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An Assessment of the Potential Success of Translocation as a Conservation Strategy for Western Toads (Anaxyrus boreas) at the Robert J. Bernard Biological Field Station

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An Assessment of the Potential Success of Translocation as a Conservation Strategy for Western Toads (*Anaxyrus boreas*) at the Robert J. Bernard Biological Field Station

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by

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Abstract

In response to imminent habitat destruction at the Robert J. Bernard Biological Field Station (BFS), translocation was assessed as a conservation strategy for a population of Western toads (*Anaxyrus boreas*). Currently, the BFS is home to a relatively unstudied population of Western toads, which rely on the existence of a seasonal breeding pond in open land owned by Harvey Mudd College on the west side of the field station. Unfortunately, there are plans to develop this plot of land within the next few years and so the breeding pond will be destroyed. In an effort to protect the Western toads, which are listed as Near Threatened by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species, a new breeding pond was developed within the protected portion of the field station. Then, the potential of the new pond to be used as a habitat for Western toads was assessed. Pond temperatures, light intensities, algae growth, and suspended material were measured in both the original and the new ponds from January to April, 2010 and were found to be suitable in both locations for the development of Western toad tadpoles. When eggs were laid in the original breeding pond, egg and then later tadpole development were monitored in the field. Additionally, nearly 400 tadpoles were captured and raised in the laboratory in water from both the new and old ponds (as well as dechloraminated tap water) in order to determine how and to what degree the different pond water types affected the development and survival of the tadpoles. In the laboratory, tadpole survival and the percent of tadpoles to achieve full metamorphosis was higher in water from the new pond than water from the original breeding pond, suggesting that there is nothing apparent about the water chemistry in the new pond that would limit tadpole development. Lastly, a mini-translocation of 400 young tadpoles was completed as a trial for a full-scale relocation attempt in the future. These tadpoles developed normally in the new pond when compared to tadpoles from the original pond and also had a high survival rate (at least 75%) to full metamorphosis. Translocation success cannot be determined without long-term monitoring and unfortunately, although the aquatic habitat of the new pond seems suitable for relocation, the terrestrial habitat as well as the predator density surrounding the new pond may limit its success in the future. However, due to the lack of time before the original breeding pond is destroyed, full-scale translocation of eggs and tadpoles during the next breeding season is recommended.

Introduction

Over the past several decades, there has been an enormous global decline in biodiversity, suggesting that Earth may be on the brink of the largest mass extinction in 65 million years (Cushman 2006). Currently, there are more than 17,300 known threatened species (Baillie *et al*. 2004). Of these, amphibians constitute the highest proportion of threatened species, with one in three amphibian species threatened or endangered (Cushman

2006). Since the late 1950's, over 122 amphibian species have disappeared (Mendelson III *et al.* 2006). Unfortunately, the severity of the threats facing amphibian populations worldwide was not internationally recognized until the First World Congress of Herpetology in 1989, at which point massive amphibian decline had already occurred (Collins and Storfer 2003; Houlahan *et al.* 2000).

Although not the only threatened group of species, amphibians are particularly vulnerable to environmental changes due to their: (1) low vagilities, (2) high vulnerability to pathogens, invasive species, and climate change/increased UV exposure, (3) susceptibility to both terrestrial and aquatic pollution (heightened by the extreme permeability of their skin), (4) herbivorous and carnivorous diet at varying life stages, relying on both floral and faunal health, and (5) need for both a suitable aquatic and a suitable terrestrial habitat with ample dispersal area and protection in order to be able to survive and successfully reproduce (Cushman 2006).

Currently, anthropogenic impacts make up the majority of the threats to the overall health of world ecosystems and to the survival of individual species. Introduction of invasive species, over-exploitation of resources, dramatic land-use changes, increased use of pesticides and toxic chemicals, and increased pollution, are some of the many anthropogenic causes of species decline (Houlahan *et al.* 2000). Of these, habitat destruction, climate change, and the spread of infectious disease are consistently cited as being the primary threats to amphibian health (Collins and Storfer 2003; Baillie *et al.* 2004; Gardner 2001; Cushman 2006). As the human population expands and competition for resources intensifies, these threats will likely increase and the number of threatened and endangered amphibians will continue to rise unless dramatic conservation measures are taken (Houlahan *et al.* 2000).

If these issues are not confronted, amphibian populations will continue to decline until it is too late to save them. The disappearance of amphibian species will undoubtedly have drastic unpredictable effects on entire ecosystems. Additionally, the loss of amphibians will directly impact humans, who currently rely on amphibians for medical advancements, protein sources, and for the reduction of malaria-ridden mosquito populations. Furthermore, with the loss of amphibian populations, humans will lose the use of amphibians as indicators of ecosystem health and environmental change (Landres *et al*. 1988).

To prevent the further deterioration of amphibian populations, conservation programs need to create strategies to mitigate the biggest threats facing amphibians. Recent research has demonstrated that habitat destruction and fragmentation are among the largest threats to amphibians (Cushman 2006). Because most amphibians need to have both a high quality terrestrial habitat, an aquatic breeding site, and also a clear dispersal zone between these two habitats, habitat loss directly affects species survival (Cushman 2006).

In response to the high frequency of habitat destruction, species translocation (introduction, relocation, and repatriation) has become increasingly popular as a conservation method for threatened amphibian populations (Dodd and Seigel 1991). Although there is some debate about the correct usage of translocation vocabulary, for the purpose of this study, the following definitions were used:

1. Translocation- the broad act of intentionally changing the location or position of a population as a means of conserving a species.
2. Introduction- the release of individuals of a species into an area not historically occupied by that species.

3. Relocation- the moving of an individual or a population away from a threat or a threatened area.
4. Repatriation- the release of a population of a species into an area formerly (or currently) occupied by that species.

Popular among marketing campaigns, translocation efforts (both of wild and captive-bred individuals) consistently gain international press and funding and are marketed as necessary solutions to dramatic species decline. In fact, the Amphibian Conservation Summit in 2007 deemed translocation to be one of three primary long-term conservation goals of the Amphibian Conservation Action Plan (Germano and Bishop 2007). However, despite its popularity, the effectiveness of translocation as a conservation strategy for anurans has been debated widely over the past few decades in response to a review by Dodd and Seigel (1991), claiming that amphibians are not suitable for translocation. In their review, Dodd and Seigel (1991) cited several examples of failed translocation attempts, including the failed attempt of researchers to establish a self-sustaining population of Houston toads, *Bufo houstonensis*, after introducing over 500,000 individuals at various life history stages between 1982 and 1990 to protected areas in Houston. Several studies have since countered their review, claiming translocation success from various amphibian and reptile conservation programs. For example, a summary of 91 amphibian and reptile translocation attempts from 1991 to 2006 concluded that 42% of these attempts were successful (meaning that they produced a viable, self-sustaining population in the wild that was monitored for a sufficient period of time in order to accurately determine success), with an additional 29% having uncertain but potential long-term success (Germano and Bishop 2007). Of the 91 summarized translocation attempts, only 28% had completely failed. Dodd and Siegel themselves later

argued that translocation could be a useful species-specific conservation strategy (Siegel and Dodd 2002).

Regardless of the general success or failure of conservation programs, species are continuing to disappear each year and vital resources, such as water and food, are becoming increasingly limited. Without the implementation of conservation strategies, habitats will continue to be destroyed and more species are going to disappear over the next several decades. On the brink of complete habitat destruction, translocation may be necessary as a final attempt to save a population living in an area that can no longer be protected and so is no longer a viable habitat for a population's survival (Bloxam and Tonge 1995).

For example, in response to looming habitat alteration, relocation may be the only way to save the population of Western toads at the Robert J. Bernard Biological Field Station (BFS), a biological research and teaching center serving students and faculty of The Claremont Colleges in Claremont, California. In response to the dire need for population relocation, the existing habitat used by the Western toad at the BFS was assessed in order to determine translocation requirements and predict the potential future success of relocation as a conservation strategy for their population.

With a moderate size (30.4 hectares) and a wide range of vegetation types, the BFS is heavily used by over 350 animal species (BFS website). Centered in the middle of an urban area, the BFS serves as the primary habitat for several wildlife populations that are searching for open and protected patches of land in a highly developed area. Included in its list of inhabitants, the BFS is home to three known species of anurans: the Western toad, *Anaxyrus boreas halophilus* (formerly *Bufo boreas halophilus*; Crother 2008), the American Bullfrog, *Lithobates catesbeianus*, and the recently reported Baja California Treefrog, *Pseudacris*

hypochondriaca. However, little is currently known about their local population ecologies, as no studies have been done on these three species within the BFS.

Found along the Pacific Coast of North America eastward to central Montana, Wyoming, Utah, Colorado, and New Mexico (Figure 1), Western toads were temporarily listed as Endangered in 1996 (and still are listed as endangered in the state of Colorado) and have been listed as Near Threatened since 2004 when it was noticed that Western toad populations were again in significant decline (Baillie *et al.* 2004).

http://www.iucnredlist.org/apps/redlist/details/3179/0

Figure 1. Known distribution of Western toads throughout the world.

Recently, populations of Western toads have declined drastically in Colorado, Wyoming, and areas of California, likely due to disease/fungi, increased ultraviolet radiation, extreme predation, habitat destruction, competition with other species, and environmental changes leading to immunosuppression (Baillie *et al.* 2004).

Adult Western toads tend to vary in coloration from brown to black but commonly have a white or yellow dorsal stripe and a pale throat (Figures 2 and 3). Adult females range in size from 75 to 100 mm in length and adult males range from 60 to 80 mm in length (Keinath and McGee 2005).

Figures 2 and 3. Photographs of the study species, *Anaxyrus boreas* (Western toad), taken at the Bernard Field Station on January 28th, 2010.

With females breeding only once every 2 years starting at the age of six, each laid clutch is large, ranging from 3,000-11,000 eggs depending on female body size and geographic location (Keinath and McGee 2005). Eggs, encased in two jelly layers, can range from 1.5 to 1.8 mm in diameter (Figures 4 and 5). Typically, two adjacent egg strands are deposited from a single female in shallow water.

Figures 4 and 5. Egg strands found at the Bernard Field Station; photographed on February 1st, 2010.

After approximately 10 days of egg development, Western toad tadpoles will hatch, emerging at a size of about 6 mm and then growing up to approximately 37 mm before metamorphosing. Tadpoles typically develop in breeding sites with temperatures ranging from 15 to 30°C (Campbell 1970). Metamorphosis can occur within 6 weeks after hatching, but can also take up to 13 weeks in cold water habitats. Throughout the development process, mortality is extremely high, with one study estimating the mortality rate between egg deposition and maturity to the adult stage being as high as 99% (Nussbaum *et al.* 1983). Mortality at young life stages can be caused by several factors, but is commonly the result of pond desiccation, predation, and habitat fragmentation (Bartelt 2000; Campbell 1970).

The Western toads at the BFS rely on a small seasonal pond as the location for the development of their eggs and larvae into juveniles. Unfortunately, the current location of the only known breeding pond used by the Bernard Field Station's population of Western toads lies adjacent to the main part of the BFS property in an area owned by Harvey Mudd College (HMC). Since HMC does not intend to preserve these 4.5 hectares of land as a conservation or research area, this breeding pond will likely be destroyed within the next few years.

With potential habitat destruction in the upcoming years leaving them nowhere to breed (as the only other previously existing body of water in the BFS is a large lake full of predators and competitors such as the American bullfrog), this population of Western toads will likely become locally extinct within the BFS unless immediate conservation actions are taken. With the aim of preventing the loss of this species from the BFS, the habitat ecology (temperature, light intensity, algae growth, and suspended matter) of their existing breeding pond was assessed, and the possibility of future large scale relocation (into a newly

constructed breeding pond in the protected portion of the BFS) was evaluated. Over a 12 week period, water temperatures, light intensities, and mass of suspended matter were measured in both the old and newly constructed breeding ponds and were then compared. Upon the appearance of egg clutches in the old breeding pond, eggs were photographed daily and monitored for development. When the tadpoles hatched, tadpole growth and development in the old pond was also monitored.

Additionally, tadpoles were captured and reared in the laboratory in order to document growth, development, and survival. These tadpoles were reared in three different water treatments (water from the old breeding pond, water from the newly constructed breeding pond, and dechloraminated tap water as a control) in order to determine their water chemistry requirements. This helped to predict future relocation success into the newly constructed pond by comparing development and survival in water from the existing pond and from the ideal release site. Lastly, after it was shown in the laboratory that the tadpoles could survive in water from the new pond, 400 tadpoles were transferred from the old to the new pond. The developmental rates of the relocated tadpoles were compared to those of the tadpoles remaining in the old pond and their percent metamorphosis was recorded. Upon project completion, tadpoles and metamorphs were released at the new pond in order to encourage future use of the protected area since time may not permit continued research before species translocation is needed.

METHODS

Site Description

This study was conducted at the Robert J. Bernard Biological Field Station (BFS), located north of Harvey Mudd College's (HMC) campus in Claremont, California (Figure 6). Constituting approximately 30.4 hectares of land, the BFS serves as a research and educational facility for members of the Claremont Colleges as well as the surrounding community. Adjacent to the western border of the BFS, there are 4.5 hectares of land which were owned by the Keck Graduate Institute (KGI) from 2000 to 2009, but were leased to the BFS for several years. In the spring of 2009, KGI sold the land to HMC, which did not continue to lease the land to the BFS. HMC is currently making plans to develop this plot of land, thus its future is uncertain.

The topography of the BFS is mainly flat with an average elevation of 318 meters above sea level. Although it is dominated by coastal sage scrub (as one of the largest remaining areas of coastal sage scrub in Los Angeles County), the BFS also consists of grassland, Riversidian alluvial fan scrub, and live oak forest. The BFS receives an average of 25 cm of rainfall per year, 90% of which is received between November and April. Temperatures in the area can range from -3°C to 42°C throughout the year. The BFS is home to over 350 species of wildlife, including the Western toad, *Anaxyrus boreas halophilus* (BFS website).

In the center of the BFS there is a 0.4 hectare, 6 meter deep artificial lake, "pHake Lake" (constructed in 1978), which provides an aquatic and marsh habitat for numerous birds, plants, and fish at the field station. Additionally, a small seasonal pond lies west of the lake in the area now owned by HMC, which serves as a habitat for fairy shrimp and for

tadpoles. This existing breeding pond for Western toads is filled with rain water and, as necessary, manually with a hose from December to July to facilitate tadpole development. This pond (which for the purposes of this study was labeled as ‘old’) fluctuates in size but has a maximum circumference of 36 meters and a maximum depth of 30.5 cm.

In the summer of 2009, an area in the BFS south of pHake Lake was selected for the creation of a new pond (‘new’) at the BFS to replace the old pond as a breeding area for Western toads once HMC develops the land adjacent to the BFS (Figure 6).

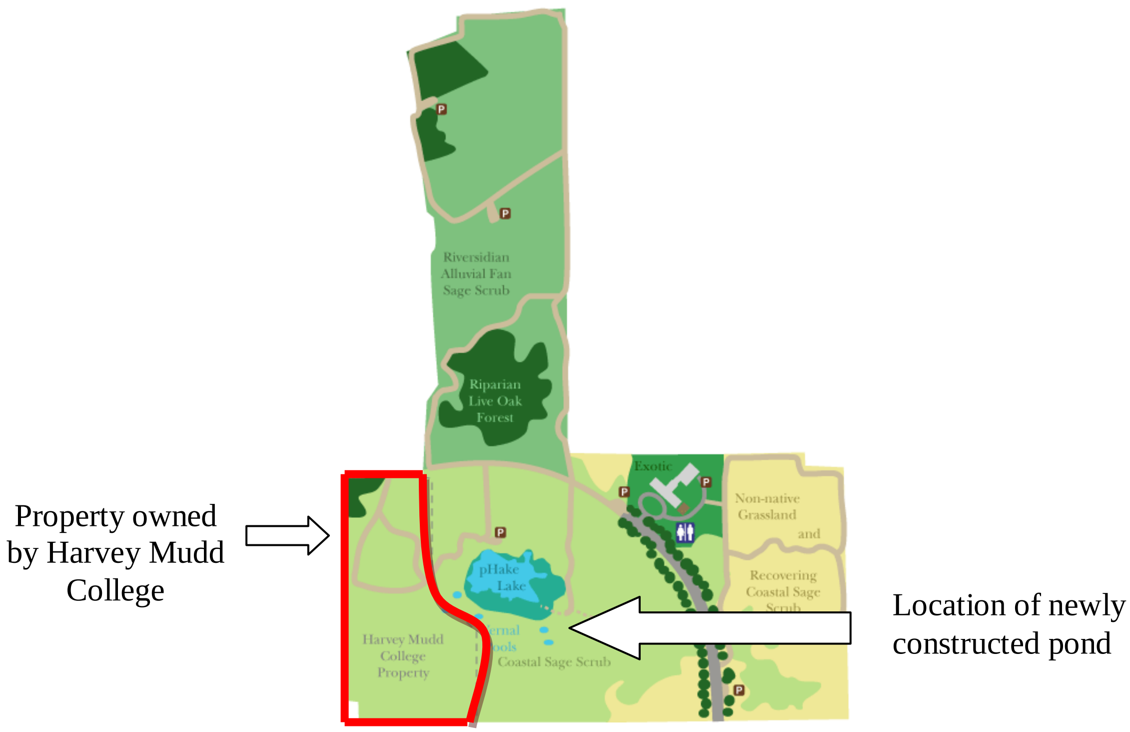

Figure 6. Map of the BFS indicating the property owned by Harvey Mudd College highlighted in red and the location of the newly constructed pond south of pHake Lake.

In October and November of 2009, the vegetation and the top layer of soil in the area were removed. Then, the area was reshaped to form a deeper pond base and a thick rubber liner was placed across the cleared surface in order to prevent seepage of the pond water into the absorbent base soil. The top layer of soil was replaced over the liner to keep it in place

and to provide a bottom layer of soil for the pond. While constructing the pond, a berm walkway was created in order to split the pond into two areas. By splitting the pond into two sections, one pond could then be used as a tadpole introduction site and the other could be used for toadlet release following metamorphosis. Furthermore, the berm facilitated movement of researchers across the pond in order to collect water, measure temperatures, and observe the tadpoles without having to walk through the water and disturb the bottom layer of soil and the tadpoles. The completed pond was 45.7 cm deep and had a maximum circumference of 43 meters (Figure 7). Once the pond was completely constructed, it was filled naturally by rainfall over several months. Initially the water was a murky brown color as the rainfall washed sediment into the pond. However, after several weeks without rain in March, the pond began to clear up, with the pond bottom slowly becoming visible.

Figure 7. Photograph of the newly constructed breeding pond at the BFS taken January 26th, 2010.

Habitat Assessment

Field Methods

From January 31st to April 8th, 2010, air and water temperature measurements were made in both the old and new breeding ponds every 1-3 days using a Fisher Scientific Dual Channel Thermometer. Temperature was measured in both shallow and deep areas of each pond and the depth of measurement as well as the temperatures (°C) were recorded and later compared between ponds. Measurements were made at different times during the day, but were usually taken between 10 AM and 4 PM. Light intensity measurements were also taken in shallow and deep areas of both ponds every week using a Milwaukee Smart Luxmeter.

As a means of measuring food availability in both ponds, six algae growth plates were deployed in each pond on February 20th, 2010 and left for 5 weeks in the field in order to allow time for natural algae in the ponds to grow on the plates. Algae plates were constructed by cutting a large piece of 5.5 mm thick acrylic plexi glass into 10 x 10 cm^2 squares. Then, pieces of hard plastic were placed on each corner of the plexi glass square. Mesh was glued around the plastic corners using a calking gun in order to create a protective covering of the plexi glass. The covering was created in order to prevent tadpoles from eating the algae growth on the plates; however, the mesh was not so small that it kept algae spores from entering. After 5 weeks, all algae growth plates were collected. Due to the large amount of mud on all of the plates as well as the destruction of several plates by coyotes while in the field, algae growth on the plates could not be quantitatively compared. However, pictures of the plates from the new and old ponds were taken and the presence or absence of algae on the plates (determined by the existence of green vegetation growth on the plates) was recorded.

Laboratory Methods

The mass of suspended matter in water from both the old and new ponds was compared. A 27 cm diameter piece of filter paper was dried in a drying oven, weighed, and then folded into a funnel shape. This filter paper was put inside a glass funnel and 4 liters of pond water from a specific treatment (new or old pond water collected from undisturbed areas of the pond) was poured into it. Once all of the water had passed through, the filter paper was again placed into the drying oven so that any remaining water would evaporate. The filter paper was then reweighed and the amount of sediment left on the filter was determined by subtracting the original weight from the new weight. For each pond (old and new), ten filter papers were used.

Tadpole Development

Tadpole Monitoring and Development in the Field

Beginning on January 18th, 2010, daily scouting for egg clutches in the old breeding pond occurred. Upon the appearance of egg clutches between January 26th and January 30th, cages were constructed using hard plastic mesh and were placed over the egg strands. These cages were designed in order to prevent the adult breeding toads from laying additional egg clutches in the same place so that the age of each egg clutch could be determined and egg clutch development could be monitored. However, three adult toads managed to get inside one of the cages on one occasion and so, although the toads were removed from the cage, the exact ages of each egg strand are not known. Egg strands were photographed daily in order to document and monitor egg development (Appendix 1).

When the first tadpoles emerged on February 11th, 2010, their presence was recorded and their behavior was observed (i.e. preferred location in the pond and level of activity). Every 7-12 days, 100 tadpoles were collected from the old pond (using thin mesh fishing nets so as to avoid contact with human flesh in an attempt to minimize disease transmission) and brought back to the laboratory to be photographed (Appendix 2). When photographed, tadpoles were placed individually into 60 x 15 mm sterile petri dishes atop a white board underneath two large white lights. Tadpoles were placed atop 1 x 1 mm^2 graph paper and were photographed when they were not moving and their tail was fully extended. Later, the size of each tadpole was measured on the computer by comparing the tadpole size (from the tip of the head to the tip of the tail) to the graph paper beneath it. After being photographed, tadpoles were immediately returned to the field so as to minimize stress and their time away from their home environment.

On March 3rd, 2010, 400 tadpoles were collected using mesh fishing nets and were moved to the newly established breeding pond in order to help determine if the tadpoles could survive in the translocation site. A wall consisting of cinder blocks spaced every 0.6 meters covered with heavy duty plastic (held down using small pebbles and large rocks) was built to completely surround the new pond so that when the tadpoles transformed into metamorphs, they would not be able to leave the immediate area (Figure 8). This facilitated toadlet capture, allowing percent survival to later be determined. When tadpoles were nearing metamorphosis, untreated wood boards (0.6 m^2) were placed around the edges of the new pond and the old pond in order to provide shelters for the vulnerable metamorphs with the hopes of increasing their survival.

Figure 8. Wall constructed in March, 2010 to surround the smaller portion of the newly constructed breeding pond at the Bernard Field Station.

When metamorphs emerged from the water into the enclosed area around the new pond, they were captured every 2-3 days and brought back to the laboratory. Once in the laboratory, the metamorphs were gently dried using a kim wipe and weighed. Weights were recorded and the metamorphs were returned to the field and released on the unenclosed side of the new pond.

Tadpole Collection and Care in the Laboratory

On February 14th, 2010, 36 plastic shoe boxes (30.5 x 16.5 mm^2) with a mesh-covered hole along the top edge were lined up in rows on two laboratory benches in room B42 in the Keck Science Center. On each bench, three 120 V lamps were placed 61 cm above the shoe boxes. The lamps were controlled by timers and were set to be on daily from 7 AM to 6 PM. Water was then collected from both the new and old ponds at the BFS and

returned to the laboratory in buckets. Fairy shrimp and large pieces of debris were filtered out of the water using a fine fishing net. Then, 2400 mL of either new, old, or tap (dechloraminated) water were poured into each shoebox and the lids were left off in order to allow complete circulation of air. Colored tape was used to label the boxes according to treatment and each box was given a unique number. Each treatment (tap, old, or new) consisted of 12 uncovered shoeboxes which were placed randomly on both laboratory benches. Once the 36 boxes were set-up, 360 tadpoles were collected from the old breeding pond at the BFS and 10 tadpoles were placed randomly into each box. Water temperatures were recorded three different times throughout the study.

Tadpoles were fed several times per day and were given constant access to food throughout the entire study. Their food consisted of boiled organic lettuce which was stored in the refrigerator before feeding. The water in each box was initially changed two times per week but starting on March 1st, the water was changed every 3 days. One week later, in response to noticeable tadpole growth, we began changing the water every 2 days in order to prevent death from high concentrations of nitrogenous compounds in the water. Water for water changes was collected from undisturbed areas of both the new and old ponds in buckets, usually on the same day as scheduled water changes. This water was filtered to remove debris and then covered with a lid until needed. Tap water was collected from a faucet connected to a chloramine filter in the laboratory. When changing the water, fecal matter was removed using a disposable transfer pipette. Then, the majority of water was dumped into a bucket, using a fishing net as necessary to capture tadpoles. When 80% or more of the water was removed, new filtered water from the same treatment was slowly poured into the box, so as to minimize tadpole stress, and the box was returned to a random

position on the bench once the correct amount of water was added (determined by prepositioned tape on the side of the box).

Tadpoles were observed and any developmental abnormalities or visible illnesses were recorded daily. When dead tadpoles were found, they were removed immediately in order to prevent the spread of diseases, and then were placed into 70% EtOH. Once the tadpoles developed all four of their legs, tadpoles were moved into new enclosures which gave them access to both aquatic and terrestrial habitats. These enclosures were larger and were placed on a slant with water at one end and moist paper towels at the other end. Once in the larger enclosure, metamorphs were fed both lettuce and fruit flies.

Tadpole Monitoring and Development in the Laboratory

The number of tadpoles in each box was counted daily and tadpole deaths were recorded. Since cannibalism was observed throughout the development process, if tadpoles were missing they were assumed to have been cannibalized, which was also noted. Some tadpoles appeared to have large edemas in the first 2 weeks of development and the number of tadpoles living with edemas was recorded. When mass mortality occurred within a box, the water was tested for high levels of ammonia in order to rule out high urine concentration as the cause of death. Ultimately, percent survival after every week for each box and each treatment was determined using a Microsoft Excel Spreadsheet.

Tadpole growth rates were determined by photographing the tadpoles from six boxes from each treatment every 7-12 days and then measuring the tadpole sizes from the photographs according to the method described in the "*Tadpole Monitoring and Development in the Field*" section (Appendix 2). When mass mortality occurred and

tadpoles in photographed boxes died, additional boxes were photographed in order to increase the sample size for each treatment.

In order to compare developmental rates, the first appearance of hind legs and forelegs of the laboratory tadpoles was recorded for every box and compared between treatments. Additionally, dates of toadlet emergence from the water were also recorded and toadlets were dried and weighed once they had lost their tails and become true metamorphs. Once weighed, the metamorphs were released to the unenclosed side of the new pond in the field.

Data Analysis

All data were entered into a Microsoft Excel 2007 Spreadsheet. Dates were converted to Julian Days with January 1st, 2010 representing Julian Day 1 and each consecutive day representing each successive number. Using Excel, means and percentages were calculated. When statistical tests were run, Statview 4.5 was used, except when 2-Way ANOVAs were run, at which point SuperANOVA 1.1 was used.

Habitat Assessment

Temperature and Light Intensity

Mean temperatures ± SD for each treatment (old shallow, old deep, new shallow, new deep) were calculated using Excel. Then, correlation analyses were used to compare mean daily air temperatures with time and also to compare mean daily air temperatures with mean daily water temperatures within each treatment. Correlation analyses were also used to compare time with mean daily water temperatures from each treatment. Excel was used to produce scatterplot graphs of the changes in water temperatures according to Julian Day. A

2-Way ANOVA was conducted to determine the effects of pond (old or new) and depth (shallow or deep) on mean water temperature as well as to determine if there was an interaction effect between pond location and depth. Once in an Excel spreadsheet, light intensity values were used to make a scatterplot representing the changes in light intensities over time. No statistical tests were run on the light intensity data because not enough measurements were made.

Algae Growth Plates and Suspended Material

Due to the mass destruction of the algae growth plates in the field and because a large amount of mud settled on the growth plates making it difficult to distinguish between mass changes from algae growth or from sediment on the plates, no quantitative measure of the amount of algae that grew on the plates in each pond in the field could be made. Rather, algae growth plates were photographed and their coloring was compared, with the greener plates determined to have more algae growth.

The weights (g) of the filter papers before and after pond water filtration were entered into Excel. Then, changes in filter paper mass were determined by subtracting the original filter paper weight from the mass of the filter paper after filtration. This change in mass was assumed to be representative of the amount of suspended material captured in the filter paper from the 4 L of water filtered through. An unpaired t-test was used to look at the effect of water source on mass difference of suspended material and a bar graph was made to facilitate the comparison of the mean mass of suspended water from each water treatment.

Tadpole Development

Once tadpoles were photographed and their sizes were measured, mean tadpole body length per box per week was calculated using Excel. Then, although the boxes did not always have the same number of tadpoles, they were assumed to each be representative of the same proportion of the total tadpole population for their treatment and the grand mean was calculated. Individual lengths could not be used to calculate the overall mean body length per treatment because tadpoles were grouped into separate boxes and so were expected to be more similar to others in their box than others from a similar treatment.

Separate 2-Way ANOVAs were run to determine the effects of treatment type (old, new, tap, and field (old/new)) on tadpole body length for each set of photographs. Tukey-Kramer Post Hoc tests were run to test which treatments had a significant effect. When there was no significant effect of laboratory treatment type on tadpole body length (for the first set of photographs), an unpaired t-test was used to determine the effect of treatment type (laboratory or field) on tadpole body length. An unpaired t-test was also used with the fifth set of photographs (which were only taken of tadpoles from the old and new pond in the field) to determine whether there was a significant effect of field pond (new or old) on body length.

After the percent tadpole survival was calculated each week for each box, a 2-Way ANOVA was used to determine the effect of time and treatment on percent survival. The percentage of tadpoles with edemas was determined by dividing the number of tadpoles with edemas for each treatment by the total number of starting tadpoles for each treatment. The percentage of tadpoles cannibalized and the percentage achieving full metamorphosis were also determined using the same method described above. In order to determine the percent of

surviving tadpoles to develop four legs, the number of tadpoles with four legs each day was divided by the remaining number of tadpoles. When tadpoles died and the number of surviving tadpoles decreased, the following day's percentage with four legs was determined using the newest numbers of remaining tadpoles. A 2-Way ANOVA was used to determine the effects of treatment and time on the development of all four legs. A 2-Way ANOVA was also used to determine the effect of treatment type in the laboratory on body mass at metamorphosis. Then, since there was no significant effect of laboratory treatment type on body mass, an unpaired t-test was used to determine the effect of being raised in the laboratory or in the field on body mass at metamorphosis. Lastly, the percent tadpole survival after relocation to the new pond was determined by dividing the number of captured metamorphs by the total number of relocated tadpoles.

RESULTS

Habitat Assessment

Between January 31st and April 6th, 2010, air temperature increased significantly in the study area ($t = 2.72$, $df = 26$, $P = 0.011$). Overall, the minimum air temperature was 12.5°C and the maximum air temperature was 29.5°C (Figure 9).

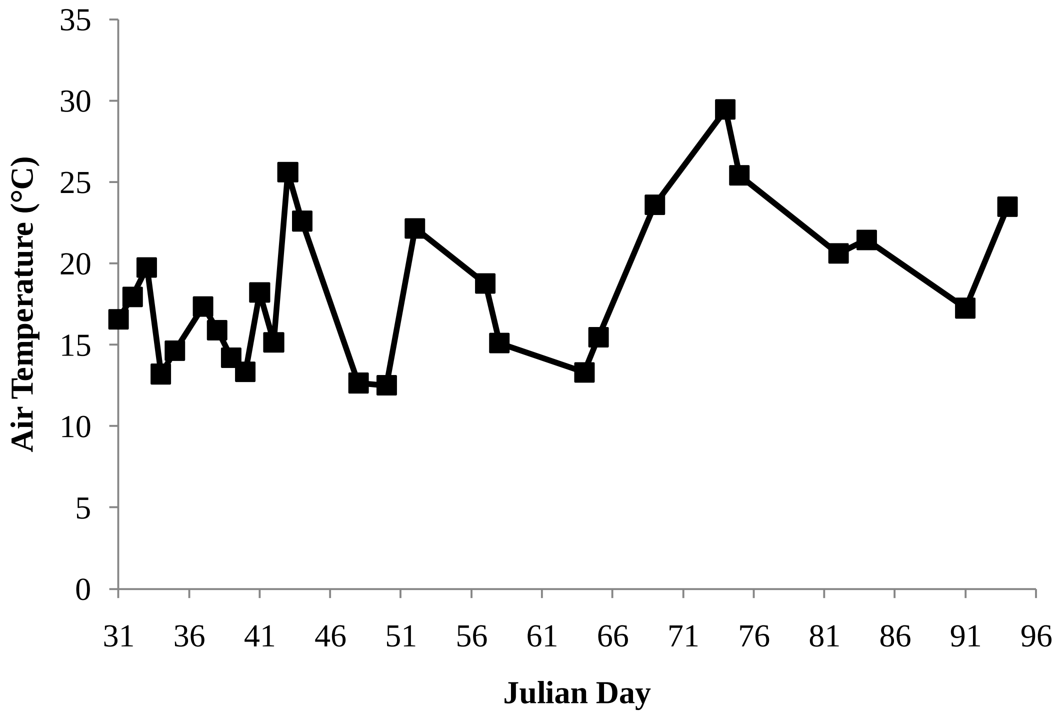

Figure 9. Mean air temperature (°C) at both field pond sites vs. Julian Day.

Water temperatures ranged from 10-30°C throughout the study, with the mean water temperature in both ponds being 18-19°C. Air temperature was found to be correlated with the mean water temperatures of the shallow area of the new pond (r = 0.722, $P < 0.0001$), the deep area of the new pond (r = 0.705, $P < 0.0001$), the shallow area of the old pond (r = 0.782, $P < 0.0001$), and the deep area of the old pond (r = 0.740, $P < 0.0001$). Julian Day was also found to be correlated with the mean temperatures of the shallow and deep areas of the new pond (r = 0.698, $P < 0.0001$ and r = 0.680, $P < 0.0001$), as well as the mean temperatures of the shallow and deep areas of the old pond (r = 0.700, $P < 0.0001$ and r = 0.587, $P = 0.0010$). Overall, there was no significant effect of the pond (old or new) or the depth (shallow or deep) on the mean water temperatures ($F_{1,107} = 0.323$, $P = 0.571$ and $F_{1,107} = 1.939$, $P = 0.167$ respectively; Figure 10). There was also no interaction effect between pond and depth ($F_{1, 107} = 0.009$, $P = 0.923$).

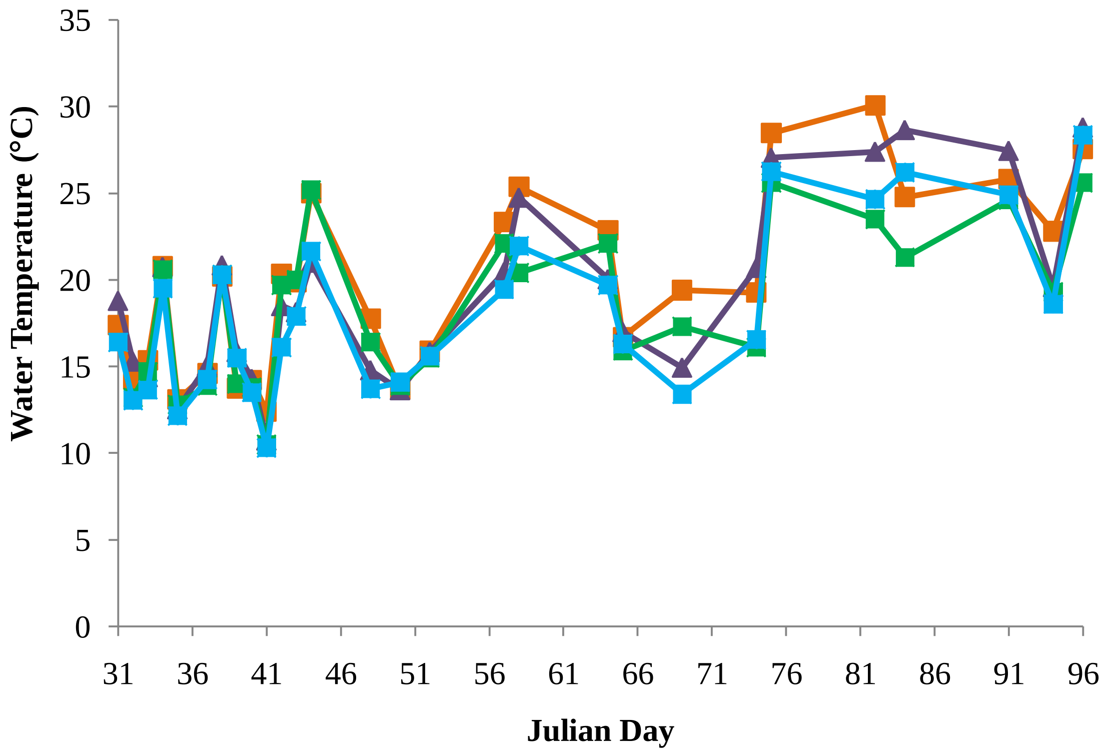

Figure 10. Mean water temperature (°C) in the shallow and deep areas of the new and old ponds at the BFS with respect to Julian Day (**Old Shallow**, **Old Deep**, **New Shallow**, **New Deep**).

Although light intensity varied according to cloud cover and date, overall the deep area of the new pond tended to have the lowest light intensities and the shallow area of the old pond tended to have the highest light intensities (Figure 11). As the shallow portion of the new pond cleared throughout the study, the light intensity at the bottom of the pond appeared to increase, becoming more similar to the light intensity in the shallow area of the old pond.

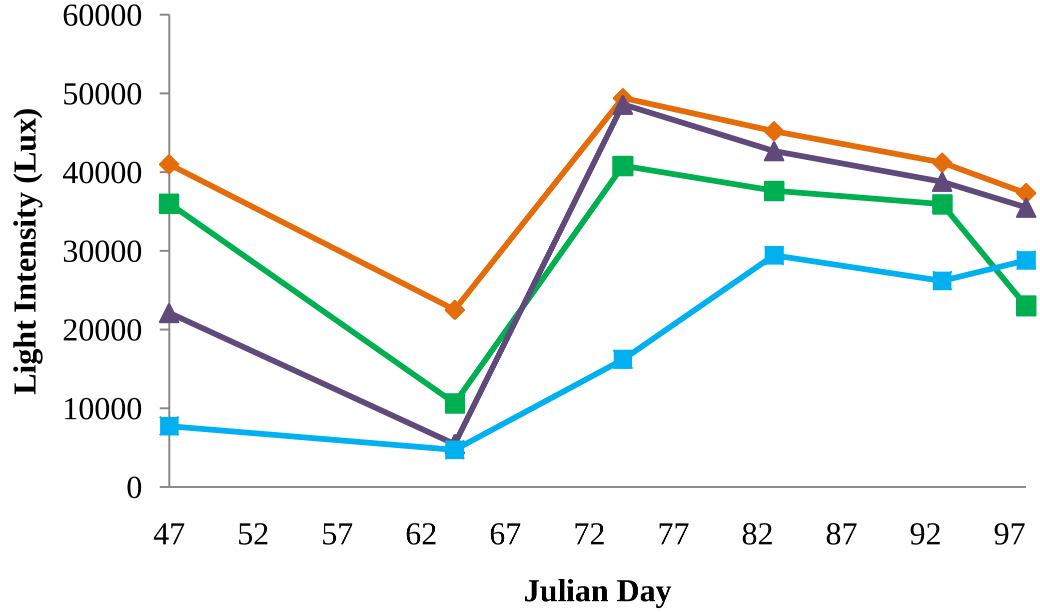

Figure 11. Light intensity (lux) in the shallow and deep areas of both field ponds versus Julian Day (**Old Shallow, Old Deep, New Shallow, New Deep**).

More algae appeared to grow on the plates in the old pond, as demonstrated by their greener coloring, whereas the plates from the new pond tended to be more filled with mud (Figure 12).

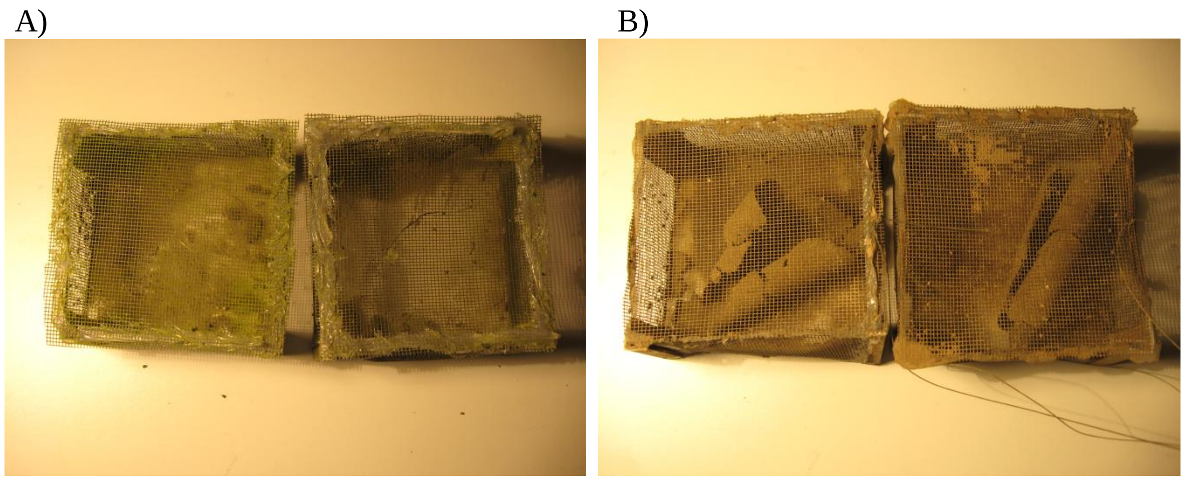

Figure 12. Algae growth plates from the old (A) and new (B) ponds after 5 weeks in the field.

In addition to the algae growth plates from the new pond having a higher proportion of mud on them than those from the old pond, there was over twice as much suspended matter filtered from water from the new pond than from water from the old pond (t = 3.753, df = 18, P = 0.0015; Figure 13).

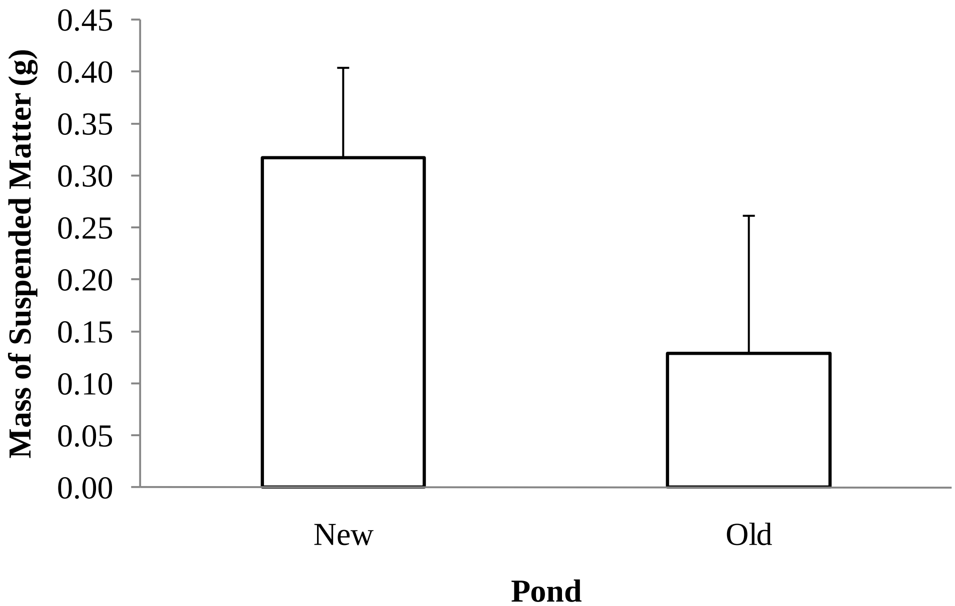

Figure 13. Mean mass of suspended matter + SD (g) filtered from water taken from the new (N=10) and old (N=10) ponds.

Tadpole Development

At least five different egg clutches were laid in the old pond at the BFS between Julian Day 26 and Julian Day 30. On Julian Day 42, the first tadpoles emerged and by Julian Day 45, 360 tadpoles had been moved to the laboratory. Tadpole development proceeded in both the laboratory and the field but was accelerated by approximately 1-2 weeks in the laboratory (Table 1).

Table 1. Dates of developmental changes of tadpoles in the laboratory and in the field.

Developmental Stage	Date first noticed in the field (Julian Day)	Date first noticed in the laboratory (Julian Day)
Egg	26-30	N/A
Tadpole	42	N/A
Hindleg Development	64	59
Foreleg Development	82	74
Metamorph (development of 4 legs and complete loss of tail)	93	80

As of April 8th, 2010, 56% of tadpoles raised in water from the new pond, 34% of tadpoles raised in water from the old pond, and 62% of tadpoles raised in tap water had survived. Overall, the total mortality in the laboratory was 51%. There was a significant effect of time and water treatment on tadpole survival ($F_{7, 264}$ = 8.734, P = 0.0001 and $F_{2, 264}$ = 7.424, P = 0.0007 respectively), however there was no interaction effect between time and treatment ($F_{14, 264}$ = 0.863, P = 0.5998). In all of the treatments, 10-20% of tadpoles died within the first week. In the fourth week, all treatments suffered high mortality and by the beginning of the fifth week, over half of the remaining tadpoles in the water from the old pond had died. However, after this period of high mortality, tadpole survival seemed to level off and very few deaths occurred between weeks five and eight (Figure 14).

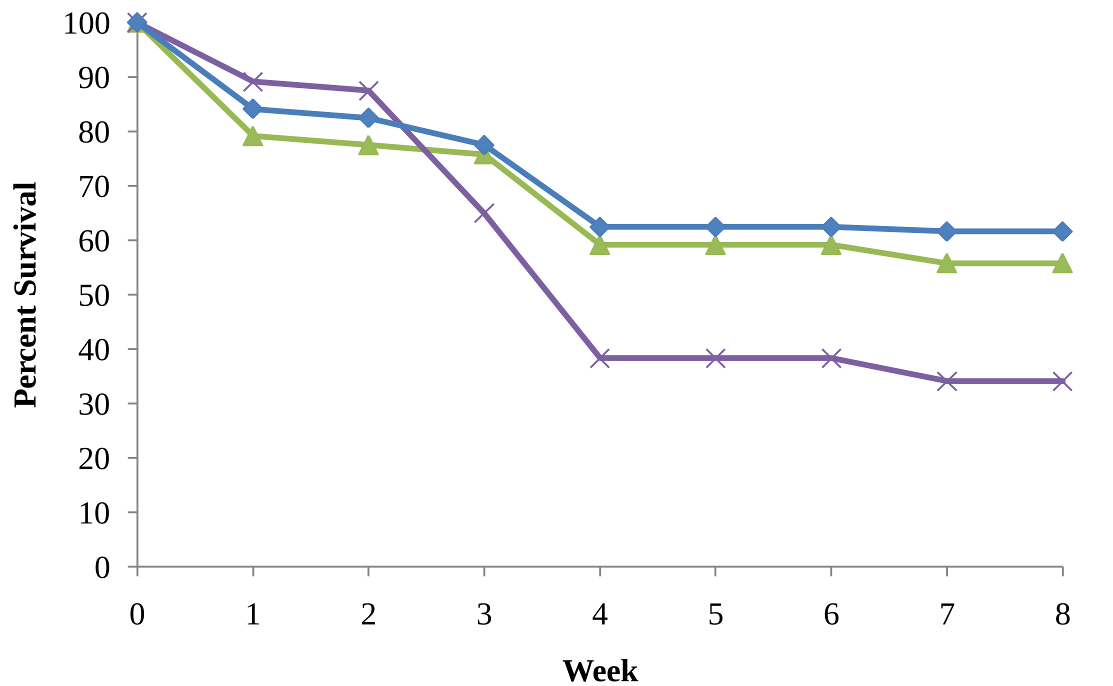

Figure 14. Mean percent survival of tadpoles raised in the three treatment types per week (**New, Old, Tap**).

Causes of death are unknown but overall, 4% of tadpoles were cannibalized, the majority of which were from either the tap or the new water treatments (Figure 15).

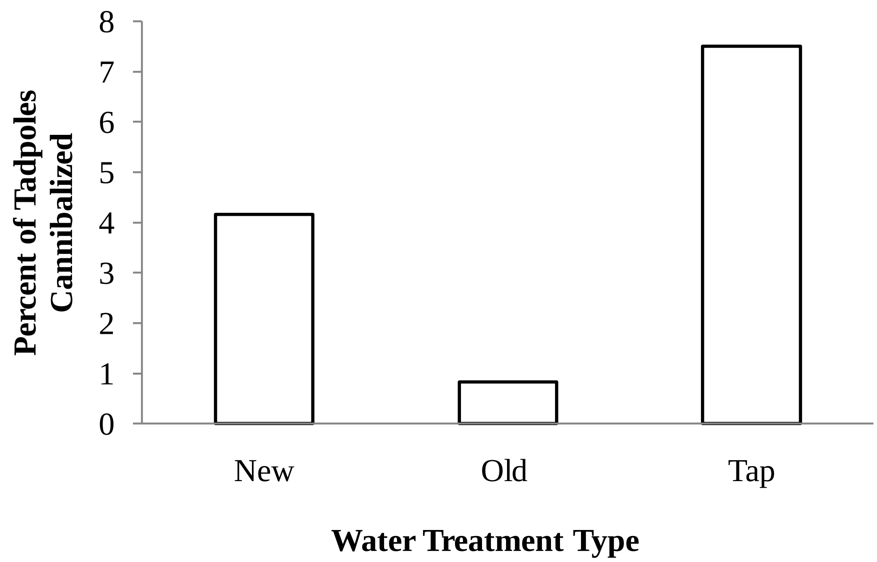

Figure 15. Percent of tadpoles cannibalized within each water treatment type.

Additionally, 17% of all tadpoles developed edemas on their bodies and ultimately died from these edemas. Again, slightly more tadpoles from the tap and the new water treatments developed edemas than tadpoles from the old water treatment (Figure 16).

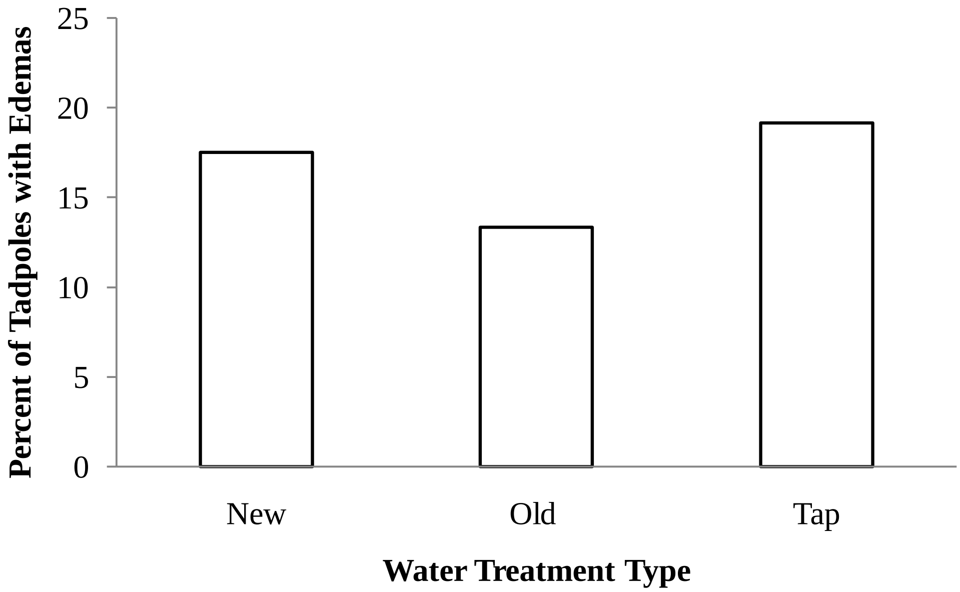

Figure 16. Percent of tadpoles with edemas per water treatment type.

There was also an effect of both time and treatment on the percentage of tadpoles that developed all four legs during the study ($F_{24,675}$ = 59.375, *P* = 0.0001 and $F_{2,675}$ = 47.769, *P* = 0.0001), with no interaction effect between time and treatment ($F_{48,675}$ = 0.796, *P* = 0.8376). Of the tadpoles that survived through week eight, 91% of those raised in new water, 67% of those raised in old pond water, and 82% of those raised in tap water had developed all four legs, with the majority of foreleg development occurring during the last 12 days of the study (Figure 17).

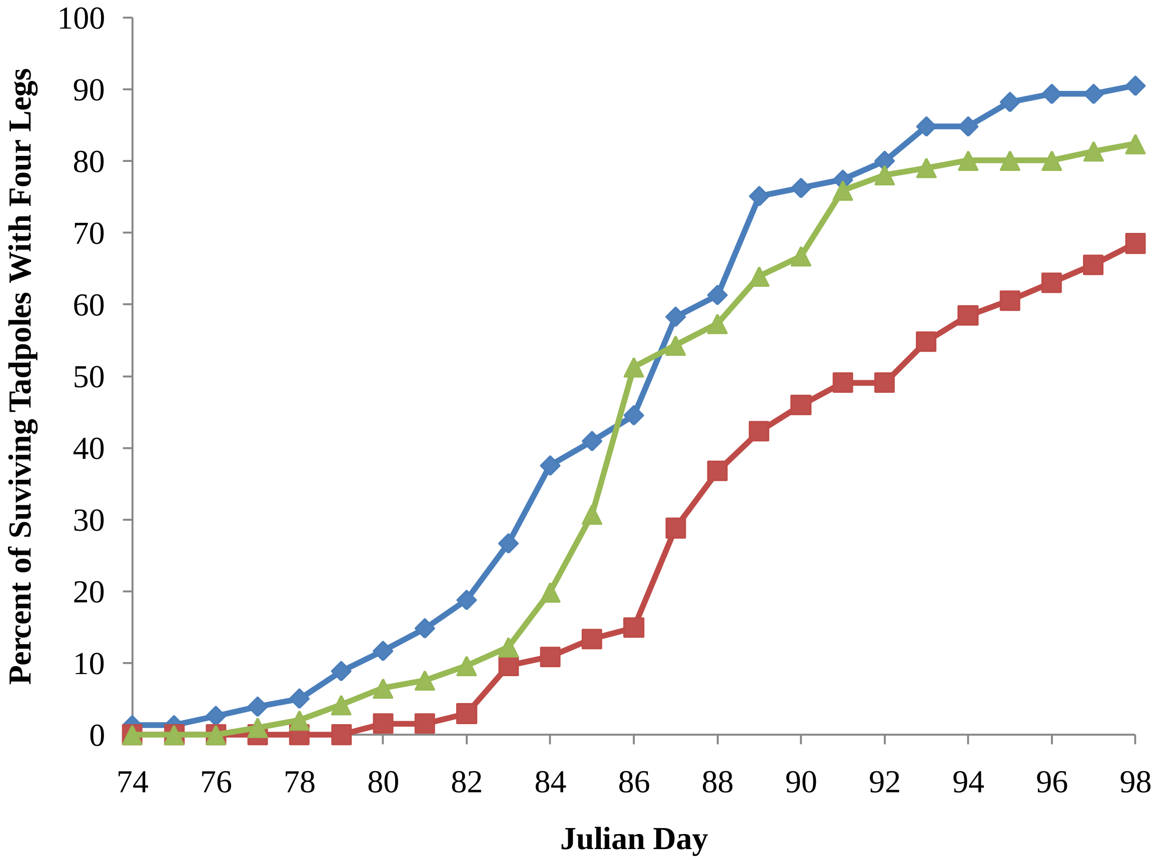

Figure 17. Percent of surviving tadpoles per treatment that developed all four legs during the study time period versus Julian Day (**New, Old, Tap**).

Overall, 42% of all tadpoles raised in new pond water, 14% of all tadpoles raised in old pond water, and 36% of all tadpoles raised in tap water completed full metamorphosis with the development of all four legs and the complete loss of their tail. Of the surviving tadpoles in the laboratory, 80% achieved metamorphosis by Julian Day 98. Additionally, at least 300 out of the 400 tadpoles that were moved to the new pond achieved full metamorphosis by Julian Day 108, suggesting a minimum survival and metamorphosis rate for the translocated tadpoles of 75%.

By the twelfth day of growth in the laboratory (Julian Day 57), the body length (mm) of tadpoles raised in the laboratory did not differ significantly depending on water treatment

($F_{2,15}$ = 1.407, P = 0.276; Figure 18). However, the pooled mean length of the tadpoles raised in the laboratory was 51% longer than the mean length of those raised in the old pond in the field (t = 20.05, df =119, P < 0.0001).

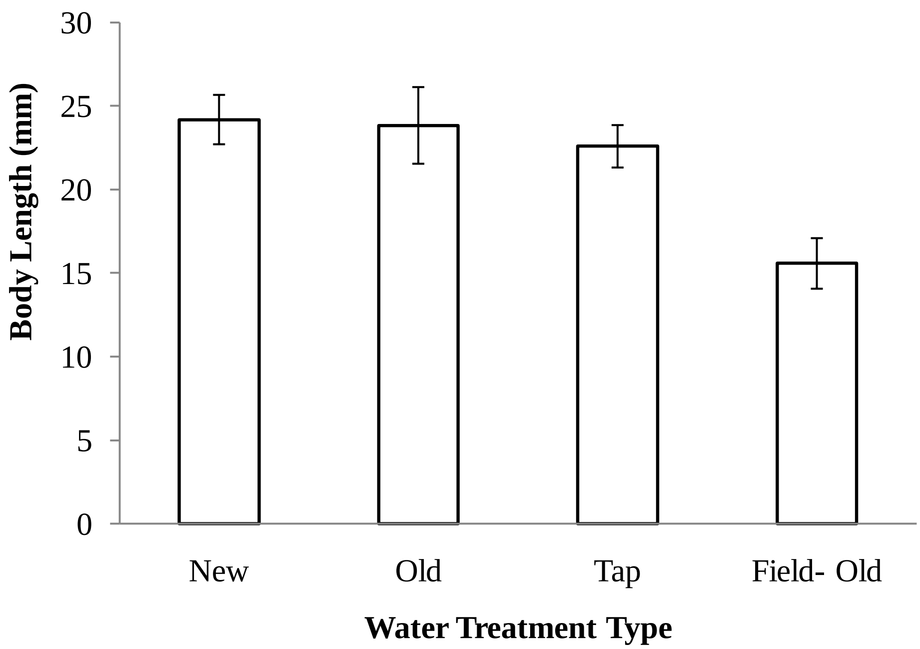

Figure 18. Mean tadpole body length ± SD (mm) as of Julian Day 57 versus water treatment type (N = 47, 56, 46, and 103 for New, Old, Tap, and Field-Old respectively).

After 19 and 26 days in the laboratory (Julian Days 64 and 71), tadpoles raised in new pond water were significantly longer than those raised in old pond water ($F_{2,17}$ = 16.284, P = 0.0001, Tukey-Kramer P < 0.05; $F_{2,16}$ = 5.274, P = 0.017, Tukey-Kramer P < 0.05) but there was no difference between the size of tadpoles raised in tap water and those raised in old or new pond water (Tukey-Kramer P > 0.05; Figures 19 and 20). Again, tadpoles raised in the laboratory were about 50% longer than those raised in the field ($F_{3,119}$ = 137.93, P = 0.0001, Tukey-Kramer P < 0.05; $F_{3,118}$ = 98.13, P = 0.0001, Tukey-Kramer P < 0.05).

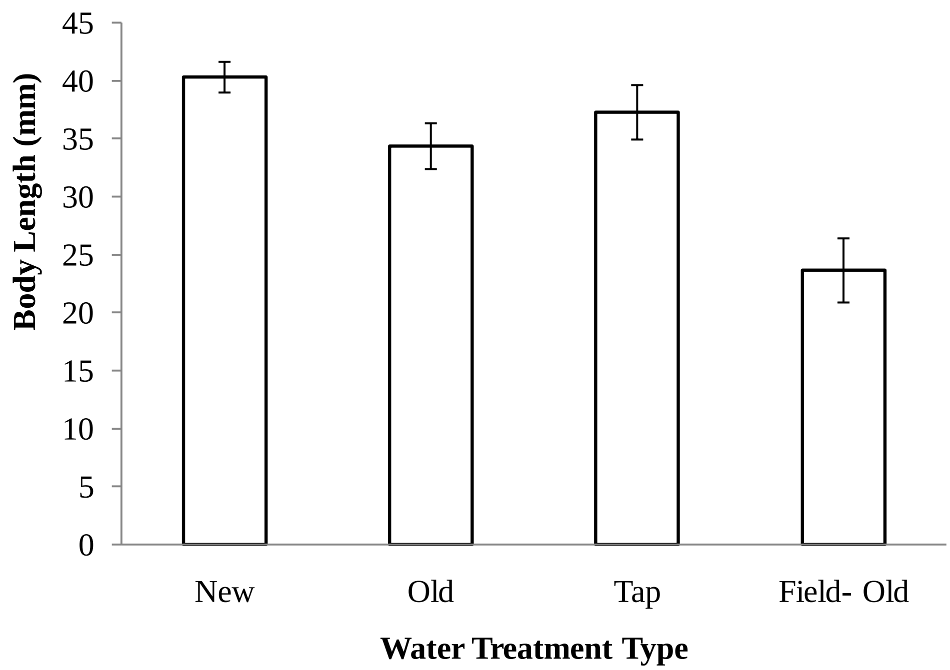

Figure 19. Mean tadpole body length ± SD (mm) as of Julian Day 64 versus water treatment type (N = 45, 55, 46, and 103 for New, Old, Tap and Field-Old respectively).

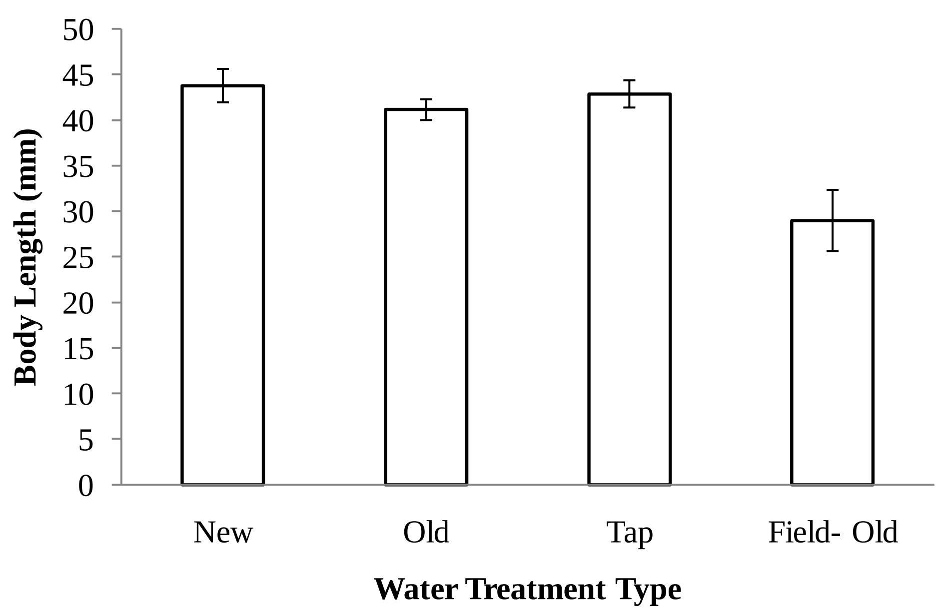

Figure 20. Mean tadpole body length ± SD (mm) as of Julian Day 71 versus water treatment type (N = 46, 48, 46, and 103 for New, Old, Tap, and Field-Old respectively).

On the 37th day of growth in the laboratory (Julian Day 82), there was again no difference between the body lengths of tadpoles raised in new, tap, or old water treatments ($F_{2,15}$ = 1.824, $P = 0.196$), with the mean tadpole body length in the laboratory being 44.8 mm (Figure 21), about twice as long as the original mean tadpole body length measured on Julian Day 57 (23.5 mm). The tadpoles raised in the laboratory were still significantly longer than those raised in the field (in either the new or old pond; $F_{4,16\ 4}$ = 25.47, $P = 0.0001$, Tukey-Kramer $P < 0.05$), however the mean body length of tadpoles raised in the laboratory (44.8 mm) was only 24% longer than the mean body length of the tadpoles raised in the field (36.4 mm; Figure 21). When the size of the tadpoles from the old pond and the relocated tadpoles from the new pond were compared on two separate occasions, there were no significant differences in their mean body lengths (Tukey-Kramer $P > 0.05$; Figures 21 and 22).

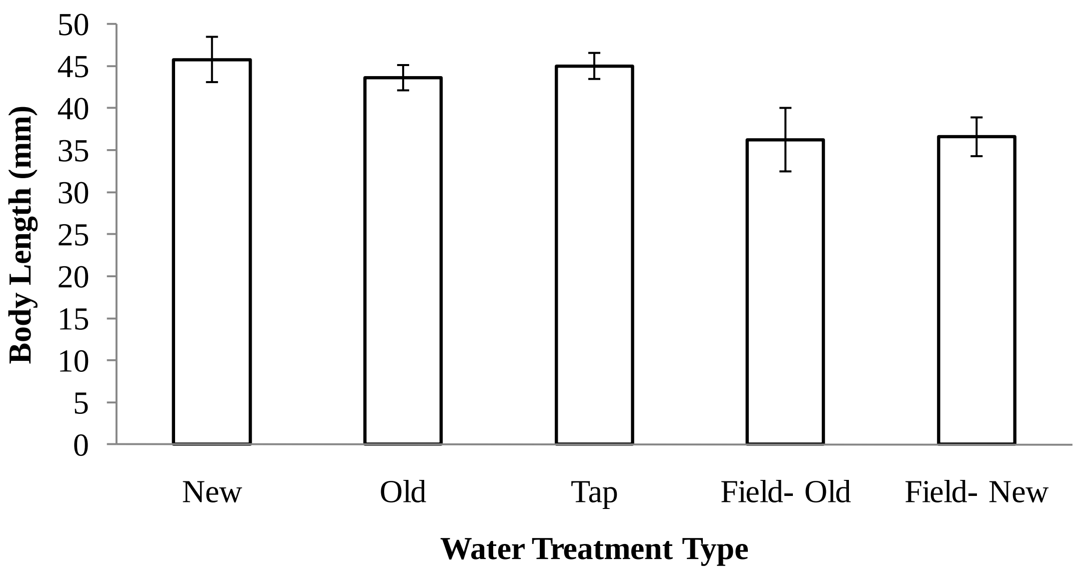

Figure 21. Mean tadpole body length ± SD (mm) as of Julian Day 82 versus water treatment type (N = 33, 42, 31, 117, and 34 for New, Old, Tap, Field-Old, and Field-New respectively).

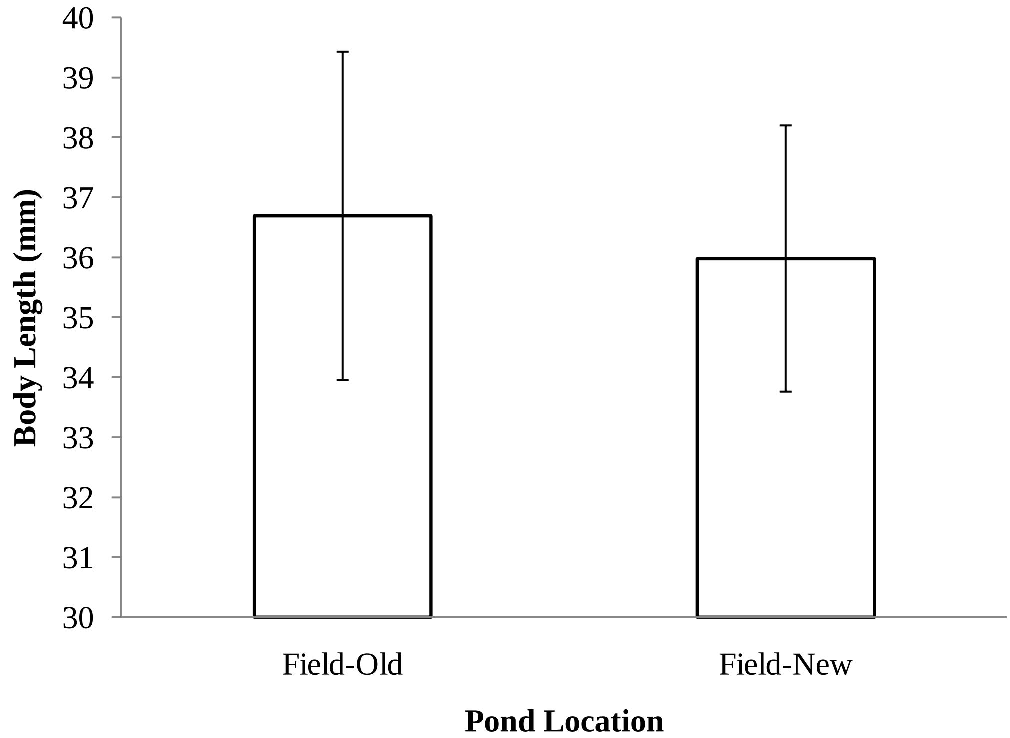

Figure 22. Mean tadpole body length ± SD (mm) as of Julian Day 93 versus pond location in the field (N = 48 and 69 for Field-Old and Field-New respectively).

Upon completion of metamorphosis (complete loss of tail), there was no significant difference between the body masses of metamorphs raised in the three different water treatments in the laboratory (t = 22.553, df = 262, $P < 0.001$; Figure 23). However, a seemingly higher proportion of tadpoles from the new and tap boxes survived and completed metamorphosis than the tadpoles in the old pond water.

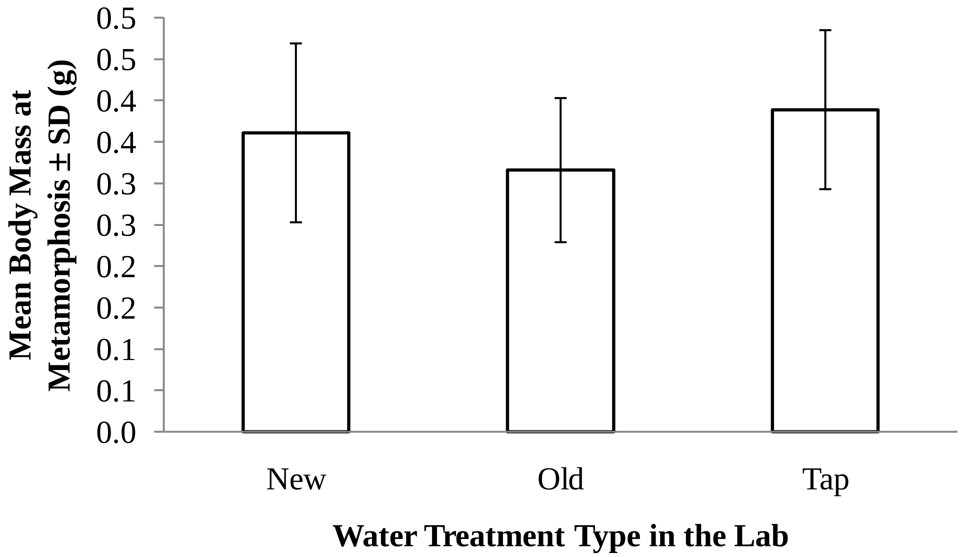

Figure 23. Mean dry toadlet body mass at full metamorphosis ± SD (g) vs. water treatment type (N = 34, 7, and 28 for New, Old, and Tap respectively).

When compared to the metamorphs captured from the new pond in the field, the metamorphs raised in the laboratory had a pooled (new, tap, and old) mean body mass nearly 90% larger than those from the field (Figure 24).

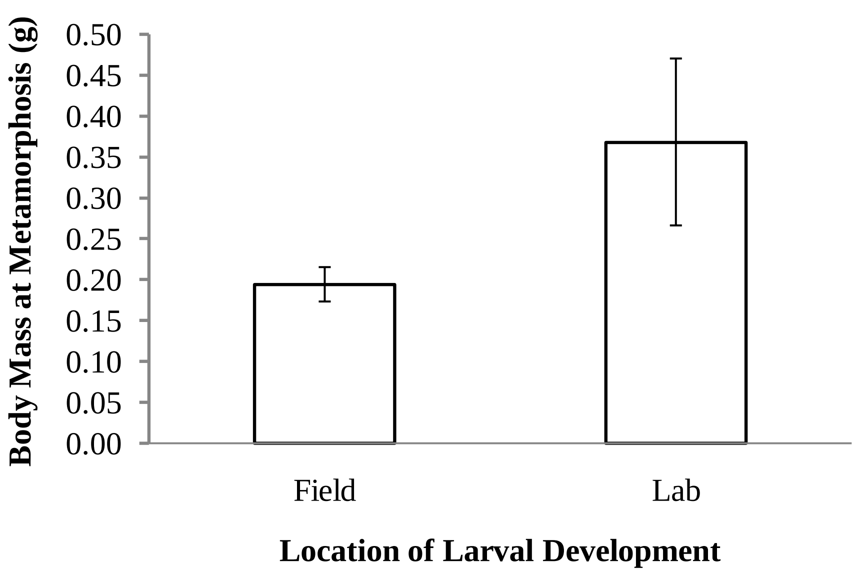

Figure 24. Mean dry toadlet body mass at full metamorphosis ± SD (g) of metamorphs raised in the laboratory (N = 69) and metamorphs raised in the field (N = 195).

DISCUSSION

Once Harvey Mudd College finalizes its plans to develop the plot of land adjacent to the BFS, the current breeding pond used by Western toads will cease to exist. Unfortunately, this means that if the Western toads do not find another area in which to breed, their population will no longer be able to reproduce and survive at the BFS. At this point in time, relocation to the newly constructed pond seems to be the only conservation strategy that could protect this population. Relocation would remove the emerging metamorphs from the immediate vicinity of the area that will soon be cleared and thus will separate them from the influx of humans that will need to enter HMC land in order to develop the new area. Furthermore, it will facilitate the metamorphs' discovery and usage of the new pond as a breeding area and will hopefully allow the larval tadpoles to imprint on the area, thus encouraging them to return to the same location in the future. As long as the newly built pond and the surrounding area can serve as an appropriate habitat for their development (which this study suggests that it can), relocation will hopefully be a successful conservation strategy for this Western toad population.

Without question, the likelihood of translocation success is species specific. Luckily, Western toads may be more likely to colonize newly constructed ponds than other species, as shown by their colonization of six new ponds in Oregon within 9 months of their construction (Pearl and Bowerman 2006). However, as several studies have shown, there are other factors that can influence translocation success regardless of the species (Reinert 1991; Pearman 1993; Bloxam and Tonge 1995; Pearman 1995; Germano and Bishop 2008). The main factors that were considered when determining the potential of relocation as a conservation strategy at the BFS, were the quality and location of the new pond (the release

habitat), as well as the new habitat's ability to mitigate the threats facing the Western toad population. Since the new pond is in the protected area of the BFS, it will not be facing habitat destruction in the near future and so can successfully mitigate the current threat facing the population. Additionally, the biological constraints of the new habitat (such as daily temperature, sunlight intensity, food availability, and water chemistry) were assessed so that recommendations could be made for future conservation attempts.

Temperature

As ectotherms, anurans cannot efficiently thermoregulate physiologically. Because of this, tadpoles are extremely sensitive to variations in habitat temperature. It has been shown that tadpole growth (and therefore translocation success) is strongly dependent on water temperatures, with consistently low temperatures resulting in delayed development (Alvarez and Nicieza 2002). Western toads most commonly lay eggs in shallow pond areas which are exposed to large fluctuations in temperature but which can reach up to 30°C in shallow, sunlit areas. Although water temperatures can drop significantly in these areas overnight, tadpole development can occur as long as the tadpoles are exposed to high water temperatures for at least part of the day (Carey *et al.* 2005). Colder water temperatures can delay development, but if tadpoles are sporadically exposed to warm temperatures, their development will proceed normally.

When the new pond was compared to the old breeding pond, it was found that there was no significant difference between the water temperatures of the two ponds, with temperatures ranging from 10-30°C (an ideal range for tadpole development) in both areas (Figure 10). Since it is already known that Western toad tadpoles can survive in the old pond, this demonstrates that temperature will likely not be a factor limiting the new pond's

adequacy as a breeding habitat for Western toads. As long as the tadpoles in the new pond can aggregate in shallow, sunlit areas during the day in order to thermoregulate (as they were repeatedly observed doing in the old pond), their development in the new pond should not be dramatically affected by temperature. Therefore, water temperatures should not have a large impact on future translocation success.

Light Intensity/Food Availability

Another factor that can influence tadpole survival and development, and so was assessed at the new pond, is the quality and quantity of available food throughout the larval stages (Alvarez and Nicieza 2002). In the BFS, tadpoles primarily appear to feed on algae. Unfortunately, algae growth in the two ponds could not be sufficiently quantitatively or qualitatively compared. However, algae growth was observed in both ponds throughout the study. Furthermore, although light intensities differed between the two ponds (likely because of the larger amount of suspended material in the new pond), in general both ponds had relatively high light intensities (above 25,000 lux) throughout the last 25 days of the study (Figure 11), suggesting that algae growth in both ponds is probably not limited by light availability. Additionally, the new pond became increasingly clear throughout the study once the amount of rain decreased and the suspended material had time to settle. The water will continue to clear once additional layers of decomposed gravel are applied to the pond edges to prevent further runoff into the pond, and so light intensity should only increase in the area, which will be beneficial for future algae growth.

Although more algae appeared to grow in the old pond based on observations of the algae plates (Figure 12), large clumps of algae were found on the edges of the new pond and some algae did grow on the new pond's algae plates. Since algae was constantly observed in

the new pond and the 400 translocated tadpoles clearly had access to food in the new pond since over 75% of them grew and morphed during the study, it seems as though food availability would likely not limit the survival of future translocated tadpoles. However, additional studies will need to determine whether or not there is sufficient food in the new pond to support the entire population of Western toad larvae since the quantity of food in the new pond is currently unknown.

Water Chemistry

Unexpectedly, tadpole survival in the laboratory was lowest in water from the old pond. Initially, tadpoles raised in old pond water had the highest survival rates of the laboratory-raised tadpoles but within a 2 week time span, several boxes of tadpoles in the old water treatment suffered mass mortality (Figure 14). This result was surprising because tadpoles were expected to thrive in their natural water habitat. Since the old pond is filled by rain water and also by tap water when necessary, it would be easy to assume that the addition of tap water to the pond was not beneficial for tadpole survival and growth and so was limiting development in the water from the old pond. However, tadpoles did well in the tap water treatment in the laboratory (Figure 14), showing that the addition of tap water to the old pond was likely not responsible for hindering tadpole development in the old pond water treatment.

Since amphibian larvae are known to excrete ammonia as a way of disposing nitrogenous waste and since ammonia can be toxic at high concentrations (Grafe *et al.* 2005), it was initially predicted that ammonia toxicity was the cause of the mass mortality in the old treatment boxes (since at the time those boxes had large numbers of surviving tadpoles and therefore likely had higher concentrations of ammonia secretion in the water). However,

water chemistry sampling indicated that ammonia levels were low in all of the tadpole boxes, suggesting that ammonia concentration may not have been at the toxic level (although the water sampling kit used was not very accurate or sensitive and so ammonia toxicity cannot be ruled out as a cause of mass mortality).

If not the result of ammonia toxicity, one of the causes of tadpole mortality could have been water contamination, a common cause of tadpole death when exposed to human populations (Seigel and Dodd 2002). Since all of the water from the old pond was collected in the same two buckets and since the mass mortality occurred so suddenly within the two week time frame, it may have been that human error resulted in water contamination which ultimately led to mass mortality. Unfortunately, causes of death cannot be determined at this point in the study. Although it is unlikely that mass mortality was the result of existing pathogens in the field since tadpoles in the field did not seem to be suffering mass mortality, it must be mentioned that the water taken from the old pond in the field may not have been entirely contaminant-free and so may have also been the cause of higher levels of tadpole mortality in the laboratory.

Not only was overall tadpole survival in water from the old pond the lowest of all three treatments in the laboratory (with tadpoles in new pond water being 1.6 times more likely to survive than tadpoles in the old pond water), but also a significantly smaller proportion of surviving tadpoles in old pond water developed all four legs by the final data collection day than the proportion of tadpoles in the new or tap treatments that developed all four legs during the study (Figure 17). Overall, development in new pond water was faster and despite there being no difference in body mass at metamorphosis depending on treatment, development to the toadlet phase proved more successful (in terms of percent

morphed) in new pond water than in old pond water, with up to 42% of all original tadpoles in new pond water achieving full metamorphosis and only 14% of tadpoles in the old pond water achieving metamorphosis. When these statistics are compared, it would appear that the overall water quality (in terms of its success at providing a suitable habitat for tadpole development and survival) was higher in the new pond than the water quality in the old pond. This would suggest that there are no limiting factors in terms of water quality preventing successful translocation to the new pond.

Dispersal Areas and Competitor/Predator Density

Although the quality of the surrounding terrestrial habitat is equally important when determining biological limitations of an area, time did not allow for the assessment of the dispersal areas surrounding the ponds. Rather, to provide temporary protection for the metamorphs at both ponds, wooden planks were placed on the ground to give them something to hide under. Additionally, signs were placed around the new pond warning people to stay away from the area.

Predator density also could not be assessed but no aquatic predators were found in the old pond. In the midst of the study (following a period of rain), an American bullfrog, a species known to eat tadpoles (Lawler *et al.* 1999), was discovered in the new pond. The bullfrog was immediately removed from the area and was taken back to the laboratory where it was placed in an aquarium. Western toad tadpoles from the field were placed in the water with the bullfrog to determine if the bullfrog would eat them and affect their survival rate in the field. However, after 2 weeks in the laboratory, the bullfrog had only eaten one out of the three tadpoles. It is possible that the bullfrog was stressed in the laboratory and not eating, but it should also be noted that the bullfrog did not prefer to eat the tadpoles over the other

pellet food (Nasco frog brittle) provided in the aquarium, and so may not actually be a major threat to tadpole survival in the field. After the 2 weeks in the laboratory, the bullfrog was relocated to another location in the BFS away from the new pond. The relocation of the bullfrog will likely not prevent it or other bullfrogs from returning to the new pond during the next period of rain, and so the presence of bullfrogs may serve as a threat to future tadpole survival in the new pond. The impact of their presence on tadpole survival should be assessed in future studies.

Overall Habitat Summary/Predicted Translocation Success

Due to time constraints and the inability to track newly morphed toadlets due to their small sizes, this study was not able to fully determine the suitability of the new pond for future translocation efforts. However, as described above, results from the assessment of the characteristics of the new habitat suggest that the new pond would be able to support the population of Western toads, and in fact, tadpoles in the laboratory actually did the best in the new water treatment. Additionally, when the mini-relocation project was conducted and 400 young tadpoles were transferred to the new pond, initial results were positive, with a 75% survival rate of tadpoles to the metamorph stage by Julian Day 108. This high survival rate can partially be attributed to the fact that these tadpoles were relocated at an older larval stage and so may have passed the period of highest vulnerability while larvae. However, there were still many tadpoles alive in the pond at the project's completion and it is likely that there would have been an even higher percent survival to metamorphosis if the study was extended for a few additional weeks. This high survival rate (even though it was measured from a later development stage) should not be ignored and suggests that the new pond can support healthy tadpole development.

Although the tadpoles and the metamorphs at the new pond in the field were significantly smaller (both in length and mass) than the tadpoles and metamorphs in the laboratory (likely due to increased competition for food, harsher weather conditions, or frequent exposure to colder water temperatures), they did not seem any smaller than the tadpoles or metamorphs at the old pond in the field (Figures 21 and 22). This suggests that they were equally as healthy as the "untranslocated" population of tadpoles in the field and that their smaller size in comparison to laboratory tadpoles was due to excellent (and stable) laboratory growth conditions rather than poor field conditions. In fact, their mean size while tadpoles (36.6 mm) was not dramatically smaller than tadpole sizes reported in other studies (37 mm; Keinath and McGee 2005). Overall their size and high initial survival rate appear to demonstrate that the new pond may be a good future relocation site for the rest of the Western toad population.

If a large scale relocation project is organized in the future, the developmental stage of the Western toads during the time of release must be considered. Several studies suggest that it is best to release young individuals (eggs, tadpoles, and recent metamorphs), which are easier to find and move than adults (Reinert 1991; Bloxam and Tonge 1995; Germano and Bishop 2007). This argument has been supported by the claim that young tadpoles will imprint on their release site and so are more likely to remain in the new area than relocated adults (Bloxam and Tonge 1995). However, one must also consider the high mortality rates during younger life stages when considering whether or not to also release adults or juveniles and when deciding how many individuals to release at a new site. Since the adult population of Western toads is not easily visible at the BFS, it is recommended that with future translocation projects all eggs and tadpoles are transferred to the new pond. Additionally, if

any adult toads are found at the old pond at night during the breeding season, they too should be captured and transferred to the new pond. Although it is unlikely that they will stay at the new pond or choose to breed in that area, capturing the adult toads would remove them from the immediate threat of habitat destruction.

Lastly and also most importantly, when preparing for mass-translocation, all efforts must be made to minimize animal stress (Teixeira *et al.* 2006) and all precautions must be taken in order to prevent disease transmission between habitat sites and individuals during the translocation attempt (Bloxam and Tonge 1995; Germano and Bishop 2007). Despite having tadpoles in this study initially die from edemas (of unknown cause) and from having several later instances of mass mortality, the overall survival rate in all of the laboratory treatments was not extremely low and there were no clear signs of disease (such as mutations or disfigurations) in the population of tadpoles captured from the field. Because no signs of disease were observed and because precautions were taken in order to minimize disease transmission, it was assumed that no diseases were transferred from the laboratory to the field when tadpoles and metamorphs were released into the field at the end of the study. If a large-scale relocation project is planned in the future (which will likely be unavoidable), precautions must be taken to ensure that no diseases are transferred between ponds.

Suggested Project Continuation

Because little was known about the Western toads at the BFS prior to this study, there is still a lot of unknown information about their development and survival in the field. Additionally, time constraints limited our ability to completely assess the new pond as a relocation site, and so several studies are suggested to gain more knowledge about tadpole development and to prepare for future translocation attempts.

Continuation of Current Study

Since translocations cannot be deemed successful without long-term monitoring in order to proclaim reproductive success (Germano and Bishop 2007), it is essential that the new pond is visited during each breeding season within the next 5-10 years to see if any of the current adult Western toads or any of the newly released metamorphs use the new pond as a breeding area in the future. Although relocation seems like it may prove to be a successful conservation strategy at the BFS, relocation will be deemed unsuccessul unless the metamorphs reach sexual maturity (at approximately 5-6 years of age; Keinath and McGee 2005) and are able to reproduce. Without monitoring of the new pond, the success of the mini-translocation and release of metamorphs at the new pond, as well as the success of the potential future large-scale relocation, will remain unknown.

New Projects

1. Throughout this study, cannibalism was observed directly and also assumed by the disappearance of several tadpoles. The majority of cannibalism occurred in tap water treatments, with almost no cannibalism occurring in old water treatments. Tadpoles are known to exhibit opportunistic cannibalism in the field (Crump 1983) and in the laboratory (Han and Bradley 2008) when in need of a more diverse food source. It is possible that the majority of cannibalistic acts occurred in the tap water because the tadpoles raised in the tap water only had access to the lettuce that they were provided, whereas the tadpoles raised in new or old pond water likely had access to algae in the water collected from the field ponds in addition to lettuce. This can be supported by the noted differentiation in the coloring of fecal matter in the three treatments.

Whereas the tadpoles in the tap water consistently produced green fecal matter, the tadpoles raised in the other two treatments often produced darker, brown fecal matter, suggesting that they were not digesting the same food. Since levels of cannibalism were lower in the old and new water treatments, it would seem as though the tadpoles raised in water from the field had access to a more diverse array of food and did not need to rely as heavily on cannibalism. However, cannibalism rates in all three treatments were low (likely because of the constant supply of lettuce) and so the significance or causes of cannibalism in the study cannot be absolutely determined. Future projects could look into the influence of food deprivation or the lack of food diversity on the frequency of cannibalism. Additionally, the impact of cannibalism within a confined area on the development of tadpoles raised within that area could be determined.

2. Past studies have shown that temperature can directly impact development of anurans (Alvarez and Nicieza 2002). When raised in colder environments, tadpoles frequently develop more slowly (Carey *et al.* 2005). However, if tadpoles are exposed to warmer temperatures for at least part of the day, their growth will not be hindered by cold temperatures (Carey *et al.* 2005). In this study, the tadpoles were initially taken from the field in February when water temperatures were ranging from 10-18°C. They were then put into laboratory habitats which had a mean temperature of approximately 20°C. Because these tadpoles were initially exposed to warmer temperatures, they may have begun developing faster. Upon completion of the study, field water temperatures ranged from 17-30°C and so the tadpoles in the field were

actually in a warmer environment. However, it is possible that the initial boost in temperature for the tadpoles raised in the laboratory may have been the cause of the faster rate of tadpole development. It would be interesting to determine the effects of variations in temperature on rate of tadpole development and on the ultimate toadlet size at metamorphosis. In future studies, one could attempt to discover the range of time taken to develop at various temperatures. Furthermore, one could determine the length of exposure time to a higher temperature required each day in order for metamorphosis not to be slowed in response to low temperatures.

3. When observed in the field during the study, tadpoles tended to congregate in the shallowest, sunniest areas of the ponds (presumably because those areas were the warmest). These tadpoles in the field started with short days and as the season progressed, they were exposed to longer hours of sunlight. Unlike the field tadpoles, the amount of light provided to the tadpoles in the laboratory was not changed throughout the study. Similar to the temperature project described above, it would be interesting to determine the amount of light required per day for healthy tadpole development.

4. Lastly, because an American bullfrog was found at the new pond but not at the old pond, the population of predators (birds, snakes, and bullfrogs) surrounding the new pond needs to be assessed in the future. Even if the aquatic habitat at the new pond is completely suitable for tadpole development, if there are too many predators surrounding the pond, the tadpoles will not be able to survive in the area. In addition

to measuring predator density around the new pond, studies should be conducted in order to determine the effects of having predators present on tadpole development. It has been shown in past studies that predation can influence the behavior, morphology, and life history of prey species and can often force anurans to develop at different rates or different sizes (Chivers *et al*. 1999). Western toad tadpoles are known to exhibit antipredator behavior in response to chemical alarm cues released from other injured tadpoles. Chivers *et al.* (1999) found that Western toad tadpoles decrease the amount of time to reach metamorphosis in response to chemical alarm cues. This change in development rate can result in smaller sizes at metamorphosis which can ultimately negatively affect the metamorphs. If the tadpoles in the new pond are consistently exposed to predators, they may develop more rapidly and emerge as smaller metamorphs, making their chance of survival as a terrestrial organism decrease. Additional studies are needed in order to determine the impacts of visual or chemical cues of the presence of predators (ideally bullfrogs) on the development of Western toad larvae.

CONCLUSIONS

- Water temperature, light intensity, and algae growth will likely not have an impact on the future success of Western toad relocation at the BFS, as they did not differ dramatically between ponds.
- The new pond did have a larger amount of suspended material (most likely clay). However, most of the suspended material settled throughout the study and will

continue to decrease once additional layers of decomposed gravel are applied to the pond edges to prevent further runoff into the pond.

- Tadpole development in the laboratory was most successful (in terms of percent survival and percent achieving full metamorphosis) in the tap and new water habitats and least successful in the old water habitat. However, tadpole sizes did not vary much according to water type.
- As the tadpoles raised in the new pond water were more successful than the tadpoles raised in the old pond water in the laboratory, there does not seem to be any factors about the water chemistry in the new pond that would limit future translocation success.
- Field tadpoles were consistently smaller than laboratory tadpoles and they developed at a slower rate than the laboratory tadpoles. This is presumed to be the result of lower food availability and harsher conditions in the field, but future studies should be conducted to determine the effects of temperature, predator abundance, and food availability on field tadpole survival and development.
- When a trial translocation was completed with the movement of 400 tadpoles from the old to the new pond, tadpole survival in the new pond was very good, with at least 75% of all tadpoles surviving and achieving complete metamorphosis by the end of the study.
- Although terrestrial survival may be low in the area surrounding the new pond, there are no current signs suggesting that relocation will fail as a conservation strategy for the Western toad population at the BFS. Due to the lack of time before the old

breeding pond is destroyed, complete relocation of the eggs or tadpoles found in the old pond during the next breeding season is recommended.

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APPENDICES

Appendix 1. Egg Development*

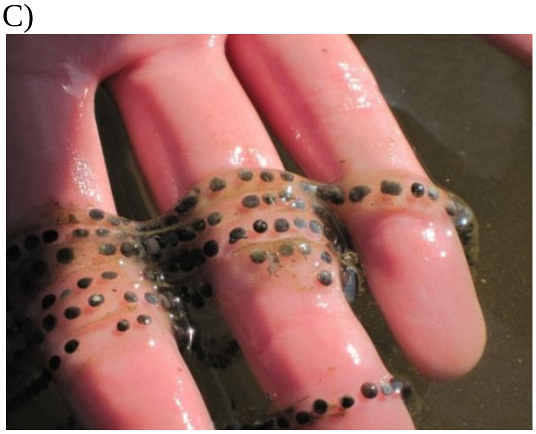
A)

Recently laid egg clutches in the old pond (January 26th, 2010).

B)

Initially, eggs are spherical and the jelly casing is fully intact (January 29th, 2010).

C)



Within a few days, the jelly casing weakens and begins to fall apart (February 1st, 2010).

D)

As they develop, the eggs elongate and the jelly casing further disintegrates (February 3rd, 2010).

E)

The eggs continue to elongate and separate from the jelly casing (February 6th, 2010).

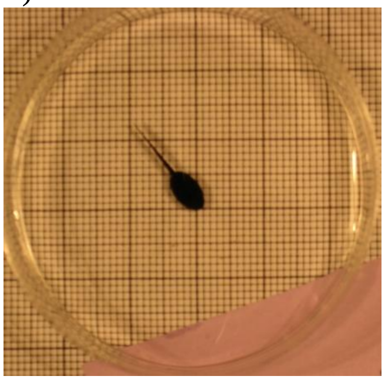
F)

Once fully elongated, the young tadpoles break through the remaining jelly casing and lay motionless while they finish their development into fully-functional tadpoles (February 10th, 2010).

*All photographs were taken by Maya Higgins and Erin Baumler at the BFS.

Appendix 2. Stages of Tadpole Development**

A)

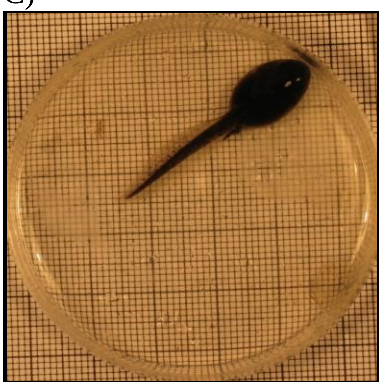


Newly born tadpole.

B)

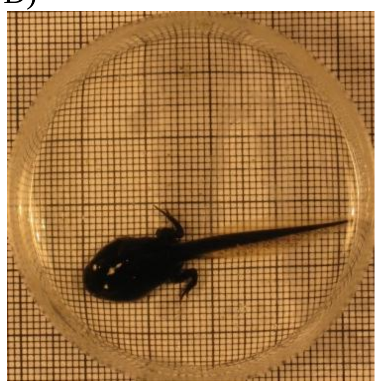
Juvenile tadpole—doubled in size.

C)



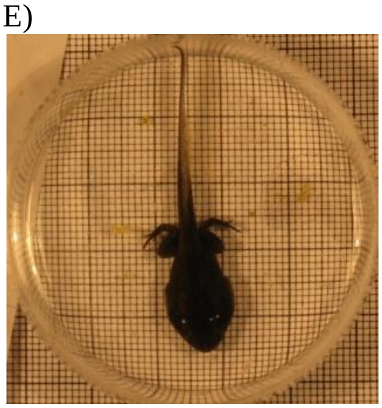
Early development of hindlegs.

D)



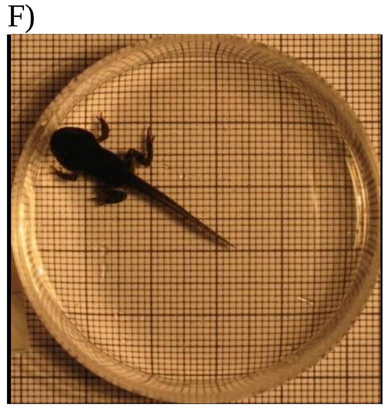
Fully developed hindlegs.

E)



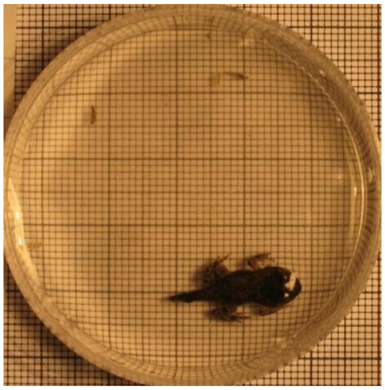
Elongated body before development of forelegs.

F)



Fully developed hindlegs and forelegs.

G)



Receding tail.

H)

Full Metamorphs/Toadlets.

**All photographs were taken by Maya Higgins and Erin Baumler between February 20th and April 6th, 2010.