

**Temporal Variation of Thermal Microhabitat Use
of Tiger Salamanders**

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Abstract

Climate change is increasing global temperatures, which forces many species to experience environments that they have not been previously exposed to. Ectotherms are particularly sensitive to temperature shifts, and previous research suggests that changes in phenology and habitat use can help species mitigate climate change. Arizona Tiger salamanders (*Ambystoma mavortium nebulosum*) are polymorphic amphibians that often live at high elevations, making them particularly susceptible to climatic shifts. In this study, we explored the temporal variation of thermal microhabitat use of tiger salamanders at the Mexican Cut Nature Preserve. By using quadrats placed in the thermal areas of permanent and semi-permanent ponds, we tested whether salamanders are using different microhabitats throughout the day based on temperature. We also used remote detection of PIT-tagged animals and temperature recordings to compare the use of vegetated and non-vegetated microhabitats based on water temperature in the quadrat. Results of the statistical analysis showed that there were significant relationships between time period and animals observed/detected, and also vegetation cover. Results showed that for visual surveys, late afternoon had a larger amount of animals observed in the quadrats. For remote detection surveys, results showed that the amount of salamanders detected/observed in vegetated quadrats was higher than the non-vegetated quadrats. In comparing data of average water temperature to animals observed for visual surveys, the water was at its warmest during the second and third visual surveys than the first. For average water temperature during remote detection surveys, more animals were detected/observed during the early afternoon and the water temperature was at its warmest. We found that vegetation cover also accounted for whether the salamanders would move into these shallow areas more frequently than areas where the water was also warm but did not provide cover. Climate change will have a significant effect on the thermoregulatory behavior of the Arizona tiger salamanders, increasing temperatures will also increase the area of the thermal areas. This might cause the salamanders to seek deeper water in order to thermoregulate.

Introduction

Climate change has been affecting ecosystems around the world and altering the environments of many species. As temperatures increase globally, amphibian species are experiencing habitat loss due to earlier snowmelt and higher pond drying frequency (Delvecchia et al. 2019). With increasing temperatures, amphibians are exposed to warmer than usual temperatures. Amphibians are ectotherms that have their internal body temperature approximately the same as the temperature of the surrounding environment (Piasečná et al. 2015). In order to maintain operative bodily functions they must thermoregulate. The tiger salamanders do this by moving in and out of shallow and deep areas around the pond. The temporal variation use of thermal microhabitat use of ectotherms is not well studied, particularly under field conditions (Heath et al. 1975, Fontenot et al. 2011). In evaluating the behavioral thermoregulation of amphibians, we can better understand the adaptive capabilities of amphibians experiencing climate change.

Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) are amphibians located at the Mexican Cut Nature Preserve (MCNP), in the vicinity of the Rocky Mountain Biological Laboratory in Gunnison County, Colorado. The MCNP is a subalpine wetland just below or at the treeline at approximately 3400 meters in elevation on the slopes of Galena Mountain (Wissinger et al 1999). The MCNP has been protected by the Nature Conservancy since 1966. Tiger salamanders are polymorphic, with a terrestrial adult form called a metamorph and an aquatic gilled adult form called a paedomorph (Whiteman et al. 2012). The process by which an individual larva becomes one morph or the other is determined by several environmental factors such as resource competition and whether the pond the reside in is temporary or permanent (Whiteman et al. 2012). Salamanders can be found in different types of ponds: permanent, semi-permanent, and temporary. Permanent ponds have water year-round, semi-permanent usually dry each fall but occasionally remain permanent, and temporary ponds dry every year. Metamorphic salamanders can be found in any type of pond while paedomorphic salamanders are only found in permanent ponds (Whiteman et al. 1996).

Here, we explored three metrics of behavioral thermoregulation in these salamanders. First, we observed whether tiger salamanders move into shallow shallow microhabitats around the edge of ponds during the day based on water temperature warming as the day progresses. Second, we quantified the thermal environment of salamanders by pairing remote detection of animals using previously implanted PIT tags and non-tagged animals with temperature readings of the water vegetated and non-vegetated areas in the ponds. We predicted that salamander density would increase in warmer microhabitats in the afternoon following solar heating of shallow areas, and that salamander density would be higher in vegetated thermal areas than in non-vegetated areas.

Materials and Methods

We studied the Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) found in the ponds at the MCNP, Colorado. In order to test the salamanders thermoregulation behavior, we surveyed thermal pools in six ponds. Thermal pools are warm shallow areas in ponds that receive the most sunlight and provide thermal variation for salamanders to do basic bodily functions. In this study the ponds were categorized as permanent or semi-permanent. Ponds 1, 5, and 12 are permanent ponds and ponds 6, 8, 9, are semi-permanent. We placed 1x2 meter quadrats marked by four pin flags in the thermal pools of the ponds. Quadrats were measured by meter rulers. Two pairs of quadrats, vegetated and non-vegetated, were placed in ponds 1, 5, 8, 9, and 12. The smallest semi-permanent pond of this study, pond 6, had only one pair of quadrats. Vegetation cover in vegetated and non-vegetated quadrats was ranked on a cover class system by Daubenmire (Baxter 2014).

In order to keep the sampling order of quadrat surveys randomized with respect to order and time of day, we first assigned each quadrat a number picked from a random list of generated numbers. Each survey was picked by writing down each pond number and being placed into a hat and drawn. Ponds where salamanders were being captured by the Whiteman lab that day were excluded from the draw because of the lower density of

salamanders thermoregulating would skew our results. If animals were returned to the ponds before the next survey then the ponds were included into the draw again. To record the water temperature of the quadrats a thermocouple was threaded through a PVC pipe with one end of the thermocouple reading the temperature and the other holding the reader. A remote detection device (Biomark reader/HPR Plus PIT Tag Reader) was used to scan the PIT tags in salamanders that had previously been tagged for H. Whiteman's long-term study. PIT tags are a small radio transponder that contain a unique 10 or 15 digit alphanumeric identification number, which is assigned to individual salamanders. In order for the PIT tags to be scanned, the tag scanner/reader sends out a radio frequency and when a tag is within range, it will relay the identification code back to the receiver. Because salamanders are difficult to see in vegetated areas and our visual estimates of abundance would have been confounded by detectability issues, we took advantage of this technology and the high proportion of tagged salamanders in our study ponds.

The visual and remote detection surveys were staggered for this study. The visual surveys were conducted three times a day, in the mid-morning, early afternoon, and afternoon. The remote detection surveys were conducted twice a day, after the first and second visual surveys. Ponds that were categorized as non-vegetated were only part of the Visual Surveys. The visual surveys consisted of two surveyors in order to have two independent counts of animals. For our statistical analysis we took the independent count with the most observed animals at each time period. The temperature of the water in the quadrat was recorded as well as the weather at each pond during the surveys. During the remote deception surveys, vegetated and non-vegetated quadrats were scanned for animals with PIT tags. In non-vegetated quadrats animals that were in the quadrats but were not tagged were recorded as paedomorphic, metamorphic or larvae.

Throughout the study many of the quadrats had to be moved as the water levels dropped naturally over time. For ponds 1, 5, 6, 8, 9, and 12 at least one or both vegetated quadrats had to be moved to keep up with the retreating water line. In ponds 8, 9, and 12 both vegetated quadrats were moved and one or more vegetated quadrats were unable to be counted in the study since they had completely dried up. Towards the end of the study, average water depth of each quadrat was also recorded (Table 2). Quadrats that had dried up were considered as n/a. Most of the data collected for the full duration of the study came from permanent ponds. Since the majority of the population in permanent ponds are paedomorphic salamanders, our data are skewed towards paedomorphic salamanders.

For our data analysis R 4.0.1 and R studio 1.3 were used as well as fitdistrplus package, lme4, multcomp, and nlme9. In analyzing the data for the visual surveys and remotes detection, we first wanted to compare the temperature at different time periods. First we used a repeated measures ANOVA test. Since the data for the visual surveys did not follow a normal distribution, we used a Poisson distribution to examine the data. Since we found that time period had a significant effect on temperature in each quadrat, we then used a post-hoc multiple comparison of means Tukey test. To analyze the temperature of the visual surveys during different time periods in vegetated and unvegetated quadrats a Shapiro-Wilk test was performed to test whether the data followed a normal distribution. The results were significant (unvegetated: $W=0.83281$, $p<0.0001$; vegetated: $W=0.97021$, $p<0.05$, Figure 5), meaning the data did not follow a

normal distribution and histograms showed the data followed a Poisson distribution. A Wilcoxon test showed that the temperature data from vegetated and non-vegetated quadrats were significantly different from each other. To further prove this a T test was performed on the same data and similar results were shown. For the temperature comparison at different times periods with the remote detection data, a Shapiro-Wilk test showed a significance for both time periods, meaning the data were not normally distributed (time period 1: $W=0.92212$, $p<0.0001$; time period 2: $W=0.90212$, $p<0.0001$). Further testing using histograms showed the data followed a Poisson distribution.

To analyze the data of the number of salamanders observed during visual surveys we used the *fitdistr* distribution to see what kind of distribution the data followed and it was similar to a Poisson distribution. A generalized mixed model was used to analyze the data of the number of salamanders counted in each quadrat and a Poisson error distribution accounted for the number of zeros in the data.

For the remote detection survey data, we also wanted to find whether there was a significant relationship between vegetated and non-vegetated quadrats and the number of salamanders. We used a generalized mixed model and a Poisson error distribution to test this. A Poisson error distribution tells R that the data is not following a normal distribution and accounts for that. We found that quadrat type, such as vegetated and non-vegetated, had a significant effect on the number of salamanders detected. A repeated measures Anova test and a Poisson distribution error test was used to also test for significance on vegetation cover and the number of salamanders detected. We found that vegetation did not have a significant effect on the number of animals detected. We also wanted to test whether time periods such as early and late afternoon had an effect on the number of animals detected. Another generalized mixed model was used and a Poisson distribution error test. Using the data from the visual and remote detection surveys, and the temperature recordings, we were able to better understand how the salamanders thermoregulate by moving between the thermal microhabitats in each pond throughout the day.

Results

Temperature at Different Time Periods

The average temperature was taken for each time period by taking all of the temperature data from each quadrat and averaging it. The average temperature of each time period showed that the temperature was highest during time period 2 for the visual surveys (23.5°C, Figure 1) and remote detection surveys (25.2°C, Figure 2).

A repeated measures ANOVA test with temperature as the response variable, time period as a fixed effect, pond and quadrat set as random errors, and a Poisson distribution error was used to examine the effect that time period had on the water temperature during the visual surveys. Time period had a significant effect on the temperature within each quadrat (Df Sum square=1, mean square=277.5, F value=42.15, $p<0.0001$, Figure 1). We then used a post-hoc multiple comparison of means Tukey test to see how significant the temperature difference was between each of the 3 time periods. The test showed that the temperature during time period 1 was significantly different from time periods 2 and 3 (1 and 2: estimate=4.0213, standard

error=0.4158, z value=9.671, $p<0.0001$; 1 and 3: estimate=2.9852, standard error=0.4238, z value=7.050, $p<0.0001$), but there was no significant difference between time periods 2 and 3 (estimate=-1.0332, standard error=0.4324, z value=-2.390, $p>0.05$).

For the vegetated and unvegetated temperature data, a Wilcoxon test also showed that the temperature data from the vegetated and unvegetated data sets were statistically different from each other ($W=4283$, $p<0.05$, Figure 5). The T test performed on the temperature data were also significant, meaning that vegetation had a significant effect on the temperature ($t = -2.2442$, $df = 200.53$, $p<0.05$).

For the temperature and time period data for the remote detection surveys, the Wilcoxon test also yielded significant results, meaning that the temperature data from time period 1 and time period 2 were statistically different ($W=4278$, $p<0.05$). The results from the T test done on the data were significant, showing that time period also had a significant effect on the temperature ($t = -2.1709$, $df = 176.11$, $p<0.05$, Figure 2).

Visual Survey Time Periods

The data are similar to a Poisson distribution using the `fitdistrplus` package in RStudio. A generalized mixed model was used with salamander count as the response variable, time period as the fixed effect, pond and quadrat as random effects, and a Poisson error distribution. The results showed that time periods 1 and 3 had a significant effect on the number of salamanders observed while time period 2 did not (time period 1: estimate=-1.7934, standard error=0.6501, Z value=-2.759, $p<0.01$; time period 2: estimate=0.3666, standard error=0.2762, Z value=1.328, $p>0.1$; time period 3: estimate=0.7776, standard error=0.2576, Z value=3.018, $p<0.01$, Figure 3).

Remote Detection Survey Vegetated vs Unvegetated Areas

We used a generalized mixed model with salamander count data as the response variable, quadrat type as the fixed effect, pond and quadrat as random effects, and a Poisson error distribution to test the relationship between quadrat type (vegetated and unvegetated) and the number of salamanders that were detected (Figure 6). The results showed that quadrat type had a significant effect on the number of salamanders detected (unvegetated: estimate=-3.3694, standard error=1.0963, Z value=-3.073, $p<0.005$; vegetated: estimate=1.8934, standard error=0.8348, Z value= 2.268, $p<0.05$).

A repeated measures ANOVA test with salamander count data as the response variable, vegetation cover and time period as fixed effects, pond and quadrat as random effects, and a Poisson distribution error was used to determine whether the amount of vegetation cover had a significant effect on the number of salamanders detected (Figure 6). The results showed that the vegetation category did not have a significant effect on the number of salamanders detected ($p>0.1$).

Remote Detection Survey Time Period 1 vs Time Period 2

A generalized mixed model with salamander count data as the response variable, time period as the fixed effect, pond and quadrat set as random effects, and a Poisson distribution error was used to test the significance of time period on the number of salamanders detected. The results

revealed that time period did have a significant effect on the number of salamanders detected (estimate=0.4387, standard error=0.1440, z value=3.047, $p<0.005$, Figure 4).

Discussion

Based on the temperature data collected during the study, Arizona tiger salamanders used the thermal pools within the ponds mostly during the mid to late afternoon when the temperature of the water is the highest for both visual and remote detection surveys (Figure 1, Figure 2). This supports our hypothesis that salamanders are moving within the pond based on the water temperature in order to thermoregulate. We created histograms showing that salamanders are using thermal pools within a certain temperature range. These graphs and tests could be used to determine the preferred, optimal, and minimum and maximum temperatures for the tiger salamanders.

Based on our results, climate change will have a significant effect on the thermoregulatory behavior of Arizona tiger salamanders. With temperatures increasing, the way that salamanders thermoregulate will change. Higher temperatures in the environment will increase the temperature of the ponds, making the thermal pools larger and deeper. Since salamanders have to thermoregulate based on the water temperature, they might start to stay in the deeper areas of the ponds because the shallows will be too hot for them to thermoregulate properly. Our results suggest that salamanders will start to stay in deeper water as temperatures increase because the shallow areas of the ponds will become too hot.

By using remote detection, we found that salamanders were more often in vegetated thermal pools than non-vegetated thermal pools. This could be due to temperature differences since the average temperature was higher in vegetated areas than unvegetated areas (Figure 5). For the most part, the vegetated areas are shallower than the unvegetated ones, meaning they would be warmer than unvegetated areas (Table 1). Other possible reasons for this trend are that vegetated areas might have more food resources or that salamanders could better camouflage themselves from larger salamanders or other predators.

Both the visual and remote detection surveys worked well for this study. For future research, remote detection is a great way to survey the tiger salamanders since most of the animals in each pond have PIT tags and it can be used to survey vegetated areas since it is not possible to do so with visual surveys. However, visual surveys work well if you are focusing on unvegetated areas of the ponds. For this type of research, we recommend starting surveys around 9 o'clock in the morning. If you plan on doing both visual and remote detection surveys, it would be best to switch between them throughout the day in order to get more data.

In the future, we could take the temperature of the water next to individual salamanders using the thermocouple to determine what the selected temperatures for tiger salamanders are. By matching temperature data with PIT tag information, we could test whether or not there is a size structure in the population for use of thermal pools. A size structure can

form in the population due to the possibility of cannibalism. Tiger salamanders become cannibalistic due to a variety of environmental cues (Denoël et al 2006). These specialized morphs develop teeth and larger heads in order to eat other salamanders, larvae, and eggs. Cannibalism also increases intraspecific competition, with larger individuals being more dominant. Since larger salamanders would be more dominant, it is possible that they would use the thermal pools more and exclude smaller salamanders from the warmer areas in the ponds.

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Tables and Figures

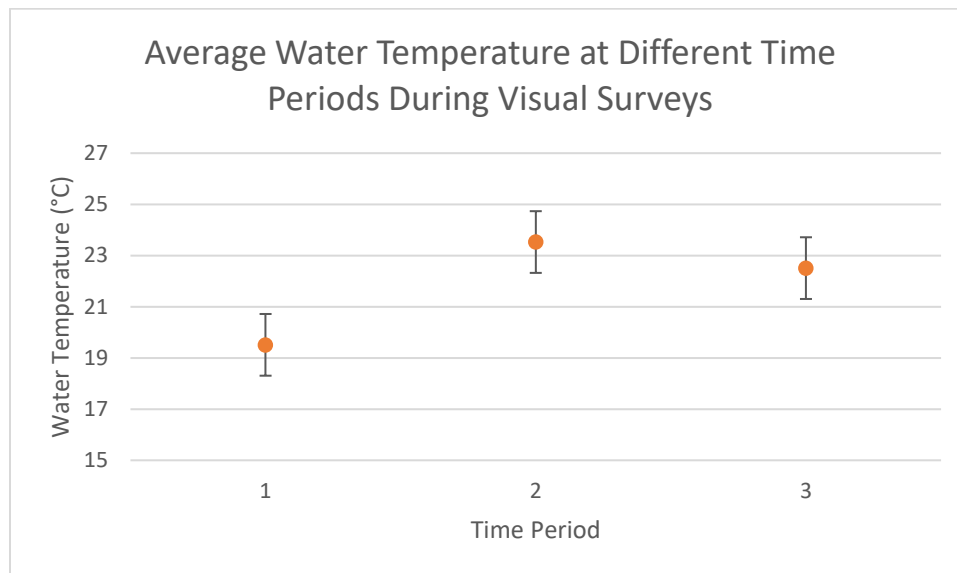


Figure 1. Average water temperature at different time periods during visual surveys

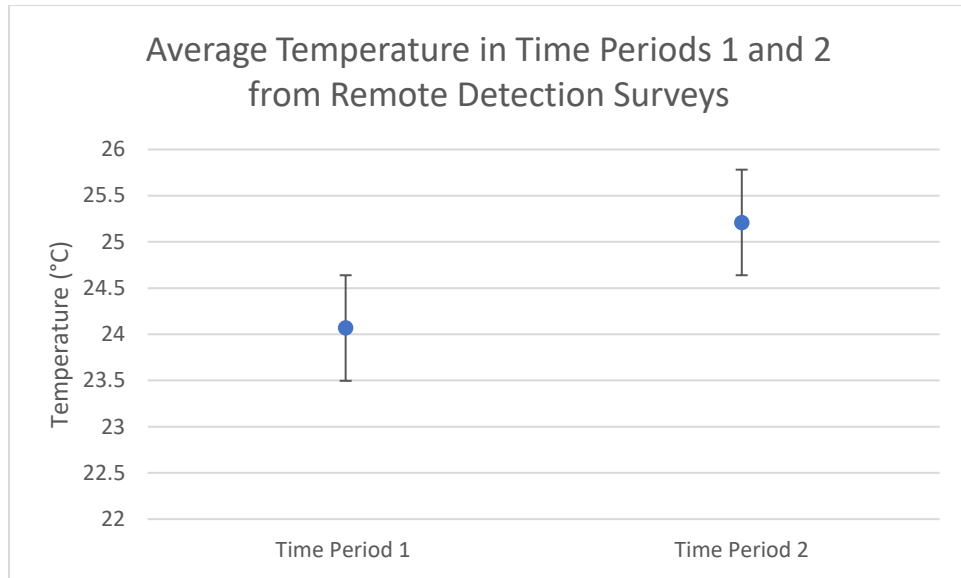


Figure 2. Average water temperature at different time periods during remote detection surveys

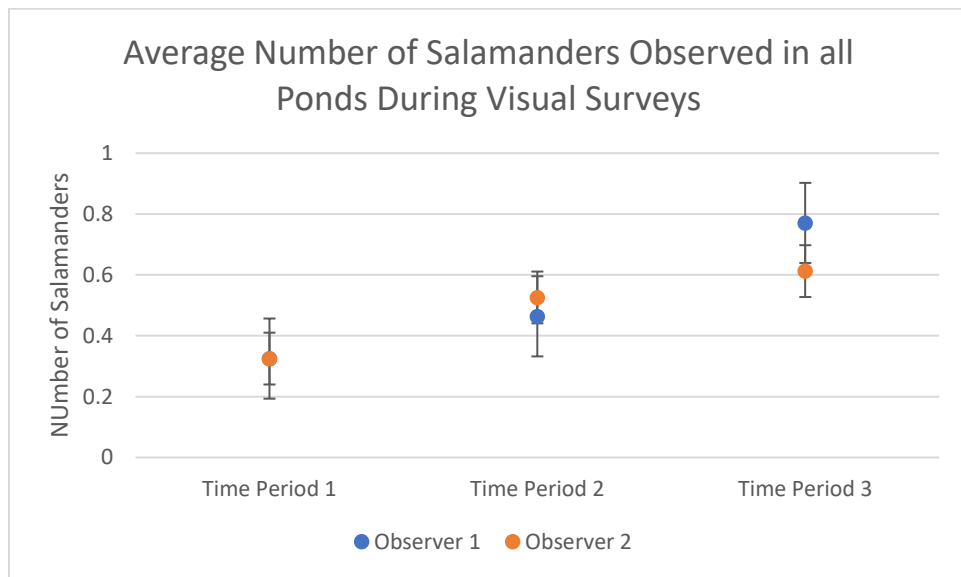


Figure 3. Average number of salamanders observed by both observers during visual surveys

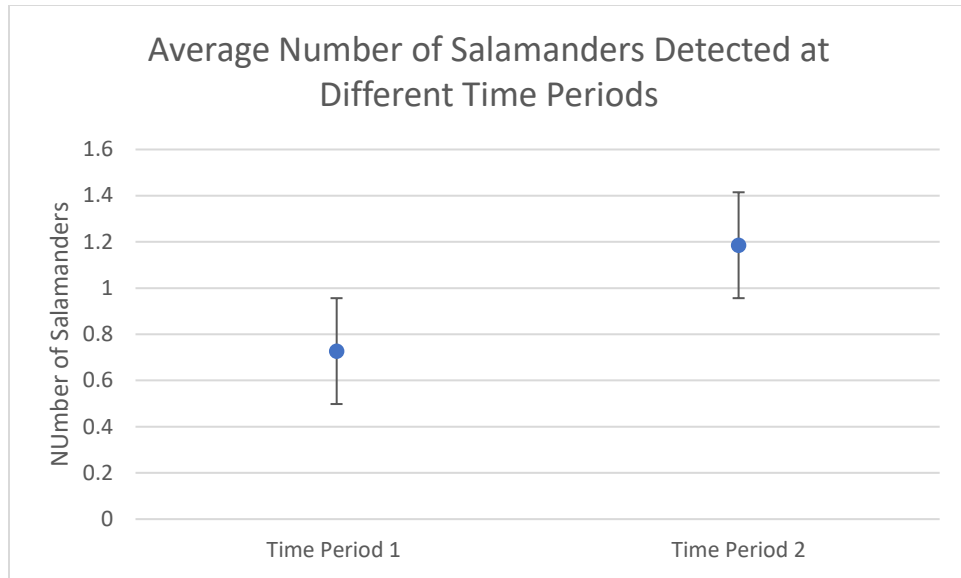


Figure 4. Average number of salamanders detected at different time periods during remote detection surveys

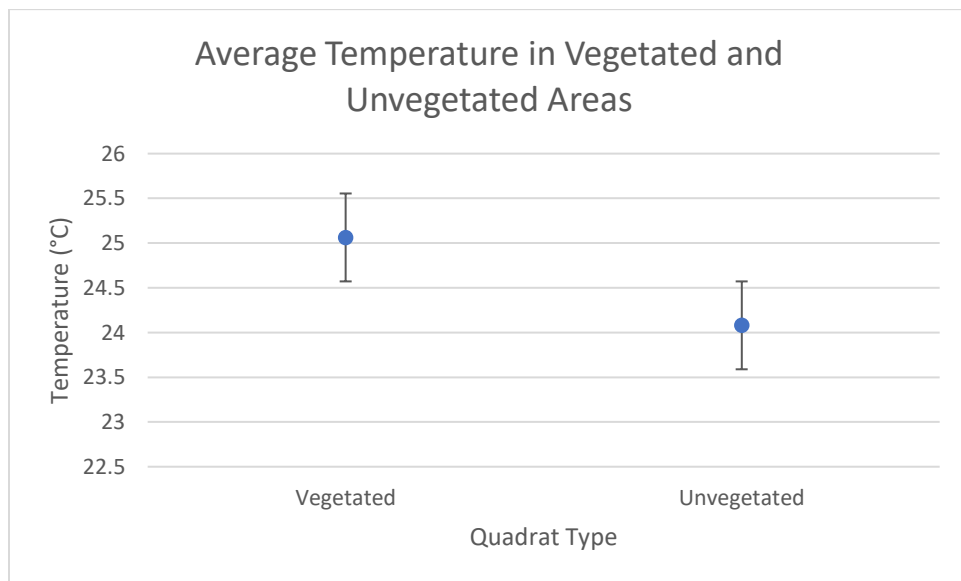


Figure 5. Average temperature in vegetated and unvegetated areas during remote detection surveys. Data includes both time periods for each quadrat type.

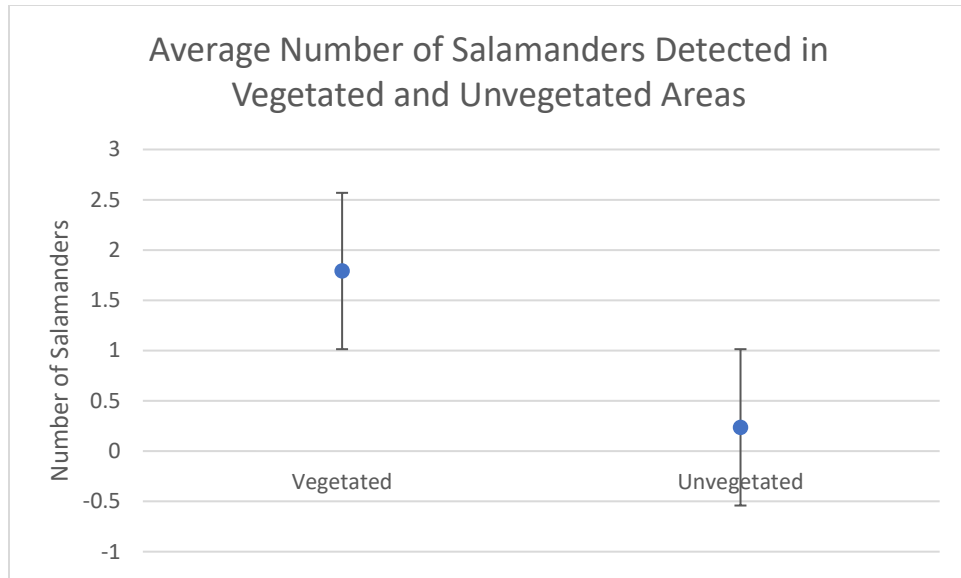


Figure 6. Average number of salamanders detected in vegetated and unvegetated areas. Data includes both time periods for each quadrat type.

Quadrat	Depth 1 (cm)	Depth 2 (cm)	Depth 3 (cm)	Depth 4 (cm)	Average Depth (cm)	Standard Deviation
1-10 V	3.9	8.0	26.1	28.1	16.525	10.697
1-14 V	4.0	5.7	14.7	18.3	10.65	5.992
1-17 NV	13.0	16.8	27.5	20.1	19.35	5.334
1-21 NV	6.0	3.0	13.2	12.0	8.55	4.208
5-1 V	6.5	15.9	46	21.5	22.475	14.601
5-4 V	12.1	10.0	30.3	24.5	19.225	8.461
5-12 NV	9.9	25	34.1	22.3	22.825	6.486
5-19 NV	22.0	11.9	31.5	32.1	24.375	8.242
6-6 V	6.5	8.0	38.4	35.9	22.2	14.895
6-8 NV	13	4.0	14.8	12.8	11.15	4.201
8-3 V	N/A	N/A	N/A	N/A	N/A	N/A
8-9 NV	5.2	4.0	7.5	16.0	8.175	4.690
8-20 V	N/A	N/A	N/A	N/A	N/A	N/A
8-22 NV	9.5	8.0	22.0	24.5	16.0	7.323
9-2 V	2.5	5.0	1.5	12.0	5.25	4.100
9-5 V	4.0	6.8	11.0	5.9	6.925	2.560
9-13 NV	4.5	6.9	17.5	19.4	12.075	6.466
9-18 NV	4.0	6.0	19.3	21.5	12.7	7.771
12-11 V	N/A	N/A	N/A	N/A	N/A	N/A
12-15 V	3.5	16.9	17.4	4.5	10.575	6.587
12-16 NV	17.9	10.0	24.5	29.6	20.5	7.345
12-25 NV	10.5	6.1	11.0	140	10.4	2.820

Table 1. Depth measurements for each quadrat. Some quadrats had dried up by the time the depth was taken.

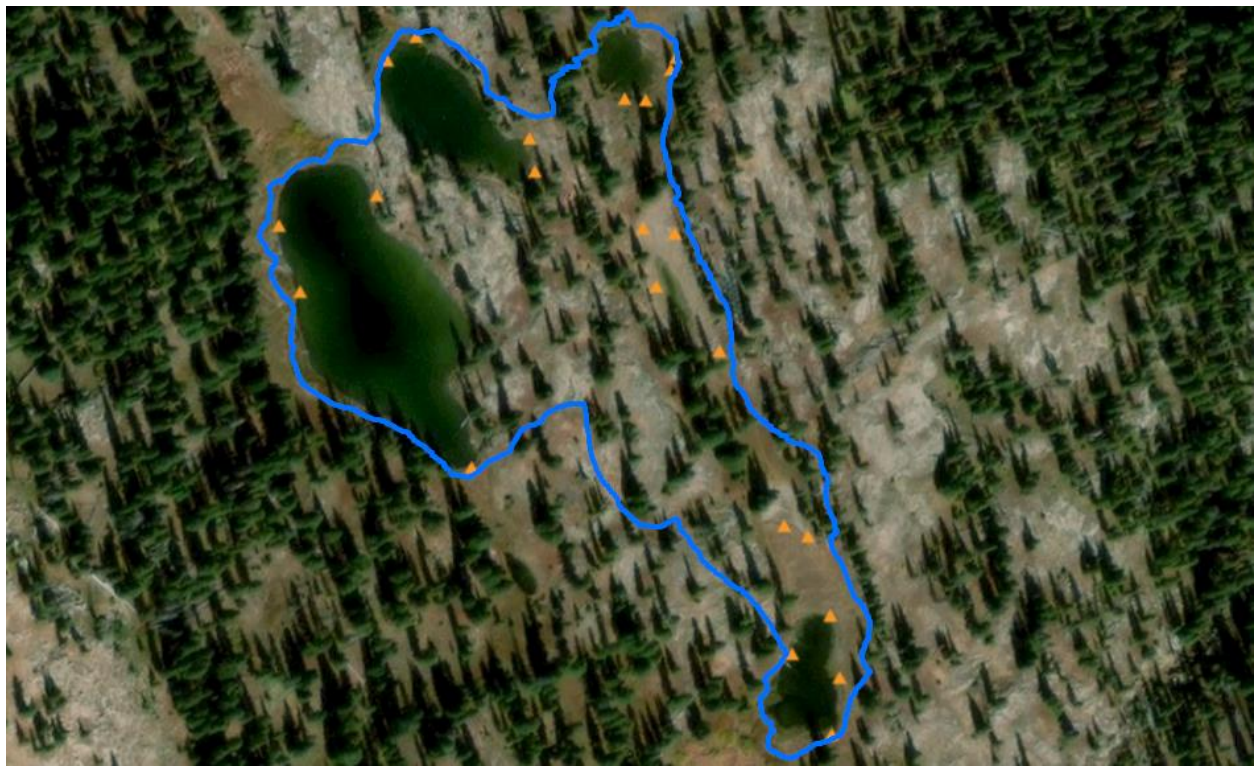


Figure 7. Map of the study site. Orange triangles indicate where quadrats were placed in each pond.