as an established predator preys upon that

that established predator (Walls 2001). Traditionally, coexistence between a predator and its prey is promoted by the presence of alternative prey species, predator avoidance behaviors, or refuge. Without these factors, predators are capable of suppressing populations of their prey to the point of extinction. However, intraguild predators may increase the abundance of their intraguild prey. By consuming a competitor that is exploitatively superior in its use of shared prey an intraguild predator may relax the predation pressure on the shared prey, thus increasing its abundance.

In many freshwater communities larval salamanders, such as *Ambystoma*, are generalist predators who may cannibalize conspecifics (Walls 2001). In fishless habitats some amphibian species can be the primary predator of the system (Walls 2001). Complexities in amphibian community food webs are often related to time of hatching where early hatchers will prey on the developing embryos of late hatchers (Walls 2001). Multiple amphibian species may prey on the same conspecific prey item, however, all amphibian species present in the community also prey on small invertebrates and microcrustaceans; thus consisting of a group of intraguild predators (Walls 2001).

The loss of individual species from these communities may have large impacts on how the system functions as a whole (Colón-Guad 2009). Losing intraguild predators may put too much stress on prey species and lead to their localized extinction from excessive predation (Walls 2001). The loss of amphibians could lead to a negative impact on local biodiversity. Local biodiversity has been linked to system stability, resistance to disease, resilience to disturbance, and vulnerability to invasion by exotic species (Chapin et al 2000, Loreau et al 2001).

Unfortunately, predicting specific impacts from pesticide and fertilizer exposure is difficult because survival rates are often species dependent, (i.e., not every species is impacted in the same way from chemical contaminants; Boone 2001). As such, there is potential at sites regularly exposed to pesticides and fertilizers to select for resistant species (Boone 2002). Additionally, there are often energetic trade-offs between resistance to chemical contamination and increased susceptibility to disease and viruses via immunosuppression (Carey et al 1999). Contaminants which artificially select for specific species could lead to a decrease in local biodiversity and alter the community as a whole (Boone 2002).

Pesticide and fertilizer effects on growth were not as pronounced as they were on survival; we observed a 7.7% decrease in growth in amphibians that were exposed to pesticides and fertilizers relative to controls. Only two chemical classes resulted in significantly decreased growth: organophosphates and triazines. As mentioned previously, these are common chemical classes and they made up approximately 31% of the total growth observations. Effects on growth may be more difficult to measure in an experimental setting as many confounding factors may be contributing these results (Relyea 2005*b*). There are many different factors that can lead to reductions in growth, including density, resource availability, and disease (Altwegg 2002, Relyea 2004, Werner 2006).

Negative impacts of chemical contaminants on amphibian growth rates may result in delayed metamorphosis and/or smaller size at metamorphosis. Any delay in metamorphosis has the potential to result in failure to outgrow potential predators as well as failure to emerge from an ephemeral water body (Berril 1993). Smaller size at metamorphosis can increase susceptibility to predation and can have negative impacts on reproductive success (Berrill 1993).

Varying chemical exposure rates throughout the spring and early summer could have profound impacts on amphibian survival. Amphibians have stage dependent responses to many pesticides, thus it is important to consider developmental stage when designing contaminant sensitivity experiments (Harris 2000). Most studies looking at amphibian sensitivity to pesticides and fertilizers focus on the larval stage; this is evident in our analysis as approximately 93% of the studies quantified larval response variables. More work is needed on the embryonic and post-metamorphic life stages before a comprehensive analysis on these life history stages can be completed (Relyea 2005*a*).

While many of the studies used in our analysis test for direct effects of pesticides and fertilizers on amphibian growth and survival, published studies quantifying indirect effects are sorely lacking. Furthermore, interactions between contaminants and other environmental factors have been shown to negatively impact amphibians and more realistically depict complex habitat conditions (Bancroft et al. 2008). For example, several studies suggest that the presence of predator cues can interact synergistically with pesticides (Relyea 2001, Relyea 2005*a*, Relyea 2005*b*, Boone 2006). Nitrates have been shown to act synergistically with ultraviolet-B radiation to lower growth in the Pacific treefrog (*Pseudacris regilla*) (Hatch 2003). Pesticides may also increase the risk of parasitic infection; amphibians exposed to agricultural runoff had a higher proportion of parasitic cysts relative to controls (Kiesecker 2002).

Most of the experiments conducted thus far have focused on a single amphibian species or genera, single contaminants, and one life stage. Research programs are only now beginning to address the need to test multiple species in naturally realistic conditions. Low-concentration cocktails of several chemical classes in addition to naturally occurring biotic and abiotic stressors would vastly increase the applicability of pesticide research to amphibian populations. In addition, we must address how pesticides and fertilizers impact communities as a whole (Relyea 2005*b*). Insecticides, herbicides, and fertilizers in aquatic communities may interact with species and ecosystem processes in non-intuitive ways. Insecticides used to kill terrestrial invertebrate pests can have non-target impacts on aquatic invertebrates, (Relyea *et al.* 2005) while herbicides accidentally applied to freshwater systems can have potentially severe bottom-up effects and disrupt entire ecosystems (Relyea 2005*b*).

Our study underscores the importance of conducting meta-analyses on globally important stressors and their impacts on amphibians. Significant impacts from pesticides and fertilizers were observed on both survival and growth of amphibians. Understanding how different chemical classes of pesticides interact with amphibian populations globally will enhance our ability to monitor and establish conservation efforts to help minimize amphibian population declines.

Table 3.1: Summary of pesticides and fertilizers organized by chemical classes, chemicals, and expected environmental concentrations (EEC) represented as mg/L.

Chemical Class	Chemical	EEC	References
Carbamate	Carbaryl	5	Boone 2003a
Chloro-nicotinyl	Imidaclorpid	42	EPA 1992
Chlorophenoxy Acid	2,4-D	0.12	Relyea 2005b
Chloropyridinyl	Release	5.77	Wojtaszek 2005
Dithiocarbamate	Mancozeb	0.008	Harris 2000
Inorganic Fertilizers	Calcium	15	Hammer 2004
	Phosphate	50	WHO 2007
	Nitrate	50	WHO 2007
Organochloride	Endosulfan	10	Harris 2000
Organophosphorus	Malathion	1.8	Relyea 2008
	Abate	0.05	EPA 1998
	Chlorpyrifos	0.0037	Wood 2002
	Diazinon	0.082	EPA 2005
Organotin	Triphenyltin	0.002	Fioramonti 2007
Phenol	Octylphenol	0.05	Rohr 2003
Polyalkyloxy Compound	POEA	0.6	Howe 2004
Pyrethroid	Permethrin	0.05	Johansson 2006
	alpha-cypermethrin	0.006	Gruelich
Triazine	Atrazine	0.002	Johansson 2006
	Cyanazine	0.9	Boone 2006
Urea	Urea	154	Schuytema 1999
	Diuron	10	Schuytema 1998
Other	Methoprene	0.05	Chu 1997
	Azadirachtin	0.5	Punzo 1997

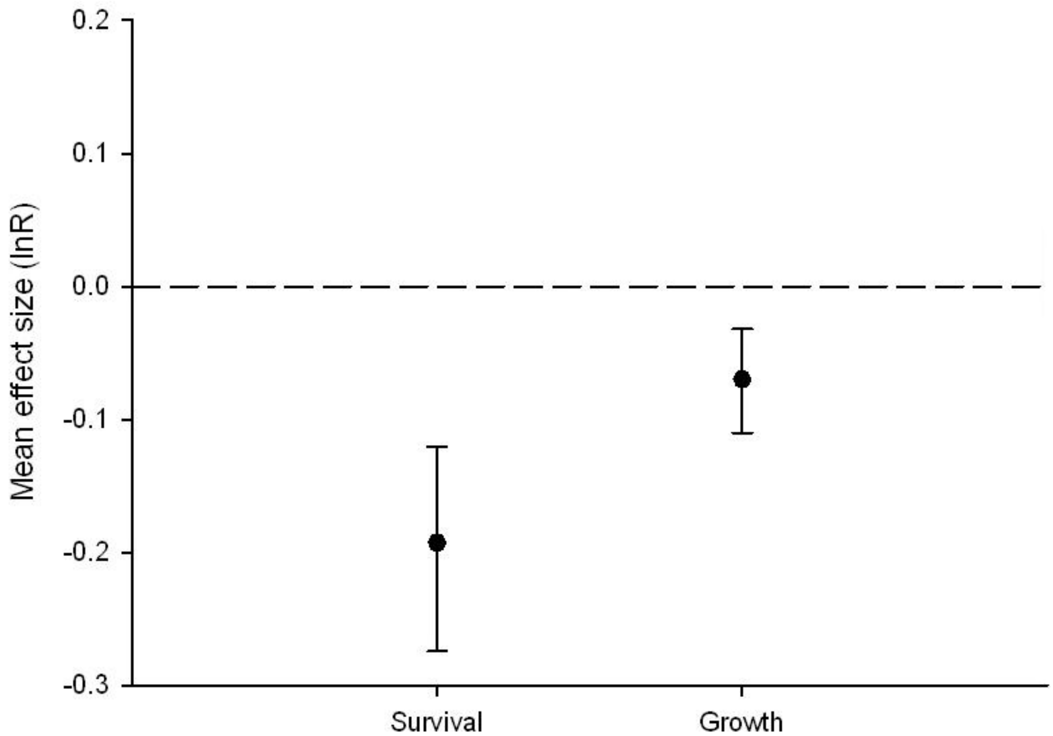

Figure 3.1. Grand mean effect size (log response ratio) and 95% confidence intervals of survival and growth. Dashed line represents zero effect. Survival and growth are both significantly lower when amphibians are exposed to pesticides and fertilizers.

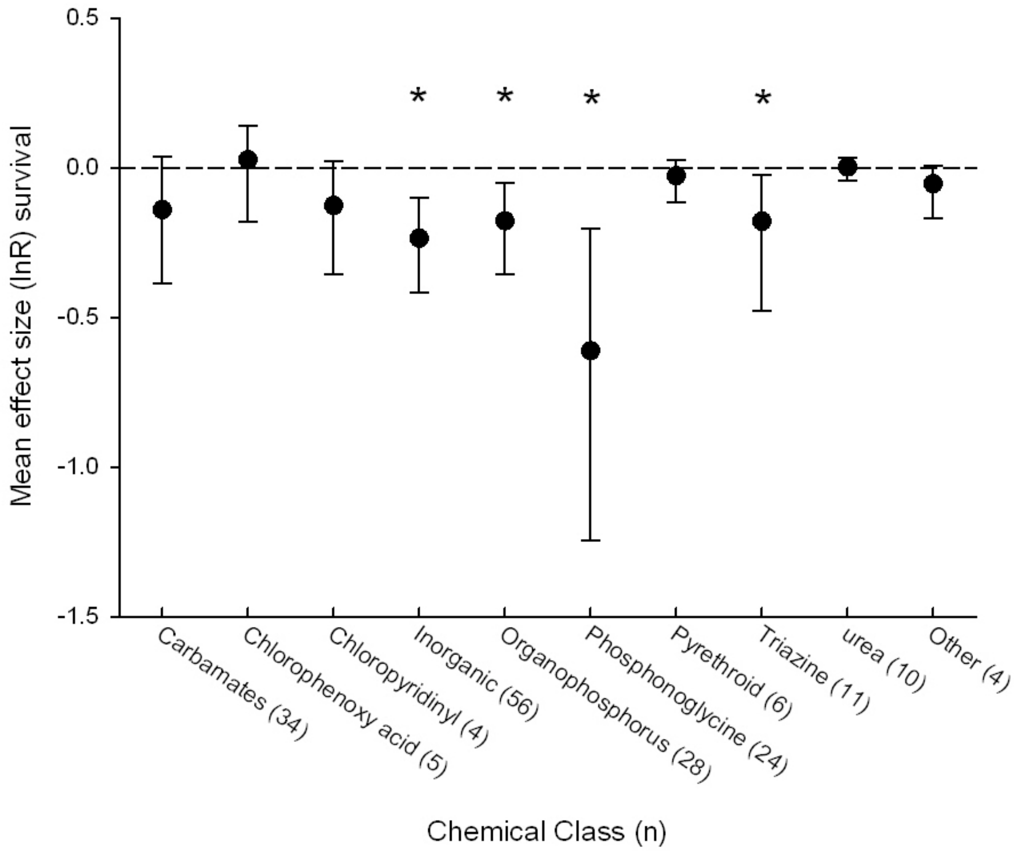

Figure 3.2. Grand mean effect size (log response ratio) and 95% confidence intervals of chemical classes on survival. Number of comparisons is indicated by the value in the parentheses. Significant effect sizes are denoted by an asterisk.

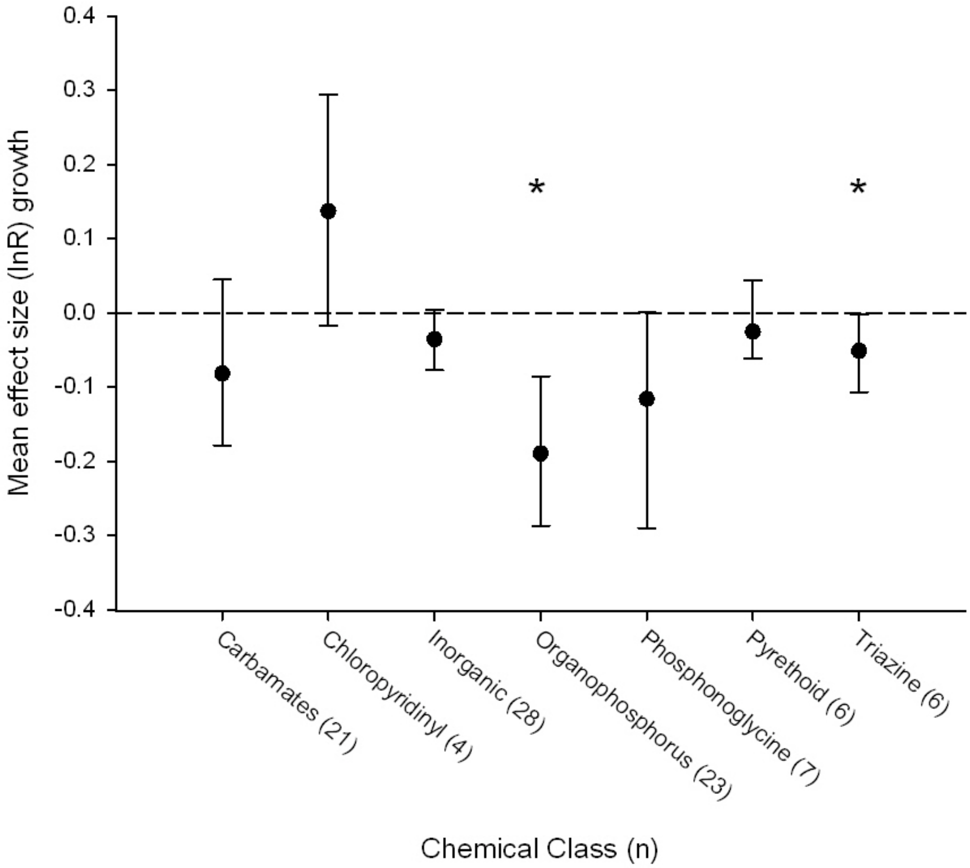

Figure 3.3. Grand mean effect size (log response ratio) and 95% confidence intervals of chemical classes on growth. Number of comparisons is indicated by the value in the parentheses. Significant effect sizes are denoted by an asterisk.

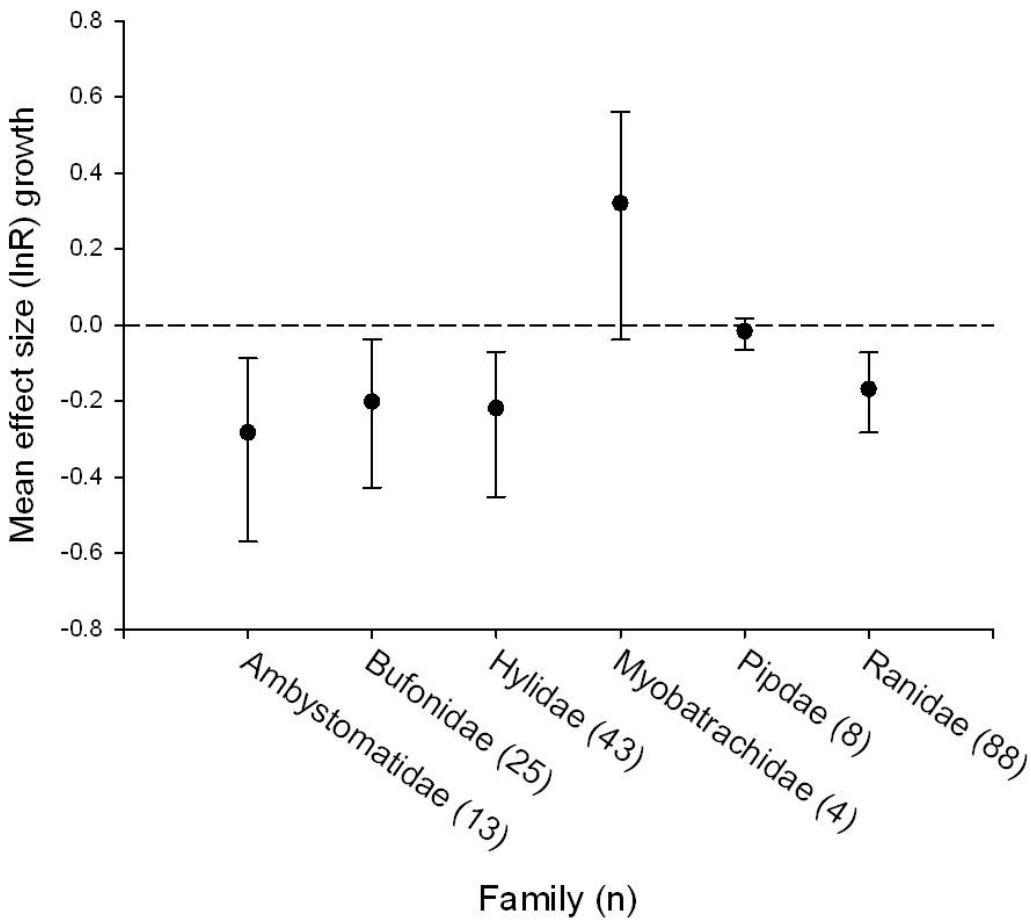

Figure 3.4. Grand mean effect size (log response ratio) and 95% confidence intervals of survival for the amphibian families present in this study. Dashed line represents zero effect. Survival for Myobatrachidae and Pipdae were not significantly affected by pesticides and fertilizers while survival for Ambystomatidae, Bufonidae, Hylidae, and Ranidae was significantly negative. Number of comparisons is indicated by the value in the parentheses.

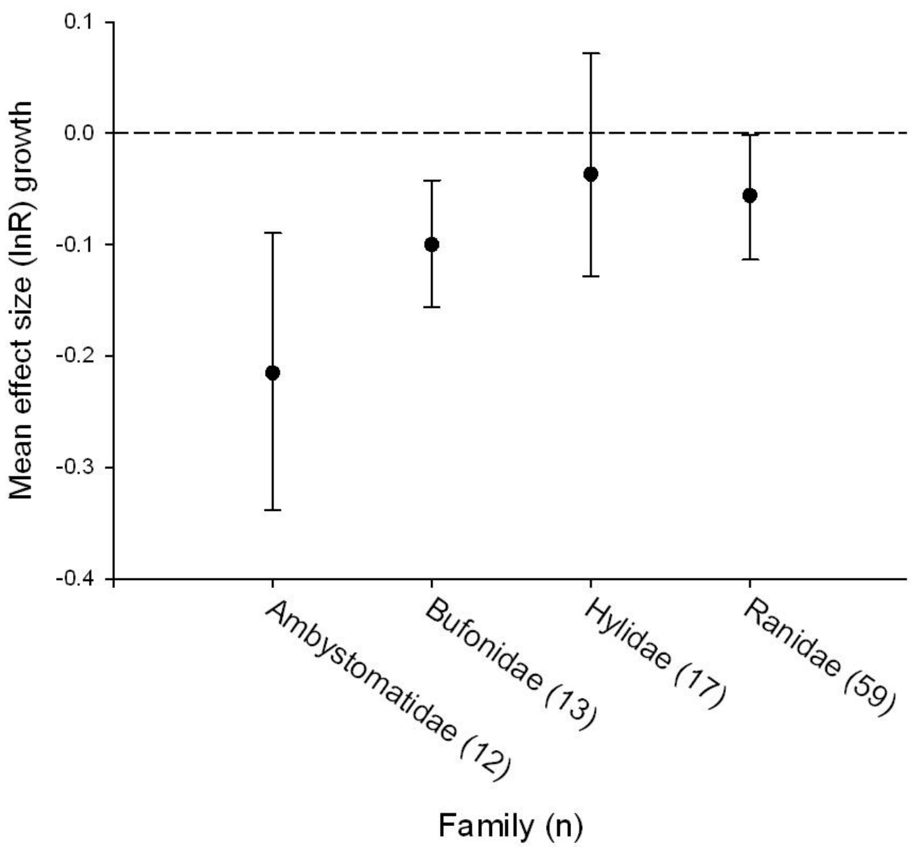

Figure 3.5. Grand mean effect size (log response ratio) and 95% confidence intervals of growth for the amphibian families present in this study. Dashed line represents zero effect. Growth for Hylidae was not significantly affected by pesticides and fertilizers while growth for Ambystomatidae, Bufonidae, and Ranidae was significantly negative. Number of comparisons is indicated by the value in the parentheses.

CHAPTER 4: CONCLUSIONS

My research explored the ecology of amphibians in agriculturally dominated habitats. Understanding the impacts of agricultural practices on amphibians is of particular importance because amphibians are currently experiencing global populations declines (Stuart 2004). I conducted field surveys and collected detailed habitat data to understand what habitats amphibians are using in agriculturally dominated landscapes. In addition to the field surveys, conservation efforts were documented and mapped across the study watershed. This information allowed me to look at amphibian species diversity in response to established conservation efforts and determine their effectiveness. I also quantified the effects of pesticides and fertilizers, common stressors on amphibian survival and growth in agricultural lands.

I found that amphibian species diversity was positively correlated with increased field level conservation effort (Chapter 2). Furthermore, amphibian species distribution analyses in the Calapooia watershed showed that Long-toed salamanders and Pacific treefrogs occupied habitats that Red-legged frogs and Rough-skinned newts did not. Long-toed salamanders tended to be associated with increased stream channel cover (Chapter 2). Pacific treefrogs showed strong associations to increased understory and ground cover vegetation structure (Chapter 2). Rough-skinned newts and Red-legged frogs had similar habitat relationships and both species occurred in established riparian or densely vegetated areas near ponds and wetlands (Chapter 2).

In chapter 3, I used meta-analytic techniques to quantify the effects of pesticides and fertilizers on amphibians. I synthesized the results of 111 articles on the effects of pesticide and fertilizer exposure on amphibians and found a 19% reduction in survival and an 8% reduction in growth of amphibians. Pesticide and fertilizer chemical classes differed in their effect on amphibian survival with organophosphates, triazines, inorganic fertilizers, and phosphonoglycines impacting survival more than the other chemical classes. Effects on growth were most prominent in the organophosphates and triazine chemical classes. Differences in chemical classes can lead to important ecological effects. By selecting chemical classes that don't have as large an impact on amphibians, we can potentially reduce amphibian declines in areas of intense agricultural production.

My thesis work demonstrates that pesticides and fertilizers are potential factors that can reduce amphibian survival and growth in agriculturally dominated systems. Additionally, I also showed a positive relationship between amphibian species diversity and field level conservation efforts, suggesting that amphibians respond well to these practices. Habitat associations followed species life history traits, with Long-toed salamanders and Pacific treefrogs showing habitat associations that varied from densely vegetated areas to sparse stream channels. Additionally, Red-legged frogs and Rough-skinned newts displayed habitat associations with high levels of. By taking into consideration these habitat associations, we can choose appropriate conservation efforts to maximize benefits for specific species. Ultimately, it is important to understand how agricultural stressors and conservation efforts interact with amphibian assemblages. This understanding is an important step for maintaining amphibian populations in agriculturally dominated landscapes.

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APPENDICES

APPENDIX A

Appendix A. Amphibian species, chemical class, life history stage, and taxonomic order used in the survival meta-analysis.

Species	Chemical Class	Life History Stage	Taxonomic Order	Reference
Ambystoma barbouri	Carbamate	Larva	Caudata	Rohr 2003
Ambystoma barbouri	Organochloride	Larva	Caudata	Rohr 2003
Ambystoma barbouri	Triazine	Larva	Caudata	Rohr 2003
Ambystoma gracile	Inorganic Fertilizer	Larva	Caudata	Nebeker 2000
Ambystoma gracile	Inorganic Fertilizer	Larva	Caudata	Marco1999
Ambystoma gracile	Inorganic Fertilizer	Larva	Caudata	Romansic 2006
Ambystoma macrodactylum	Carbamate	Larva	Caudata	Metts 2005
Ambystoma macrodactylum	Triazine	Larva	Caudata	Forson 2006
Ambystoma maculatum	Carbamate	Larva	Caudata	Boone 2007
Ambystoma maculatum	Inorganic Fertilizer	Larva	Caudata	Boone 2007
Ambystoma opacum	Carbamate	Larva	Caudata	Metts 2005
Ambystoma tigrinum	Inorganic Fertilizer	Larva	Caudata	Griffis-Kyle 2007
Bufo americanus	Carbamate	Larva	Anura	Relyea 2005*b*
Bufo americanus	Carbamate	Larva	Anura	Relyea 2004

Bufo americanus	Carbamate	Larva	Anura	Relyea 2003
Bufo americanus	Chlorophenoxy Acid	Larva	Anura	Relyea 2005*b*
Bufo americanus	Inorganic Fertilizer	Larva	Anura	Hecnar 1995
Bufo americanus	Organochloride	Larva	Anura	Harris 2000
Bufo americanus	Organophosphorus	Larva	Anura	Relyea 2005*b*
Bufo americanus	Organophosphorus	Larva	Anura	Relyea 2004
Bufo americanus	Organophosphorus	Larva	Anura	Relyea 2004*b*
Bufo americanus	Organophosphorus	Larva	Anura	Boone 2008
Bufo americanus	Organophosphorus	Larva	Anura	Relyea *et al.* 2005
Bufo americanus	Phosphonoglycine	Larva	Anura	Relyea 2004
Bufo americanus	Phosphonoglycine	Larva	Anura	Relyea *et al.* 2005
Bufo americanus	Phosphonoglycine	Larva	Anura	Relyea 2005*b*
Bufo americanus	Phosphonoglycine	Larva	Anura	Relyea 2005*a*
Bufo americanus	Phosphonoglycine	Larva	Anura	Relyea 2005*c*
Bufo americanus	Pyrethroid	Larva	Anura	Berrill 1993
Bufo americanus	Triazine	Larva	Anura	Storrs 2004
Bufo boreas	Inorganic Fertilizer	Larva	Anura	Marco 1999

Bufo bufo	Inorganic Fertilizer	Larva	Anura	Ortiz 2007
Bufo bufo	Inorganic Fertilizer	Larva	Anura	Marcías 2007
Bufo calamita	Inorganic Fertilizer	Larva	Anura	Ortiz 2007
Bufo quercicus	Other	Larva	Anura	Punzo 1997
Bufo woodhousii	Carbamate	Larva	Anura	Boone 2004
Crinia signifera	Inorganic Fertilizer	Larva	Anura	Hamer 2004
Discoglossus galganoi	Inorganic Fertilizer	Embryo	Anura	Ortiz 2007
Hyla arborea	Inorganic Fertilizer	Embryo	Anura	Ortiz 2007
Hyla meridionalis	Inorganic Fertilizer	Larvae	Anura	Shinn 2008
Hyla versicolor	Carbamate	Larva	Anura	Relyea 2005*b*
Hyla versicolor	Carbamate	Larva	Anura	Relyea 2004
Hyla versicolor	Carbamate	Larva	Anura	Relyea 2003
Hyla versicolor	Carbamate	Larva	Anura	Relyea 2001
Hyla versicolor	Carbamate	Larva	Anura	Boone 2006
Hyla versicolor	Chlorophen oxy Acid	Larva	Anura	Relyea 2005*b*
Hyla versicolor	Inorganic Fertilizer	Larva	Anura	Vaala 2004
Hyla versicolor	Organophos phorus	Larva	Anura	Relyea 2005*b*

Hyla versicolor	Organophosphorus	Larva	Anura	Relyea 2004
Hyla versicolor	Organophosphorus	Larva	Anura	Relyea *et al.* 2005
Hyla versicolor	Organophosphorus	Larva	Anura	Relyea 2004*b*
Hyla versicolor	Phosphonoglycine	Larva	Anura	Relyea 2005*b*
Hyla versicolor	Phosphonoglycine	Larva	Anura	Relyea 2004
Hyla versicolor	Phosphonoglycine	Larva	Anura	Relyea 2005*a*
Hyla versicolor	Phosphonoglycine	Larva	Anura	Relyea *et al.* 2005
Hyla versicolor	Phosphonoglycine	Larva	Anura	Relyea 2005*c*
Hyla versicolor	Triazine	Larva	Anura	Diana 2000
Hyla versicolor	Triazine	Larva	Anura	Boone 2006
Limnodynastes peronii	Inorganic Fertilizer	Larva	Anura	Hamer 2004
Limnonectus limnocharis	Organophosphorus	Larva	Anura	Gurushankara 2007
Litoria aurea	Inorganic Fertilizer	Larva	Anura	Hamer 2004
Litoria citropa	Organochloride	Larva	Anura	Broomhall 2002
Notophthalmus viridescens	Organophosphorus	Larva	Caudata	Relyea *et al.* 2005
Notophthalmus viridescens	Phosphonoglycine	Larva	Caudata	Relyea *et al.* 2005
Pelobates cultripes	Inorganic Fertilizer	Embryo	Anura	Ortiz 2007

Pelophylax perezi	Inorganic Fertilizer	Larva	Anura	Shinn 2008
Pseudacris crucifer	Carbamate	Larva	Anura	Relyea 2005*b*
Pseudacris crucifer	Chlorophen oxy Acid	Larva	Anura	Relyea 2005*b*
Pseudacris crucifer	Organophos phorus	Larva	Anura	Relyea 2005*b*
Pseudacris crucifer	Phosphonog lycine	Larva	Anura	Relyea 2005*b*
Pseudacris crucifer	Triazine	Larva	Anura	Storrs 2004
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Hatch 2003
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Marco 1999
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Nebeker 1998
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Romansic 2006
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Schuytema 1999*b*
Pseudacris regilla	Urea	Larva	Anura	Schuytema 1999*a*
Pseudacris regilla	Urea	Larva	Anura	Schuytema 1998
Pseudacris triseriata	Inorganic Fertilizer	Larva	Anura	Hecnar 1995
Ptychadena bibroni	Organophos phorus	Larva	Anura	Ezemonye 2007
Rana arvalis	Pyrethroid	Embryo	Anura	Greulich 2003
Rana aurora	Inorganic Fertilizer	Larva	Anura	Marco 1999

Rana aurora	Inorganic Fertilizer	Larva	Anura	Nebeker 1998
Rana aurora	Inorganic Fertilizer	Larva	Anura	Romansic 2006
Rana aurora	Inorganic Fertilizer	Larva	Anura	Schuytema 1999*a*
Rana aurora	Urea	Larva	Anura	Schuytema 1998
Rana catesbeiana	Carbamate	Larva	Anura	Relyea 2004
Rana catesbeiana	Carbamate	Larva	Anura	Relyea 2003
Rana catesbeiana	Carbamate	Larva	Anura	Boone 2003*c*
Rana catesbeiana	Inorganic Fertilizer	Larva	Anura	Smith 2004
Rana catesbeiana	Inorganic Fertilizer	Larva	Anura	Smith 2005
Rana catesbeiana	Inorganic Fertilizer	Larva	Anura	Smith 2006
Rana catesbeiana	Organophos phorus	Larva	Anura	Relyea 2004
Rana catesbeiana	Organophos phorus	Larva	Anura	Relyea 2004*b*
Rana catesbeiana	Phosphonog lycine	Larva	Anura	Relyea 2004
Rana catesbeiana	Phosphonog lycine	Larva	Anura	Relyea 2005*c*
Rana catesbeiana	Urea	Larva	Anura	Schuytema 1998
Rana clamitans	Carbamate	Larva	Anura	Relyea 2003
Rana clamitans	Carbamate	Larva	Anura	Relyea 2006

Rana clamitans	Carbamate	Larva	Anura	Boone 2003*a*
Rana clamitans	Carbamate	Larva	Anura	Boone 2004
Rana clamitans	Carbamate	Larva	Anura	Boone 2005
Rana clamitans	Chloropyridinyl	Larva	Anura	Wojtaszek 2005
Rana clamitans	Inorganic Fertilizer	Larva	Anura	Boone 2005
Rana clamitans	Inorganic Fertilizer	Larva	Anura	Hecnar 1995
Rana clamitans	Inorganic Fertilizer	Larva	Anura	Smith 2005
Rana clamitans	Inorganic Fertilizer	Larva	Anura	Smith 2006
Rana clamitans	Organophosphorus	Larva	Anura	Relyea 2004*b*
Rana clamitans	Organophosphorus	Larva	Anura	Sparling 1997
Rana clamitans	Phosphonoglycine	Larva	Anura	Relyea 2005*c*
Rana clamitans	Pyrethroid	Embryo	Anura	Berrill 1993
Rana clamitans	Triazine	Larva	Anura	Storrs 2004
Rana limnocharis	Other	Larva	Anura	Feng 2004
Rana nigro-maculata	Other	Larva	Anura	Feng 2004
Rana perezi	Inorganic Fertilizer	Larva	Anura	Marcías 2007
Rana pipiens	Carbamate	Larva	Anura	Relyea 2005*b*

Rana pipiens	Carbamate	Larva	Anura	Relyea 2004
Rana pipiens	Carbamate	Larva	Anura	Relyea 2003
Rana pipiens	Chlorophenoxy Acid	Larva	Anura	Relyea 2005*b*
Rana pipiens	Chloropyridinyl	Larva	Anura	Wojtaszek 2005
Rana pipiens	Inorganic Fertilizer	Larva	Anura	Hecnar 1995
Rana pipiens	Inorganic Fertilizer	Larva	Anura	Sparling 2006
Rana pipiens	Organochloride	Larva	Anura	Harris 2000
Rana pipiens	Organophosphorus	Larva	Anura	Relyea 2005*b*
Rana pipiens	Organophosphorus	Larva	Anura	Relyea 2004
Rana pipiens	Organophosphorus	Larva	Anura	Relyea *et al.* 2005
Rana pipiens	Organophosphorus	Larva	Anura	Relyea 2004*b*
Rana pipiens	Organophosphorus	Embryo	Anura	Gaizick 2001
Rana pipiens	Organophosphorus	Larva	Anura	Relyea 2008
Rana pipiens	Other	Larva	Anura	Ankley 1998
Rana pipiens	Phosphonoglycine	Larva	Anura	Howe 2004
Rana pipiens	Phosphonoglycine	Larva	Anura	Relyea 2005*b*
Rana pipiens	Phosphonoglycine	Larva	Anura	Relyea 2004
Rana pipiens	Phosphonoglycine	Larva	Anura	Relyea 2005*a*
Rana pipiens	Phosphonoglycine	Larva	Anura	Relyea *et al.* 2005

Rana pipiens	Phosphonoglycine	Larva	Anura	Relyea 2005*c*
Rana pipiens	Pyrethroid	Embryo	Anura	Berrill 1993
Rana pretiosa	Inorganic Fertilizer	Larva	Anura	Marco 1999
Rana sphenocephala	Carbamate	Larva	Anura	Mills 2004
Rana sphenocephala	Carbamate	Larva	Anura	Boone 2004
Rana sphenocephala	Carbamate	Embryo	Anura	Bridges 2000
Rana sphenocephala	Carbamate	Larva	Anura	Bridges 2000
Rana sphenocephala	Triazine	Larva	Anura	Boone 2003*b*
Rana sylvatica	Carbamate	Larva	Anura	Relyea 2005*b*
Rana sylvatica	Carbamate	Larva	Anura	Relyea 2003
Rana sylvatica	Chlorophenoxy Acid	Larva	Anura	Relyea 2005*b*
Rana sylvatica	Inorganic Fertilizer	Larva	Anura	Burgett 2007
Rana sylvatica	Inorganic Fertilizer	Larva	Anura	Griffis-Kyle 2007
Rana sylvatica	Organophosphorus	Larva	Anura	Relyea 2005*b*
Rana sylvatica	Organophosphorus	Larva	Anura	Relyea 2004*b*
Rana sylvatica	Organophosphorus	Larva	Anura	Relyea 2008
Rana sylvatica	Phosphonoglycine	Larva	Anura	Relyea 2005*b*
Rana sylvatica	Phosphonoglycine	Larva	Anura	Relyea 2005*c*

Rana sylvatica	Pyrethroid	Embryo	Anura	Berrill 1993
Rana sylvatica	Triazine	Larva	Anura	Storrs 2004
Rana temporaria	Pyrethroid	Larva	Anura	Johansson 2006
Rana temporaria	Triazine	Larva	Anura	Johansson 2006
Scinax nasicus	Phosphonoglycine	Larva	Anura	Lajmanovich 2003
Triturus pygmaeus	Inorganic Fertilizer	Embryo	Caudata	Ortiz 2006
Xenopus laevis	Carbamate	Larva	Anura	Zaga 1998
Xenopus laevis	Inorganic Fertilizer	Larva	Anura	Schuytema 1999*b*
Xenopus laevis	Organophosphorus	Embryo	Anura	Bonfanti 2003
Xenopus laevis	Triazine	Larva	Anura	Oka 2008
Xenopus laevis	Urea	Larva	Anura	Schuytema 1999*a*
Xenopus laevis	Urea	Larva	Anura	Schuytema 1998

APPENDIX B

Appendix B. Amphibian species, chemical class, life history stage, and taxonomic order used in the growth meta-analysis.

Species	Chemical Class	Life History Stage	Taxonomic Order	Reference
Acris crepitans	Organophosphorus	Larva	Anura	Widder 2008
Ambystoma barbouri	Carbamate	Larva	Caudata	Rohr 2003
Ambystoma barbouri	Organochlorine	Larva	Caudata	Rohr 2003
Ambystoma barbouri	Phenol	Larva	Caudata	Rohr 2003
Ambystoma barbouri	Triazine	Larva	Caudata	Rohr 2003
Ambystoma Gracile	Inorganic Fertilizer	Larva	Caudata	Nebeker 2000
Ambystoma Gracile	Organophosphorus	Larva	Caudata	Nebeker 1998
Ambystoma macrodactylum	Carbamate	Larva	Caudata	Boone 2003*b*
Ambystoma macrodactylum	Inorganic Fertilizer	Larva	Caudata	Hatch 2003
Ambystoma macrodactylum	Inorganic Fertilizer	Larva	Caudata	Hatch 2003
Ambystoma macrodactylum	Triazine	Larva	Caudata	Forson 2006
Ambystoma maculatum	Carbamate	Larva	Caudata	Metts 2005
Ambystoma opacum	Carbamate	Larva	Caudata	Metts 2005
Bufo americanus	Carbamate	Larva	Anura	Relyea 2004
Bufo americanus	Carbamate	Larva	Anura	Boone 2008
Bufo americanus	Carbamate	Larva	Anura	Boone 2007

Bufo americanus	Inorganic Fertilizer	Larva	Anura	Boone 2007
Bufo americanus	Organophosphorus	Larva	Anura	Relyea 2004
Bufo americanus	Organophosphorus	Larva	Anura	Relyea 2004
Bufo americanus	Organophosphorus	Larva	Anura	Boone 2008
Bufo americanus	Phosphonoglycine	Larva	Anura	Relyea 2004
Bufo americanus	Pyrethoid	Larva	Anura	Boone 2008
Bufo bufo	Inorganic Fertilizer	Larva	Anura	Ortiz 2007
Bufo calamita	Inorganic Fertilizer	Larva	Anura	Ortiz 2007
Bufo terrestris	Inorganic Fertilizer	Larva	Anura	Edwards 2006
Bufo woodhousii	Carbamate	Larva	Anura	Boone 2004
Discoglossus galganoi	Inorganic Fertilizer	Larva	Anura	Ortiz 2007
Discoglossus galganoi	Inorganic Fertilizer	Larva	Anura	Ortiz 2006
Gastrophryne olivacea	Organophosphorus	Larva	Anura	Widder 2008
Hyla arborea	Inorganic Fertilizer	Larva	Anura	Ortiz 2007
Hyla chrysoscelis	Organophosphorus	Larva	Anura	Widder 2008
Hyla versicolor	Carbamate	Larva	Anura	Relyea 2004
Hyla versicolor	Inorganic Fertilizer	Larva	Anura	Vaala 2004
Hyla versicolor	Organophosphorus	Larva	Anura	Relyea 2004

Hyla versicolor	Organophosphorus	Larva	Anura	Relyea 2004
Hyla versicolor	Phosphonoglycine	Larva	Anura	Relyea 2004
Hyla versicolor	Triazine	Larva	Anura	Diana 2000
Limnonectus limnocharis	Organophosphorus	Larva	Anura	Gurushankara 2007
Notophthalmus viridescens	Organophosphorus	Larva	Caudata	Relyea 2005
Notophthalmus viridescens	Phosphonoglycine	Larva	Caudata	Relyea 2005
Pelobates cultripes	Inorganic Fertilizer	Larva	Anura	Ortiz 2007
Pleurodeles waltl	Inorganic Fertilizer	Larva	Urodela	Ortiz 2007
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Hatch 2003
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Nebeker 2000
Pseudacris regilla	Inorganic Fertilizer	Larva	Anura	Schuytema 1999*b*
Pseudacris regilla	Organophosphorus	Larva	Anura	Nebeker 1998
Pseudacris regilla	Urea	Larva	Anura	Schuytema 1999
Pseudacris triseriata	Inorganic Fertilizer	Larva	Anura	Hecnar 1995
Rana arvalis	Pyrethoid	Larva	Anura	Greulich 2003
Rana aurora	Inorganic Fertilizer	Larva	Anura	Nebeker 2000
Rana aurora	Inorganic Fertilizer	Larva	Anura	Schuytema 1999
Rana aurora	Organophosphorus	Larva	Anura	Nebeker 1998

Rana boylii	Carbamate	Larva	Anura	Davidson 2007
Rana catesbeiana	Carbamate	Larva	Anura	Relyea 2004
Rana catesbeiana	Carbamate	Larva	Anura	Relyea 2006
Rana catesbeiana	Carbamate	Larva	Anura	Boone 2003*c*
Rana catesbeiana	Carbamate	Larva	Anura	Boone 2007
Rana catesbeiana	Inorganic Fertilizer	Larva	Anura	Boone 2007
Rana catesbeiana	Inorganic Fertilizer	Larva	Anura	Smith 2004
Rana catesbeiana	Inorganic Fertilizer	Larva	Anura	Smith 2005
Rana catesbeiana	Inorganic Fertilizer	Larva	Anura	Smith 2006
Rana catesbeiana	Organophosphorus	Larva	Anura	Relyea 2004
Rana catesbeiana	Organophosphorus	Larva	Anura	Relyea 2004
Rana catesbeiana	Phosphonoglycine	Larva	Anura	Relyea 2004
Rana clamitans	Carbamate	Larva	Anura	Relyea 2004
Rana clamitans	Carbamate	Larva	Anura	Relyea 2006
Rana clamitans	Carbamate	Larva	Anura	Boone 2003a
Rana clamitans	Carbamate	Larva	Anura	Boone 2005

Rana clamitans	Chloropyridinyl	Larva	Anura	Wojtaszek 2005
Rana clamitans	Chloropyridinyl	Larva	Anura	Wojtaszek 2005
Rana clamitans	Inorganic Fertilizer	Larva	Anura	Boone 2005
Rana clamitans	Inorganic Fertilizer	Larva	Anura	Smith 2005
Rana clamitans	Inorganic Fertilizer	Larva	Anura	Smith 2006
Rana clamitans	Organophosphorus	Larva	Anura	Relyea 2004
Rana clamitans	Organophosphorus	Larva	Anura	Relyea 2004
Rana clamitans	Phosphonoglycine	Larva	Anura	Relyea 2004
Rana clamitans	Pyrethoid	Larva	Anura	Berrill 1993
Rana esculenta	Organotin	Larva	Anura	Fioramonti 1997
Rana pipiens	Carbamate	Larva	Anura	Relyea 2004
Rana pipiens	Chloroacetanilide	Larva	Anura	Hayes 2006
Rana pipiens	Chloroacetanilide	Larva	Anura	Hayes 2006
Rana pipiens	Chloropyridinyl	Larva	Anura	Wojtaszek 2005
Rana pipiens	Chloropyridinyl	Larva	Anura	Wojtaszek 2005
Rana pipiens	Inorganic Fertilizer	Larva	Anura	Sparling 2006
Rana pipiens	Organophosphorus	Larva	Anura	Relyea 2004
Rana pipiens	Organophosphorus	Larva	Anura	Relyea 2004

Rana pipiens	Organophosphorus	Larva	Anura	Hayes 2006
Rana pipiens	Organophosphorus	Larva	Anura	Relyea 2008
Rana pipiens	Other	Larva	Anura	Ankley 1998
Rana pipiens	Other	Larva	Anura	Hayes 2006
Rana pipiens	Phosphonoglycine	Larva	Anura	Howe 2004
Rana pipiens	Phosphonoglycine	Larva	Anura	Relyea 2004
Rana pipiens	Polyalkyloxy Compound	Larva	Anura	Howe 2004
Rana pipiens	Pyrethoid	Larva	Anura	Hayes 2006
Rana pipiens	Pyrethoid	Larva	Anura	Hayes 2006
Rana pipiens	Sulfonylurea	Larva	Anura	Hayes 2006
Rana pipiens	Triazine	Larva	Anura	Hayes 2006
Rana pipiens	Xylylalanine	Larva	Anura	Hayes 2006
Rana sphenocephala	Carbamate	Larva	Anura	Mills 2004
Rana sphenocephala	Carbamate	Larva	Anura	Boone 2003*b*
Rana sphenocephala	Organophosphorus	Larva	Anura	Widder 2006
Rana sphenocephala	Organophosphorus	Larva	Anura	Widder 2008
Rana sphenocephala	Other	Larva	Anura	Ortiz 2007
Rana sphenocephala	Triazine	Larva	Anura	Boone 2003*b*
Rana sylvatica	Organophosphorus	Larva	Anura	Relyea 2008
Rana temporaria	Pyrethoid	Larva	Anura	Johansson 2006

Rana temporaria	Strobin	Larva	Anura	Johansson 2006
Rana temporaria	Triazine	Larva	Anura	Johansson 2006
Xenopus laevis	Inorganic Fertilizer	Larva	Anura	Schuytema 1999b
Xenopus laevis	Organophosphorus	Larva	Anura	Richards 2003
Xenopus laevis	Urea	Larva	Anura	Schuytema 1999