# Larval Salamander Growth and Development across a Landscape Gradient

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**Abstract**

Marbled salamanders, *Ambystoma opacum,* utilize vernal pools for breeding and development, but many of these pools are in agricultural and urbanized habitats which can lead to polluted, toxic conditions within pools. This experiment studied the effects of road runoff and agricultural runoff on the development of marbled salamander larvae. Three agricultural vernal pools and three roadside vernal pools containing salamander larvae were visited twice, one in the beginning of the winter and once toward the end of winter. During visits, temperature, dissolved oxygen, conductivity, and pH were measured and larvae were collected using dips-nets to assess abundance and length. Water samples and sediment samples were also collected from pools to test for metal, pesticide, chlorophyll, and nutrient concentrations. During the second visits, two roadside pools no longer contained larvae, and overall, agricultural pools had slightly greater larval growth and abundance than the roadside pools. Roadside pools generally had higher conductivity, lower dissolved oxygen, and greater metal concentrations while agricultural pools had greater chlorophyll concentrations. However, there was no statistically significant difference in water quality and salamander development between the two vernal pool classifications. Based on results, the mortality of larvae in the two roadside pools was attributed to road salt applications used during the winter and general runoff of metals from roads. No pesticides were detected in agricultural pools so further research during the spring could be important to assessing the effect of landscape on local salamander populations.

**Introduction**

Amphibians are a unique group of tetrapods with biphasic life histories consisting of an aquatic larval stage and a terrestrial adult phase. Gelatinous amphibian eggs must remain in a wet environment throughout development of the embryo and larvae have external gills that must remain in water for respiration until metamorphosis. This aquatic larval stage makes amphibians highly dependent on water.

Even terrestrial adults amphibians, which have thin, scaleless skin for respiration, are susceptible to desiccation and must constantly keep their skin moist (Julian, 2000).

Salamanders are part of the order Caudata which is part of the class Amphibia. Like most amphibians, salamanders hatch from aquatic eggs as larvae with external gills and metamorphose into air-breathing, terrestrial adults. However, life histories and breeding characteristics can vary among salamander species. Marbled salamanders, *Ambystoma opacum*, utilize vernal pools for breeding and development (Turtle, 2000; Figure 1). Vernal pools are ephemeral wetlands or pools of varying size that periodically dry up. Marbled salamanders arrive at these pools during the fall, when pools are dry (Figure 2). Mating occurs on land and eggs are deposited by females under debris along pool margins, and when the pools fill with winter rain or snow melt, low oxygen levels trigger the eggs to hatch. Larvae spend the winter and spring in the pool developing, metamorphosing, and preying on other pool organisms including plants, invertebrates, and spring-breeding amphibians (Calhoun & deMaynadier, 2007).

Salamanders play vital roles in both aquatic and terrestrial ecosystems. After an extensive study, researchers Davic and Welsh (2004) found six ways in which salamanders act as dominant keystone species in the local ecosystem: (1) they provide energy and nutrients for terrestrial and aquatic consumers; (2) they regulate energy pathways and the release of minerals by maintaining invertebrate populations; (3) they increase the diversity of lower trophic taxa by reducing competitively dominant prey; (4) they serve as pathways for energy and matter between aquatic and terrestrial landscapes due to their biphasic lifestyle; (5) they facilitate soil dynamics by modifying underground refugia; (6) they enhance forest stability by converting and storing large amounts of secondary production through their biomass (Davic & Welsh, 2004). Overall, they concluded that salamanders perform key ecological roles by consuming a variety of plants, invertebrates, and vertebrates within terrestrial and aquatic environments, and that salamander density is linked to prey density, trophic cascades, nutrient cycling, and detritus-litter food webs (Davic & Welsh, 2004).Their study also shows that the loss of salamanders could have serious negative effects on ecosystem stability, and it is this ecological importance of salamanders that makes salamanders and vernal pools so important.

However, amphibian populations are declining globally and understanding the factors associated with their declines is necessary in order to preserve ecologically important species. Of the 234 identified salamander species, over two-thirds are ranked “imperiled or critically imperiled”, and yet only 13 species are protected or have been proposed for protection under the Endangered Species Act (Davic & Welsh, 2004). Dr. Calhoun of University of Maine Orono says there are five common threats to amphibians: disease, invasive species, commercial exploitation, climate change, and habitat loss and alteration (Calhoun & deMaynadier, 2007). Habitat loss and alteration is thought to be the greatest threat to amphibian species, and chemical contamination and pollution are considered forms of habitat alteration. Amphibians need moisture for respiration, reproduction, and development which makes them highly dependent on local water sources and the high permeability of amphibian skin and egg masses also makes them highly vulnerable to water contamination (Calhoun & deMaynadier, 2007). Many larval salamanders are also detritus feeders and can directly ingest contaminants in the sediment (Julian 2000).

Roadside ditches provide vernal pool-like environments that can be utilized by aquatic and wetland species such as marbled salamanders. However, water rapidly runs off the impervious surface of roads and can carry toxic materials to vernal pools. Sediments, herbicides, vehicular NOX emissions, hydrocarbons from petroleum, and metals like lead, zinc, copper, chromium, and cadmium are common contaminants in roadside vernal pools that have shown to increase amphibian mortality (Forman & Alexander, 1998). The ingestion of petroleum and silt can result in liver and respiratory issues in amphibians and other aquatic species. Deicing of roads and increased salt runoff has shown to decrease egg mass density and larval survival of salamanders (Karraker et al., 2008).

Many vernal pools are also closely associated with agricultural fields which can be sources of pollution. Agricultural runoff can result in pesticide and nutrient contamination of nearby vernal pools. Pesticides have shown to increase deformities and decrease hatching by directly affecting the nervous and endocrine system of amphibians or by indirectly affecting food sources (Rohr et al., 2003). Griffis-Kyle and Richie (2007) found that nitrogen levels are highest in surface waters in temperate climates during snowmelt, which is the same time marbled salamander eggs and larvae are developing. In their study, high nitrogen levels resulted in decreased hatching of eggs, decreased growth rates, slower development, and increased physical abnormalities. However, other studies have shown that nutrient runoff from agricultural fields can increase primary productivity and therefore increase food availability in pools which can increase salamander development rates and survival (Gray, Smith, & Brenes, 2004).

Many studies have found that contaminants have varying effects on amphibian species, and that in the field, contaminants can work together to create different effects across amphibian life stages. This experiment aims to analyze the effects of road runoff and agricultural runoff on the development of salamander species, *Ambystoma opacum*. This will be done by evaluating water quality parameters and growth of salamander larvae within two different classifications of vernal pools: agricultural and roadside, at Chino Farms in Kingstown, Maryland. The purpose of this experiment is to monitor how these different landscapes affect salamander survival in the field and to address possible conservation strategies based off its findings.

**Methods**

During the fall of 2013, potential sites were found by surveying the Chino Farms area in Kingstown, Maryland for vernal pools likely utilized by marbled salamanders. In January, after the vernal pools filled and eggs were estimated to hatch, potential sites were visited to determine which contained marbled salamanders. Of the potential sites, 6 vernal pools contained marbled salamander larvae and were used as test sites for the experiment (Figure 3, Figure 4). The distance between the pools and either an agricultural field or road was measured using base aerial imagery and measuring tools in ArcGIS. Pools were then classified as agricultural or roadside based on whether they were within 10m to an agricultural field or road. Due to the landscape of the area, no woodland or forested vernal pools were found; all salamander-containing pools were closely located near agricultural fields and roads. Each test site was visited two times within a two-month period; first during the third week of January 2014 and then during the second week of March 2014.

At each visit, larval salamanders were collected using dip-nets. Salamanders were collected for 100 dips or until 50 larvae were collected. After larvae were found in a dip-net, they were transferred to a bucket filled with water from the vernal pool. Individually, larvae were taken from the bucket, put into a plastic bag, and stretched out to measure their length in centimeters. Due to their small size, measuring snout to vent length was difficult so total length was measured; larvae with deformed tails were not measured. Larval stage of each salamander was also noted according to the R. Harrison Staging System for Salamanders from the Virginia Herpetology Society (Amphibian Development Charts). After measurements, larvae were transferred to a second bucket, also filled with pond water, and were returned to a part of the pool already surveyed to decrease the chance of collecting the same larvae twice. The total number of larvae and the total number of dips were recorded to assess salamander abundance at each pool.

Dissolved oxygen concentration, temperature, pH, and conductivity were measured using a HACH probe at the area of the pool most abundant with salamanders. Conductivity was not measured at the first visit due to a delay in obtaining a conductivity probe. Conductivity measurements were used to evaluate the potential for the presence of common deicing salts: $Na^{2+}$, $Cl^{-}$, $Mg^{2+}$ (Karraker et al., 2008). Visual assessments of each vernal pool were also conducted, noting the presence of plant material, macro invertebrates, and other organisms.

Two water samples were collected from each pool: one sample for nutrient, metal, and chlorophyll analysis and another for pesticide analysis. At the first visit to the pools, water samples were collected in 1L plastic sampling bottles at a depth of approximately 6 inches, from a part of the pool most abundant with salamanders. The bottles were labeled A1, A2, A3, R1, R2, R3 for the 3 agricultural pools and the 3 road-side pools. These samples were taken back to Washington College to test for the presence of nutrients, chlorophyll, and metals.

It is important to note that these samples were not filtered in the field and were kept in a refrigerator for 24 hours before filtration in the lab. However, due to the time of year, it was assumed that chlorophyll levels were low enough that the data would not be greatly affected by the delay in filtration. A syringe was attached to a filtration device containing a Whatman GF/F microfiber 25mm filter. For each filtration, the syringe was filled approximately with 50mL of water sample from the 1L sampling bottle and was then slowly pushed through so that the filter trapped phytoplankton cells containing chlorophyll *a*.  A blank was also made by filtering deionized water. The filters were placed into glass vials using forceps, and all seven vials were labeled (A1, A2, A3, R1, R2, R3, Blank) and filled approximately with 15mL of 90% acetone using a titration tube. The vials were then covered with aluminum foil to limit the filters' exposure to light and were placed in a freezer for 36 hours. After the fluorometer was calibrated using a fluorometric standard, samples were placed in the fluorometer and total chlorophyll (µg) measurements were recorded. These values were then converted to µg/L by dividing by the exact volume filtered to determine chlorophyll concentrations.

Total and reactive nitrate and total and reactive phosphorus concentrations were tested using the filtered water samples. In addition to the 7 filtered water samples (A1, A2, A3, R1, R2, R3, B), a standard was made for each nutrient, and another blank was made using deionized water.  Using a HACH TNT 835 kit, samples were prepared according to the instructions on the kit and were placed in a spectrometer to measure reactive nitrate. To measure total nitrate, samples were prepared by using a

HACH Total Nitrogen Test N Tube kit and were measured in a spectrometer. For total and reactive nitrate, the standard was made using a 1:10 ratio of 10mg/L $NO_3$ and deionized water. To measure total phosphorus and reactive phosphorus, samples were prepared by using a HACH TNT 843 kit and were measured in a spectrometer. For phosphorus, the standard was made using a 1:10 ratio of 10mg/L $PO_4$ and deionized water.

To measure total extractable metals, 1mL of trace metal grade nitric acid was pipetted into the remaining water left in the 1L sample bottles after chlorophyll and nutrient testing to create a pH of about 2. Then, a Nalgene polysulfone filtration apparatus was set up, a polycarbonate membrane 4µm filter soaked in 10% nitric acid was placed in the apparatus, and a hand pump was attached to filter the water samples. In between each sample, the apparatus was cleaned with nanopure water and nitric acid, and a new filter was rinsed three times with nanopure water and once with about 3mL of sample water by pumping the water through the apparatus. About 10mL of sample was filtered through the filtration apparatus and then transferred to a test tube. A duplicate of A2 was filtered and nanopure water was filtered in addition to the six water samples to serve as a blank. Next, approximately 4.5mL of 2% nitric acid ($HNO_3$) and 0.5mL of sample were pipetted and gravimetrically weighed into a tared ICMPS test tube. Specific amounts of sample and $HNO_3$ were recorded to determine dilution factor. Another test tube was filled with a standard obtained from the National Institute of Standards and Technology (1643e), another was filled with nanopure water, and another was filled with nitric acid. All eleven tubes were placed in the ICMPS and total extractable metal concentrations of aluminum, chromium, magnesium, iron, nickel, copper, cadmium, zinc, lead, and arsenic were measured.

The second water sample was collected from each pool on February 11$^{th}$, 2014 in a 1L glass sampling bottle, at a depth approximately 6 inches from a part of the pool most abundant with salamanders. These sampling bottles were provided by the Chesapeake Environmental Laboratory (CEL) in Stevensville, Maryland and were used to test for pesticides at the CEL. A list of all pesticides used on the Chino Farms properties within 2 years was obtained from the farm manager (Figure 5). Due to pricing, all agricultural vernal pool water samples were combined to create one water sample and all roadside vernal pool water samples were combined to create a second sample, and samples were only tested for simazine, S-metolachlor, atrazine, propiconazole, and metribuzin. The CEL determined concentrations of these parameters for both samples and results were mailed to Washington College.

At the first visit to the pools in January, a sediment sample was also collected from the part of the pool most abundant with salamanders. Using a plastic spoon, the top 2cm of sediment was scooped into a plastic bag. Samples were stored in a refrigerator for a week before processing. Sediment samples were transferred to 500mL beakers and the mass of the beakers with the wet sediment was found. Beakers filled with sediment were placed in an oven at approximately 90°C to dry, and after 48hours, the mass of the beakers with dry sediment was found. The dry sediments were then ground using a mortar and pestle. Ground sediment was sifted through a 425mm sieve and remaining sediment in the sieve was reground. When the majority was grounded and sieved, sediment was transferred to plastic sediment containers and labeled. Approximately 0.1 to 0.2 grams of sediment was weighed from the plastic containers into tared Teflon containers. A duplicate of A2 and a standard (1646a) obtained from the National Institute of Standards and Technology were also weighed and transferred to Teflon containers. The sediments were digested by adding 2mL of trace metal grade concentrated $HNO_3$, 6mL of trace metal grade concentrated Hydrochloric acid (HCL), and 2mL of concentrated peroxide ($H_2O_2$). To control effervescence, $H_2O_2$ was added to the standard and R2 sediment samples first and was left to sit for an hour before pipetting $HNO_3$ and HCL. Three blanks were made containing $HNO_3$, HCL, and $H_2O_2$. The following day the eleven Teflon containers were loaded into a MARS6 One Touch microwave and digested using the soil aqua regia method. After digestion, sediments were transferred from the Teflon containers to tared test tubes. The Teflon containers were rinsed three times with nanopure water and the water was also transferred to the test tubes to ensure that all sediment was transferred. The test tubes were then diluted to about 50g with nanopure water and inverted several times. About 0.5mL of each diluted sample was pipetted into a tared ICMPS test tube and then about 4.5mL of 2% $HNO_3$ was added. Exact mass of sample and $HNO_3$ were recorded to determine dilution factor. All eleven samples were placed in the ICMPS and concentrations of aluminum, chromium, magnesium, iron, nickel, copper, cadmium, zinc, lead, and arsenic were measured.

For both water and sediment metal analysis, the average blank concentration was subtracted. Values were then divided by the dilution factor, multiplied by the amount diluted, divided by the amount of water or sediment sample, and converted to µg /g. Metal concentrations found for NIST standards were evaluated and compared to expected values to assess the accuracy of the ICMPS instrument.

Statistical tests were conducted to compare basic water quality parameters, nutrient concentrations, pesticide concentrations, and metals concentrations among the six vernal pools to assess whether geographic location and landscape affected water quality. Statistical tests were also conducted to compare larval growth and abundance between pools to assess whether salamander development and survival differed. Using Microsoft Excel, t-Test: Two Sample Assuming Unequal Variances was used for statistical analysis, and p-values less than 0.05 were considered significant.

**Results**

Statistical tests showed there was no significant difference ($p>0.05$) in water quality parameters between agricultural vernal pools and roadside vernal pools, but some trends were present (Table 1). Roadside pool 2 (R2) had an average dissolved oxygen concentration of 1.70mg/L whereas the majority of the other pools had dissolved oxygen concentrations above 4mg/L (Table 2, Figure 6). Conductivity was notably higher in roadside vernal pools than agricultural pools, and R2 had the highest conductivity of 389µS/cm (Table 2, Figure 7). Average roadside conductivity was 207.97µS/cm and average agricultural conductivity was 51.33µS/cm and had a p-value of 0.115 (Table 1, Table 2). Agricultural pool 2 (A2) had higher chlorophyll concentrations and generally had higher nutrient levels than the rest of the vernal pools (Table 2, Figure 8, Figure 9).

There was a significant difference in chromium, nickel, and lead concentrations of roadside and agricultural vernal pool sediment samples (Table 3). The roadside pools had higher chromium levels than agricultural pools, and chromium concentration was highest in R2 (Table 4). Nickel was also greater in roadside pools compared to agricultural pools, and nickel concentration was highest in R2 (Table 4). Lead was greater in agricultural pools than roadside pools (Table 4). There was no statistical significance in any other metal concentrations between pools. However, R1 and R2 had noticeably greater aluminum levels and R3 had greater magnesium levels than other pools. Both R2 and R3 had the greatest concentrations for 3 of the 10 metals tested (Table 5). There was no significant difference in the metal concentrations of roadside and agricultural pool water samples (Table 6). However, R2 had the highest concentration for 7 out of 10 of the metals tested (Table 7, Table 8). No pesticides were detected by the Chesapeake Environmental Lab for the agricultural or roadside sample. The CEL's detection limit was 0.0020mg/L for all 33 parameters that were tested.

There was no significant difference between larvae growth and abundance between roadside and agricultural pools throughout the experiment (Table 9). However, comparisons in larval growth could only be considered for ponds A1, A2, A3, and R1, where salamanders survived. At initial visits to the pools in January, larvae were more abundant in roadside pools (Table 10). For two of the roadside pools, 50 larvae were collected in under 20 dips and 48 salamanders were collected at the other (Table 11). Only one agricultural pool collected 50 salamanders, and only 5 and 2 larvae were collected from the other pools (Table 11). Abundance of larvae decreased in every pool between the first and second visit, and no salamanders were found in R2 or R3 at the second visit (Table 11). The loss of salamanders in two of the roadside ponds could have affected length values and statistical comparisons of length between the vernal pool types.

**Discussion**

While there were no significant differences found in salamander growth and abundance between agricultural and roadside vernal pools, the complete loss of salamander larvae from previously abundant vernal pools R2 and R3 is noteworthy. The winter of 2014 experienced lower temperatures and greater snowfall than average. According to the Baltimore Sun, average snowfall for the Baltimore area is 20.1 inches, but during the span of this experiment, about 78 inches of snow was accumulated (Loricchio, 2014). The increase in snow accumulation likely resulted in increased road salt application which could have affected conductivity of roadside vernal pools. Results showed that conductivity was markedly greater in roadside pools than agricultural pools and was substantially higher in R2. A study by Karraker, Gibbs, and Vonesh (2008) found that larval spotted salamander survival reduced with increased conductivity. High conductivity resulted in adverse physiological effects and decreased benthic invertebrate populations, a food source for larvae (Karraker et al., 2008). Sanzo and Hecnar (2005) found that increased salt concentration affected time to metamorphosis, weight, activity, and the presence of abnormalities in amphibians due to increased osmotic dehydration. High conductivity levels found in R2 could be the cause of salamander mortality and explain the disappearance of larvae in that pool.

Roadside pool 2 (R2) had very low dissolved oxygen levels which could have affected larvae and caused mortality. Larvae respire via diffusion of gases between the water and their external gills. The concentration of oxygen within the water affects the concentration gradient and diffusion rate of oxygen from the environment into the gills. Low dissolved oxygen levels would result in lower respiration rates in larvae which can have negative physiological effects (Skei & Dolmen, 2006).

Agricultural pool 2 (A2) had the greatest chlorophyll concentration and R1 had the second greatest chlorophyll concentration while R2 had the lowest concentration. Pools A2 and R1 had the greatest abundance of larvae while R2 had the lowest abundance. These results suggest that increased chlorophyll concentration could have positively affected larval abundance. High chlorophyll concentration in A2 is likely due to high nutrient levels in the pool which could be a result of runoff from nearby agricultural fields. Increased nutrient levels can increase primary production and food availability for herbivores. Salamander larvae consume both plants and herbivores in the pool so an increase in chlorophyll could directly and indirectly increase the amount of food available to larvae.

Metal concentrations were almost always greatest in roadside pools which could explain the loss of larvae in R2 and R3. Roadside pool sediments had significantly higher concentrations of chromium and nickel, and R2 had notably high aluminum concentrations and R3 had notably high manganese concentrations. According to the United States Environmental Protection Agency's (EPA) National Recommended Water Quality Criteria and Aquatic Life Criteria Table, several pools had metal concentrations that exceeded chronic levels; aluminum concentrations exceeded standard levels in all pools but R3, manganese concentrations exceeded standards in all pools but R1, copper levels exceeded standards in R2, and lead concentrations exceeded standards in A3 and R2 (United States Environmental Protection Agency, 2014). According to an EPA Sediment Contamination Report, several pools also exceeded ERL standard, the level at which that adverse effects to aquatic life are seen. Chromium concentrations were higher than the standard in R2 and manganese concentrations were higher than the standard in R3 (United States Environmental Protection Agency, 1999). According to the RATL, a database of Reptile and Amphibian Toxicology Literature, several vernal pools have metal concentrations that exceed the 7d LC50 found by the Canadian Wildlife Service's National Wildlife Research Centre. Aluminum concentrations exceeded LC50 values in the water of R3 and in the sediment of all six vernal pools; zinc concentrations exceeded LC50 values in the water of pools A1, A2, A3, and R2 and in the sediments of all six vernal pools; lead concentrations exceeded LC50 values in the sediment of A3 (Pauli, Perrault & Money, 2000). Metals can be directly toxic to larvae and disturb respiration by affecting ion exchange between the environment and gills (Skei & Dolmen, 2006).

The lack of detectable pesticides in any of the vernal pools could be attributed to the season at which the experiment was conducted. Vernal pools were sampled during the winter after being filled with precipitation and snow melt. However, most pesticides are applied during the spring and summer months, and can run off fields after application and during spring rains. It is possible that detectable levels and adverse effects will not be seen until the spring when pesticides are applied and could potentially run off or leach into the pools. Undetectable pesticide levels could also be due to limits in testing. There were 23 pesticides sprayed within the two last years on the fields adjacent to the vernal pools but due to costs and limits of the CEL, only 5 pesticides were tested for. It is possible that another pesticide or metabolite is present in the vernal pools but was not tested or found. Several studies have shown that pesticides can reduce larval survival, lower growth rates, and increase physical abnormalities (Rohr et al., 2003; Jones et al., 2010). Pesticides are designed to kill unwanted pests but can also cause similar toxic affects to non-targeted organisms by disturbing the nervous or endocrine system.

**Conservation Implications**

Amphibians play an important role in both aquatic and terrestrial ecosystems. Salamanders specifically perform key ecological roles by consuming a variety of plants, invertebrates, and vertebrates within terrestrial and aquatic environments. Salamander density affects prey density, trophic cascades, nutrient cycling, and detritus-litter food webs, as well (Davic & Welsh, 2004). However, amphibian populations, including salamanders, are declining globally which could seriously affect the stability of many ecosystems. It is this ecological importance of salamanders that makes conservation of vernal pools necessary.

Dr. Calhoun of University of Maine Orono says there are five common threats to amphibians: disease, invasive species, commercial exploitation, climate change, and habitat loss and alteration (Calhoun & deMaynadier, 2007). Habitat loss and alteration is thought to be the greatest threat to amphibian species and chemical contamination and pollution is a form of habitat alteration. This experiment found that conductivity, dissolved oxygen, and metal concentrations likely affected larval abundance and growth in roadside pools. Increases in conductivity, decreases in dissolved oxygen, and increases in metal concentration could be results of pollution from road runoff, and it is also possible that pollutants could run off from agricultural fields later in the year which could cause changes in the water quality of nearby vernal pools. These forms of pollution have altered the habitat for larvae and this study showed how that in turn may have affected their development.

Researchers Preisser, Kefer, Lawrence and Clark (2000) from Yale University say that constraints in vernal pool conservation fall into 2 categories: ecological and social. Ecological limitations include the difficulty in the ability to identify and define vernal pools and ephemeral wetlands (Preisser, Kefer, Lawrence & Clark, 2000). Social constraints include the lack of legislation and implementation in vernal pool protection (Preisser, et al., 2000). In order to conserve these wetlands and salamander species, nation, state, and local governments along with land use organizations, environmental organizations, scientists, and educators need to be involved.

The United States Clean Water Act defines a wetland as “areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soils. Wetlands generally include swamps, marshes, bogs, and similar areas” (United States Environmental Protection Agency, 2012). This definition does not include ephemeral wetlands like vernal pools. However, the Maryland state definition of non-tidal wetlands also recognizes wetlands containing “signification plant or wildlife value” of several unique community types including vernal pools

(Department of the Environment). First, scientists and government organizations need to work together to establish complete and accurate definitions of wetland types in order to identify and conserve wetland areas on a local scale.

The Kingstown, Maryland area is mainly rural with primarily corn and soybean agriculture. However, the results of this experiment showed the importance of roads and urban impact on water quality of local vernal pools, and the area is becoming more urbanized with more suburban neighborhoods being developed. With this increase in development, results of studies like this need to be considered in order to preserve fragile wetland ecosystems. Therefore, there is a great need for more research within the local scientific community to further examine the importance of these wetland species and how anthropogenic activities affect their survival. Further research ideas could include: field studies to better understand what specific contaminants are polluting local wetlands, laboratory studies to determine how these contaminants directly affect local species, lab and field studies to determine the indirect effects of contaminants on local species and how contaminants work together, and lab and field studies to determine the importance of buffer zones and the distance contaminants can travel through groundwater and runoff. This study only looked at the effects of land use and habitat quality on the larval stage of salamanders but these parameters could have different effects on salamander egg masses or adults which eventually disperse into upland habitats. Research examining all life stages in salamander development is also needed.

It is necessary that scientific research is shared with environmental organizations and educators to facilitate awareness and with local government and land use organizations to develop appropriate conservation strategies within an area to prevent habitat destruction. Once wetlands can be defined and identified, wetland delineators and scientists can determine the watershed of individual wetlands or vernal pools which can then be considered during land use planning and zoning.

As a threatened species in the state of Massachusetts, the Department of Natural Resource Conservation and University of Massachusetts Amherst conducted a ten year study monitoring 14 vernal pools in Western Massachusetts in order to develop a conservation plan for marbled salamanders (McGarigal, Compton, & Gamble, 2008). Their first step was to identify potential vernal pools with high connectivity to other pools and upland habitats where population persistence was likely to occur. This was done by developing a resistant kernel model. This model was then made into an ArcGIS shapefile that could be used by conservation planners. Their second step was to visit high ranking potential vernal pools and confirm the presence of standing water and marbled salamanders. Step three then involved assessing the integrity of the pools, their surrounding upland habitat, and connections between pools. The researchers then brainstormed several conservation tactics and evaluated them in order to prioritize which were the most important or useful. They concluded that land acquisition/conservation restrictions, which involved preventing development between and near pools, were the most useful, followed by environmental review and regulation, education, tunnels and barrier to limit road threats, wetland creation, and then population seeding (McGarigal, Compton, & Gamble, 2008). The researchers suggested that further modeling be done to addresses parameters like seasonality, land use, and road type in order to examine how changes in land parcels affect the viability of salamander populations. They also suggest more extensive surveying to confirm existing populations by either visual walking through habitats or trapping, as well as intensive research in the breeding requirements, life histories, harbitat selection, movement ecology, and sources of mortality of salamanders (McGarigal, Compton, & Gamble, 2008). Examining studies like this could be useful to organizations or areas like Chino Farms in determining the location and role of marbled salamanders in an ecosystem and developing effective strategies for protecting specific developmental stages and overall populations of marbled salamanders.

In conclusion, this experiment found that roads can have negative impacts on the survival of marbled salamander larvae due to the runoff of metals and deicing road salts into nearby vernal pools.

Further studies during the spring and summer could provide better insight into the impacts of agriculture on marbled salamander populations and whether regulations or restrictions regarding farming practices are necessary. Findings from this study suggest that in order to preserve marbled salamander populations the frequency and magnitude of road salt applications, future development and construction of impervious surfaces, and riparian buffer zones need to be regulated within the watershed of salamander-containing vernal pools. This would reduce metals, salts, and other contaminants from leaching or running into nearby wetlands and would likely improve the habitat quality and survival rate of marbled salamander populations.

**Figures**


Figure 1. Life history stages of marbled salamander (McGarigal, Compton, & Gamble, 2008).

Figure 2. Seasonal breeding pond (McGarigal, Compton, & Gamble, 2008).


Figure 3. Map of four out of six vernal pools sampled for the experiment


Figure 4. Map of two out of six vernal pools sampled for the experiment

| Farm Field | N1 (A1 and A2) | N2 (A1 and A2) | O1 (A3) | T2 (R1) |
|---|---|---|---|---|
| **2012** | Harmony Extra SG | Aatrex Nine-O | Aatrex Nine-O | LV Ester 24D |
| | LV Ester 24D | Gramoxone Inteon | Gramoxone Inteon | Touchdown Total |
| | Select Max | Lexar | Lexar | |
| | First Rate | Princep 4L | Princep 4L | |
| | Dual II Mag 2.5 | Touchdown Total | Touchdown Total | |
| | Touchdown Total | | | |
| | Canopy DF | | | |
| | Gramoxone SL2 | | | |
| | Basis Blend | | | |
| | Simazine | | | |
| | Unison | | | |
| | Roundup Power Max | | | |
| | Cadet | | | |
| | Aatrex Nine-O | | | |
| | Gramoxone Inteon | | | |
| **2013** | Gramonone SL2 | Harmony Extra SG | Harmony Extra SG | Gramonone SL2 |
| | Lexar | Touchdown Total | Touchdown Total | Princep 4L |
| | Princep 4L | Tilt | Tilt | Lexar |
| | Basis Blend | | | |
| | Roundup Power Max | | | |
| | Cadet | | | |
| | Touchdown Total | | | |
| | Medal | | | |
| | Canopy DF | | | |
| | Unison | | | |
| | Shadow/clethodim 2EC | | | |
| | Basagran | | | |
| | Dual II Mag 2.5 | | | |

Figure 5. Pesticides used on fields adjacent to test sites during 2012 and 2013

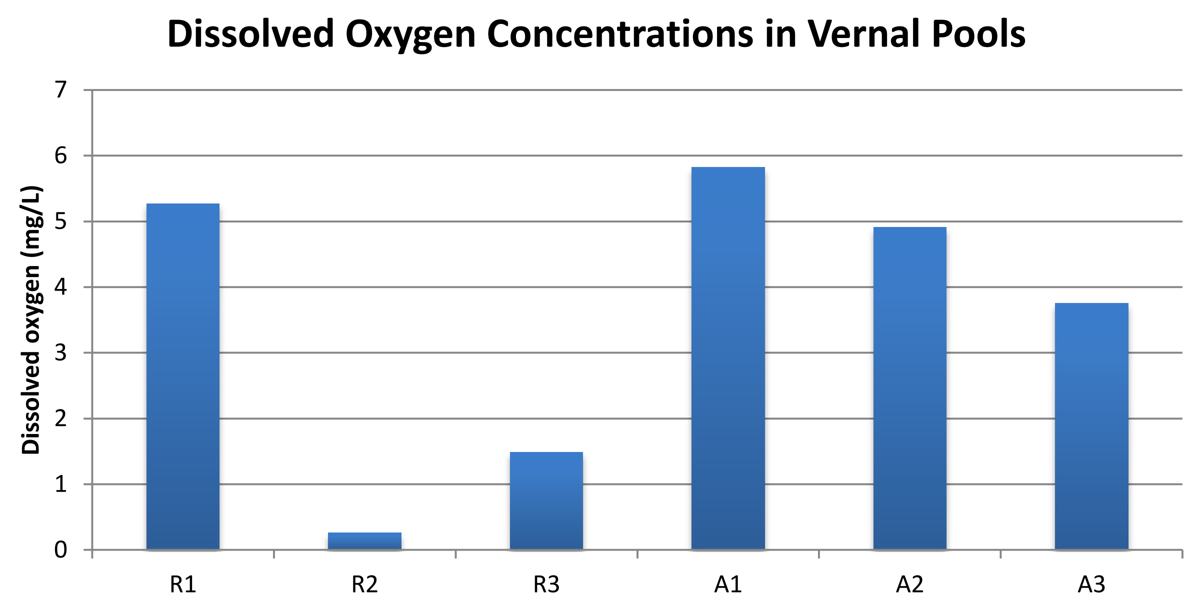


Figure 6. Dissolved oxygen concentrations of six vernal pools in Kingstown, Maryland during the second visits in March 2014 measured using a HACH probe .

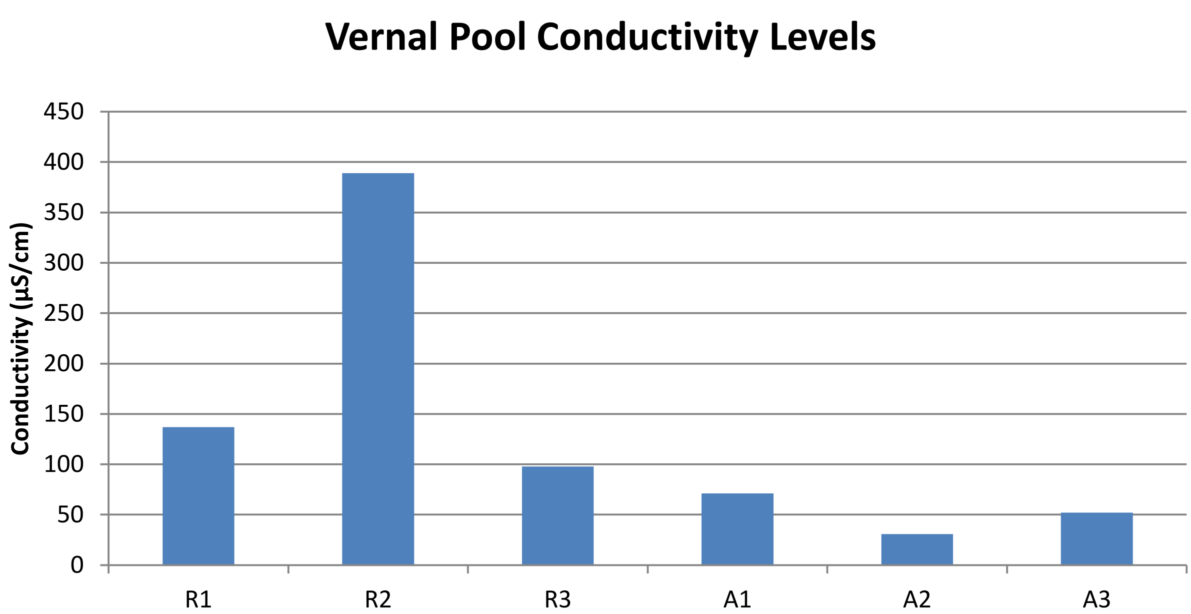


Figure 7. The conductivity of six vernal pools in Kingstown, Maryland during March 2014 measured using a HACH probe.

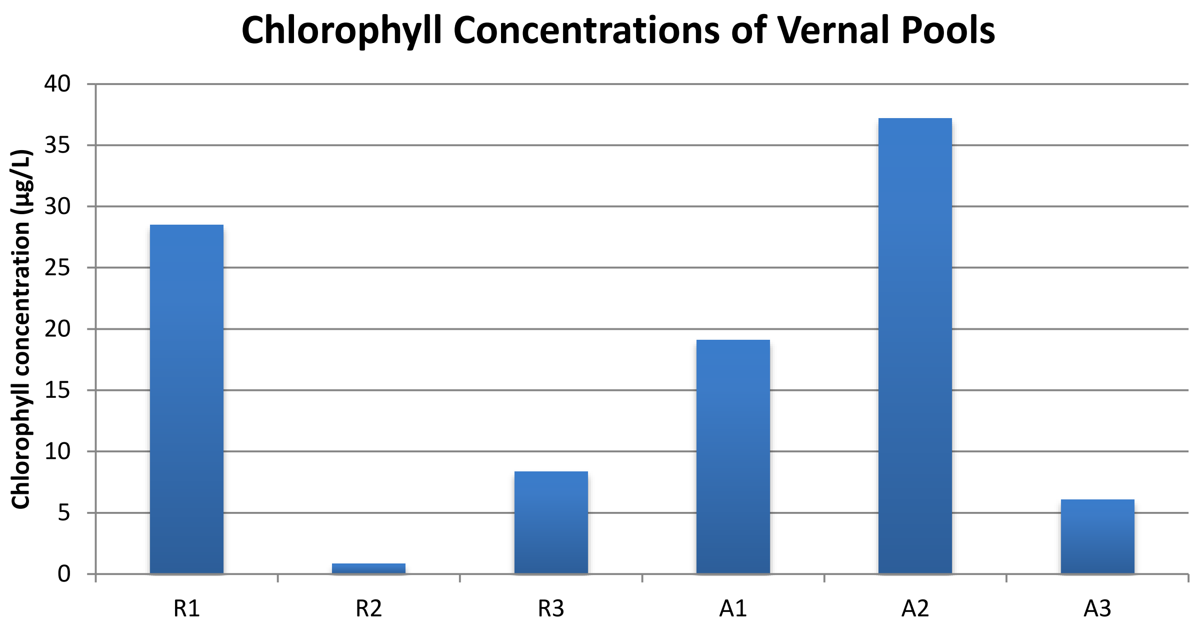


Figure 8. The chlorophyll concentrations of water samples collected from six vernal pools in Kingstown, Maryland in January 2014 was measured using fluorometry.

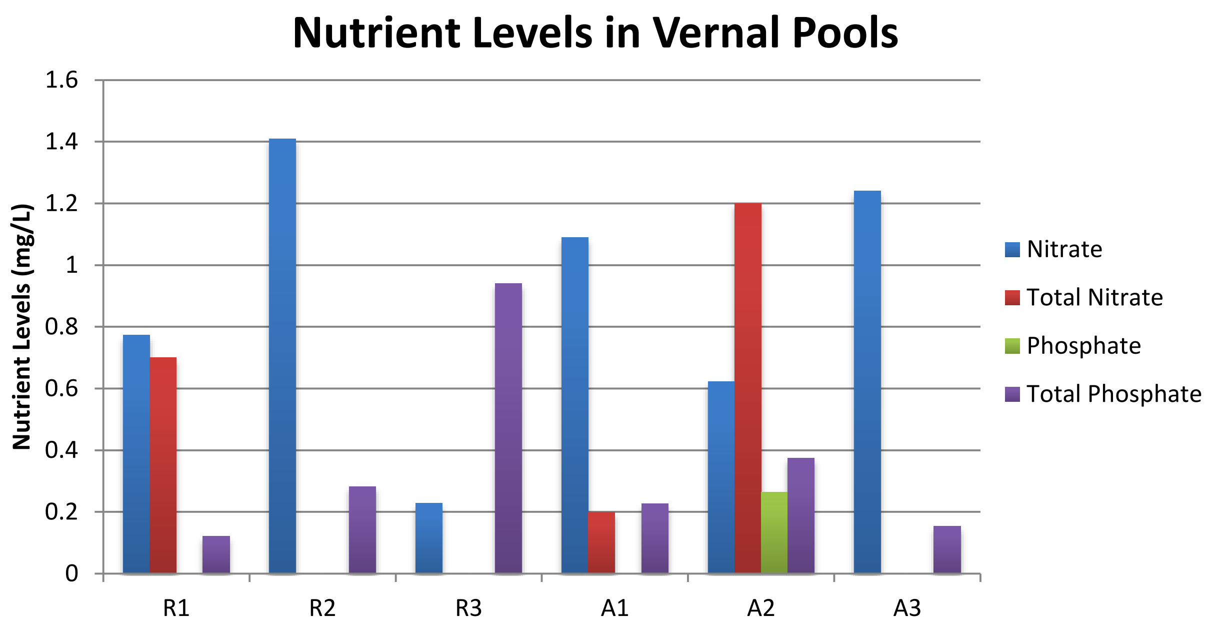


Figure 9. Nitrate, total nitrate, phosphate, and total phosphate concentrations of six vernal pools water samples during January 2014 were found using HACH TNT kits and a mass

**Tables**

Table 1. The results of t-tests showing the significance between water quality parameters of roadside vernal pools and agricultural vernal pools

| | p-value |
|---|---|
| Dissolved oxygen (mg/L) (visit 1) | 0.114263 |
| Dissolved oxygen (mg/L) (visit 2) | 0.111403 |
| Conductivity (uS/cm) | 0.115303 |
| Temperature (Celcius) (visit 1) | 0.488598 |
| Temperature (Celcius) (visit 2) | 0.252899 |
| pH (visit 1) | 0.122492 |
| pH (visit 2) | 0.220160 |
| Chlorophyll (ug/L) | 0.268915 |
| Nitrate (mg/L) | 0.336911 |
| Total Nitrate (mg/L) | 0.411534 |
| Phosphate (mg/L) | 0.211325 |
| Total Phosphate (mg/L) | 0.263249 |

Table 2. Water quality parameter values were found for six vernal pools, 3 roadside and 3 agricultural pools, in Kingstown, Maryland during the winter of 2014

| Water quality parameter | R1 | R2 | R3 | A1 | A2 | A3 | Road Average | Ag Average |
|---|---|---|---|---|---|---|---|---|
| Dissolved oxygen (mg/L) | 7.06 | 1.70 | 5.1 | 3.60 | 4.74 | 4.3 | 4.62 | 4.21 |
| Conductivity (µS/cm) | 137 | 389 | 97.9 | 71.1 | 30.9 | 52 | 207.97 | 51.33 |
| Temperature (°Celcius) | 8.45 | 9.35 | 5.2 | 7.85 | 10.2 | 7.35 | 7.67 | 8.47 |
| pH | 6.96 | 6.24 | 7.59 | 6.48 | 6.52 | 5.88 | 6.93 | 6.29 |
| Chlorophyll (µg/L) | 28.5 | 0.82 | 8.33 | 19.1 | 37.2 | 6.06 | 12.55 | 20.79 |
| Nitrate (mg/L) | 0.77 | 1.41 | 0.23 | 1.09 | 0.62 | 1.24 | 0.73 | 0.98 |
| Total Nitrate (mg/L) | 0.7 | 0 | 0 | 0.2 | 1.2 | 0 | 0.23 | 0.47 |
| Phosphate (mg/L) | 0 | 0 | 0 | 0 | 0.26 | 0 | 0 | 0.15 |
| Total Phosphate (mg/L) | 0.12 | 0.28 | 0.94 | 0.23 | 0.38 | 0.15 | 0.14 | 0.23 |

Table 3. The results of t-tests showing the significance of metal concentrations between agricultural and roadside vernal pools

| | p-values |
|---|---|
| Al | 0.163410 |
| Cr | **0.002921** |
| Mn | 0.209357 |
| Fe | 0.200562 |
| Ni | **0.028532** |
| Cu | 0.412733 |
| Zn | 0.422639 |
| Ar | 0.16194 |
| Cd | 0.16194 |
| Pb | **0.032406** |

Table 4. Metal concentrations in ppb found in sediment samples from six vernal pools and US EPA exceedance levels (ERL) and LC50 levels for metals.

| Pool | 27Al | 52Cr | 55Mn | 56Fe | 60Ni | 65Cu | 66Zn | 75As | 111Cd | 208Pb |
|---|---|---|---|---|---|---|---|---|---|---|
| A1 | 6042.6 | 2.90 | 33.03 | 2152.22 | 7.47 | 9.92 | 49.43 | 0.305 | 1.07 | 35.82 |
| A2 | 6931.5 | 3.29 | 162.28 | 4739.55 | 6.52 | 11.56 | 54.61 | 0.86 | 0.55 | 27.62 |
| A3 | 4994.7 | 1.42 | 80.16 | 10262.84 | 7.17 | 9.70 | 41.46 | 1.38 | 0.27 | 42.25 |
| R1 | 9256.7 | 7.89 | 111.21 | 9496.23 | 15.86 | 10.01 | 31.76 | 2.07 | 0.30 | 10.39 |
| R2 | 9319.0 | 8.71 | 75.83 | 9223.44 | 18.02 | 8.24 | 46.76 | 1.63 | 0.23 | 14.49 |
| R3 | 4893.3 | 6.16 | 365.01 | 6120.90 | 11.30 | 14.36 | 75.97 | 0.62 | 0.37 | 28.43 |
| ERL | unknown | 8.1 | 300 | 17000 | 21 | 34 | 150 | 8.2 | 1.2 | 47 |
| LC50 | 50 | 30 | 142 | unknown | 50 | 40 | 10 | 40 | 40 | 40 |

Table 5. Ranking of metal concentrations in sediment from six ponds, from highest concentration to lowest

| Aluminum | Chromium | Magnesium | Iron | Nickel | Copper | Zinc | Arsenic | Cadmium | Lead |
|---|---|---|---|---|---|---|---|---|---|
| R2 | R2 | R3 | A3 | R2 | R3 | R3 | R1 | A1 | A3 |
| R1 | R1 | A2 | R1 | R1 | A2 | A2 | R2 | A2 | A1 |
| A2 | R3 | R1 | R2 | R3 | R1 | A1 | A3 | R3 | R3 |
| A1 | A2 | A3 | R3 | A1 | A1 | R2 | A2 | R1 | A2 |
| A3 | A1 | R2 | A2 | A3 | A3 | A3 | R3 | A3 | R2 |
| R3 | A3 | A1 | A1 | A2 | R2 | R1 | A1 | R2 | R1 |

Table 6. The results of t-tests showing the significance of metal concentrations between water samples from agricultural and roadside vernal pools

| | p-values |
|---|---|
| Al | 0.135720 |
| Cr | 0.411263 |
| Mn | 0.157217 |
| Fe | 0.385015 |
| Ni | 0.308022 |
| Cu | 0.307349 |
| Zn | 0.176329 |
| Ar | 0.384003 |
| Cd | 0.125063 |
| Pb | 0.335073 |

Table 7. Total extractable metals in ppb found in water samples from six vernal pools and US EPA chronic levels and LC50 levels for metals.

| | 27Al | 52Cr | 55Mn | 56Fe | 60Ni | 65Cu | 66Zn | 75As | 111Cd | 208Pb |
|---|---|---|---|---|---|---|---|---|---|---|
| A1 | 433.56 | 0.57 | 110.47 | 489.15 | 3.04 | 0.96 | 20.97 | 1.15 | 0.17 | 2.52 |
| A2 | 265.22 | 1.90 | 356.36 | 98.95 | 1.24 | 0.68 | 12.01 | 0.35 | 0.09 | 0 |
| A3 | 626.08 | 0.35 | 103.42 | 1108.48 | 2.80 | 3.17 | 19.76 | 0.55 | 0.10 | 2.78 |
| R1 | 185.05 | 0 | 45.82 | 561.24 | 3.35 | 2.33 | 7.29 | 0.56 | 0.04 | 0.28 |
| R2 | 469.36 | 2.49 | 102.89 | 1427.72 | 5.60 | 5.14 | 21.43 | 1.74 | 0.13 | 3.17 |
| R3 | 39.24 | 0 | 83.08 | 145.91 | 0.67 | 0.08 | 3.61 | 0.25 | 0.02 | 0 |
| Chronic | 100 | 11 | 100 | 1000 | 52 | 5 | 120 | 150 | 0.25 | 2.6 |
| LC50 | 50 | 30 | 142 | unknown | 50 | 40 | 10 | 40 | 40 | 40 |

Table 8. Ranking of total metals in the water of six vernal pools, from highest concentration to lowest

| Aluminum | Chromium | Magnesium | Iron | Nickel | Copper | Zinc | Arsenic | Cadmium | Lead |
|---|---|---|---|---|---|---|---|---|---|
| A3 | R2 | A2 | R2 | R2 | R2 | R2 | R2 | A1 | R2 |
| R2 | A2 | A1 | A3 | R1 | A3 | A1 | A1 | R2 | A3 |
| A1 | A1 | R2 | R1 | A1 | R1 | A3 | R1 | A3 | A1 |
| A2 | A3 | A3 | A1 | A3 | A1 | A2 | A3 | A2 | R1 |
| R1 | R1 | R3 | R3 | A2 | A2 | R1 | A2 | R1 | A2 |
| R3 | R3 | R1 | A2 | R3 | R3 | R3 | R3 | R3 | R3 |

Table 9. The results of t-tests showing significance of larvae length and growth between roadside and agricultural vernal pools.

| | p-value |
|---|---|
| Length at first visit | 0.10101 |
| Length at second visit | 0.08073 |
| Change in length | 0.09306 |

Table 10. Average length of salamanders in centimeters for two visits to six vernal pools and the change in length between visits.

| Visit | R1 | R2 | R3 | A1 | A2 | A3 |
|---|---|---|---|---|---|---|
| 1 | 2.066 | 1.8544 | 2.1404 | 1.95 | 1.887 | 1.733 |
| 2 | 2.635 | - | - | 2.90 | 2.606 | 2.833 |
| Change | 0.569 | -1.8544 | -2.1404 | 0.95 | 0.719 | 1.1 |

Table 11. The total number of salamanders caught at six vernal pools for either 100 dips or until the number of dips until 50 salamanders were caught.

| Visit 1 | R1 | R2 | R3 | A1 | A2 | A3 |
|---|---|---|---|---|---|---|
| Number of dips | 19 | 100 | 16 | 100 | 15 | 100 |
| Total larvae | 50 | 48 | 50 | 2 | 50 | 5 |

| Visit 2 | R1 | R2 | R3 | A1 | A2 | A3 |
|---|---|---|---|---|---|---|
| Number of dips | 22 | 100 | 100 | 100 | 90 | 100 |
| Total larvae | 50 | 0 | 0 | 1 | 50 | 3 |

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