Green Frog (*Rana clamitans*) calling habitat associations: Are males selecting calling habitat more closely associated with egg-laying or predator protection?

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Abstract

Successful reproduction makes individuals evolutionarily fit but requires balancing costs. Literature suggests green frogs defend territories for breeding. Males will call in these territories to attract a mate. Unfortunately, calling may increase susceptibility to predation requiring males defend habitat with more protection. In contrast, females select the oviposition sites, potentially based on factors besides predation. Males defending habitat appropriate for oviposition may be more successful. We examined habitat for calling and egg-laying to determine whether territoriality is associated with defending oviposition sites or protection from predators. Our results show that calling males are more closely associated with emergent vegetation, especially medium emergent vegetation and negatively associated with open water. A comparison of the habitat at calling, non-calling, and oviposition locations suggests that there is no real difference between oviposition and calling or non-calling locations. However, calling locations had significantly more emergent vegetation (both medium as well as all combined heights) than non-calling locations. The oviposition sites had intermediate levels of emergent vegetation, suggesting that calling males may be selecting habitat more for protection than oviposition sites.

Introduction

Evolutionary fitness requires individuals to obtain the appropriate resources to survive and reproduce, thus passing their genes into the next generation. Because competition for resources is often limiting, one strategy to be successful is to find and defend a territory containing resources. Territoriality has been identified in all animal taxa (Maher and Lott 2002). However, this strategy appears to be variable across taxa and even within species (Maher and Lott 2002) reflecting potential tradeoffs associated with this strategy. Defending a territory can be costly and if the benefit comes at too high a cost, territoriality may not be an evolutionarily successful strategy (Stamps 1994).

In a review of the literature Maher and Lott (2002) recognized 10 general ecological variables thought to influence territoriality (Table 1). While each of these variables acting alone could influence the importance of territoriality, combinations of them may also play an important role. For instance, males may collect and defend mates, however, if the population density is so high (or low) where defending these mates becomes too costly, territoriality may not be a successful strategy.

Green frogs (*Rana clamitans*) are described as territorial (Harding 2000) and there is ample evidence to support that some populations do show territorial behavior (Martof 1953, Schroeder 1968, Wells 1977, Given 1990, Bee et al. 1999). Most studies seem to provide support that males are defending habitat rather than food, mates, space or other resources. For instance, in a supplemental feeding experiment, Gordon (2004) determined that territoriality may not be related to the availability of food.

Interestingly, a recent study suggests that territorial behavior does not always occur in green frogs (Shepard 2002). However, the author was specifically looking for uniform spacing (suggesting defended territories) among the males and assumed that habitat was uniform around the pond. If the cost/benefit model is correct, it is possible that males become more defensive in higher quality habitat because the benefit is higher than the cost (although population density could influence the cost). In poorer quality habitat, males may choose not to use a territorial strategy.

Wells (1977) examined habitat of the territories defended by green frogs and found that they were centered on artificial shelters, clumps of bulrushes and sedges, and occasionally an abandoned muskrat tunnel. This suggests that males are defending territories because it contains habitat better protected from predators at a time when they may be most exposed to predation (calling). However, territoriality may be a way of defending areas that increase likelihood of breeding. For instance, Martof (1953) and Wells (1977) found that egg deposition occurred in the vicinity of where males were calling.

In particular, our hypothesis is that male green frogs are defending habitat more suitable for egg-deposition. Thus we should be able to show that habitat associated with egg-deposition will be more similar to habitat associated with territorial defense. In this study, we assume that calling males are likely to be territorial. Therefore, habitat associated with calling locations should be similar to habitat where eggs are deposited. Further, we would expect locations where males were not calling (and assumed not territorial) to be less similar in habitat with either calling locations or areas where eggs are deposited.

Methods and Materials

Study Area:

This study was conducted in a meadow wetland just east of the Hyla house property, Pierce Cedar Creek Institute (PCCI) in Hastings, MI (Figure 1). The wetland is bounded by Cloverdale Road (dirt road) to the north, residential property to the west (Hyla House), and abandoned agricultural fields to the east and south. The wetland is seasonal; maintaining water throughout the spring and part of the summer during dry years while in wet years it may maintain water the entire year. There is no direct drainage (stream) that feeds into or out of this wetland. The nearest wetland appears to be seasonal wetland on private property north of Cloverdale road. To assist in systematically sampling the entire pond, 13 transects were randomly established oriented in a north/south direction (approximately 4 to 5 meters apart).

Study Species:

Green frogs are common anurans found throughout the eastern United States into the midwest states (Conant and Collins 1998). In Michigan, green frogs are commonly found in a wide variety of wetland habitats and are known to breed as early as mid-May through July or later (Harding 2000). This species is known to occur on the PCCI property from previous studies (McCurdy and Krum, 2005; McCurdy and Lupek, 2006)

Amphibian Sampling

The primary method used for identifying green frog individuals and egg masses were visual encounter surveys (VES). These surveys require observers to visually search for green frogs and egg masses while traversing the wetland. The VES were conducted after dusk between the hours of 8:30-2:00 a.m. from April 31 – June 16 for a total of 14 nights.

On nights sampled, a Kestrel 2500 Pocket Weather Meter was used to record the ambient temperature, wind speed, wind chill, and barometer. During VES, observers surveyed systematically in either a north to south or south to north direction along marked transects. The observers searched the area between two flagged transect lines, listening and watching for frogs. When an individual was spotted, calling status was noted and hand capture was used to avoid disturbing the habitat. Handling was done quickly to minimize stress.

Males were identified from a collection of characteristics including a yellow throat, large tympanum, and nuptial thumb pads. We uniquely marked each male by removing one to two toes using a marking system (Figure 2) similar to one identified by Martof (1953). No more than two toes were ever removed from an individual and never more than one toe per foot. Sharp surgical scissors were disinfected in a 70% ethanol solution and the appropriate toe(s) were clipped at the first joint (just above the webbing). Toe clipping has been shown to potentially have adverse effects (Clarke 1972), it is a commonly used and recommended form of cheaply and quickly marking individuals (Donnelly et. al 1994). In one instance, after toe clipping a calling male and returning him to his calling location, he immediately began to call again. Clipped toes were collected and frozen for potential DNA analysis in future projects.

All males were placed in a plastic bag, weighed to the nearest gram using a spring scale and the snout-vent length was measured in millimeters using a plastic ruler. To measure SVL, one person held the frog and flattened the body by pressing on the sacral joint to elongate the frog's body. Marked males were identified and unmarked males were toe-clipped. Individuals were then released in the same location of capture.

A bamboo stake with flagging tape was inserted at all locations in which a male was captured so we could revisit the location the next day and sample microhabitat. The date and ID number of the frog capture were marked on the flagging tape. Any individuals that escaped were also marked with a stake but labeled as unknowns (indicating the possibility of being a male). Males calling that escaped were still indicated as unknowns, but recorded as calling.

All individuals captured that did not have typical male characteristics were recorded as either females (larger individuals) or immature and returned to location of capture. For each green frog or egg mass identified, the location was flagged for later habitat assessment in a similar fashion as with the males. At a later date, geographic locations were determined for each point using a Magellen global position system Thales Navigation Mobile Mapper.

Habitat Sampling

Microhabitat was sampled using a 1 m^2 point frame to estimate the percent cover for various microhabitats (Table 2). The point frame was used to assess microhabitat percentages by taking data from 25 points inside the grid at 10cm intervals. At each point, a meter stick was vertically inserted to assess the immediate habitat type touching the stick. Depth measurements were also taken using a meter stick at the four corners and middle of the grid. When assessing habitat, information was taken for the bottom (underneath the surface) and top (immediately visible on surface) habitat.

Sampling occurred at every capture (or escape location) and at various points along transects (available habitat). The transect points were designated as 0-1 m from the shoreline, 0-1 m into the water from shoreline, 1-2 m into the water from shoreline, and then every 4m in between.

A map of the macrohabitat types was (Figure 3) generated by determining location of each macrohabitat using a Thales Navigation Mobile Mapper. The distribution of macrohabitat within Hyla pond was visualized using ArcGIS (ESRI).

Statistical Analysis of Data

Backwards Stepwise Logistic Regression (BSLR) was used to determine microhabitat associations. Additionally, an ANOVA was used to compare the resulting significant habitats identified in the BSLR among locations with calling frogs, locations with non-calling males, and egg masses. All statistical analyses were conducted using SPSS ver. 14.0.

Results

Amphibian Captures:

Over the course of 14 nights, we were able to capture 89 frogs. Population estimates suggest that we captured the majority of males within the pond (99 ±XX – Lincoln-Peterson, 88 ±XX – Jolly-Seber). Of the males that were captured, most were captured once (60.7%) or twice (22.5%), with a few (17.8%) captured three or more times (Table 3). We compared the size of males captured that were found to have called at some time during the survey period to those males captured that were never known to call using an Independent Samples t-test and found no significant difference (Table 4)

Over the course of the sampling period we captured 83 females. The daily average of females to males (Figure 4) was 0.72 suggesting that a total population of green frogs might be roughly 153 (89 males and 64 females). Seven egg masses were located during the 14 survey nights and during 14 subsequent days of habitat sampling.

Habitat associations:

To determine what habitat variables were associated with calling, we used BSLR with calling and non-calling as the binary measure. A location in which a captured male was known to call was considered a calling site. Other locations in which males were captured were considered non-calling sites. We included repeated captures of males (some in both calling and non-calling categories) as they appear to have moved more than 1 meter from their previous locations of capture. It is assumed that these selections would be independent of one another. The BSLR resulted in a 2 variable model explaining what habitat is associated with calling ($p=0.009$,$r^2 = 0.125$). The percentage of open water was negatively associated with the likelihood of calling ($B= -4.206$, $p=0.128$) while the percentage of medium emergent vegetation was positively associated ($B= 2.425$, $p=0.031$). Because vegetation height may be less important for calling than just having structure, we wanted to determine if emergent vegetation (grouping short, medium and tall emergent vegetation) would still be associated with calling. The second BSLR resulted in a significant model ($p=0.004$,$r^2 = 0.110$) with the combined percentage of emergent vegetation the only variable retained in the model ($B=3.098$, $p=0.007$).

Comparison of habitat – calling vs non-calling vs egg mass locations:

To determine if calling was more closely associated with egg laying, we compared average habitat where egg masses were found with calling and non-calling sites using ANOVA. It is assumed that if calling sites were more closely associated with egg-laying, there would be no difference between egg mass locations. However, there should be a significant difference between calling and non-calling habitat as well as egg mass and non-calling habitat. We only used the three habitat variables found important in the habitat model. We found no significant difference among location types (calling, non-calling, egg mass) and open water ($p = 0.095$ – Table 5). However, we did find significant differences in the percentage of medium emergent vegetation ($p= 0.041$- Table 5) and the combined emergent vegetation variable ($p=0.015$ – Table 5).

Post hoc independent samples t-tests found that the primary differences were between calling and non-calling habitat for both medium emergent vegetation ($p=0.017$) and the combined emergent vegetation (p=0.005). However, there were no significant differences between egg masses and either calling or non-calling on either variable.

Distributional Patterns:

Figure 5 shows the distribution of calling and non-calling locations across the pond for the entire sampling period. It is clear from the figure that the frogs were broadly distributed around the pond with most of the activity concentrated in the middle and southeast corner. It is interesting to note that few frogs were captured in the northeast corner (dominated by Purple Loosestrife) and the southwest corner (dominated by Reed Canary Grass). Figure 6 shows the weekly progression of calling locations. Over the course of the sampling period, the frogs appear to begin using the northwest and southeast corners of the pond for calling, at the peak of calling activity, they were distributed across the wetland, and toward the end of the activity, the frogs concentrated calling in the southeast corner.

Discussion

Size is thought to influence which males are more likely to defend territory. For instance, Wells (1977) found that larger males defended individual sites longer than smaller males (which he referred to as satellite males). In our study, however, we found no significant difference between calling behavior and size of the frog. Unfortunately, calling is not always indicative of territoriality and therefore our results may just represent no difference in sizes with reference to what males do or do not call. It is possible that some males that were classified as non-calling could have called at times when we did not sample as we have evidence of males captured two or more times that were calling during one sampling period and not in a second. However, a clear indication of territoriality was observed when a wrestling bout between two males was witnessed. The winner of the wrestling bout began to call immediately as the other fled from the location. Both males were captured and measured, and we found that the smaller male actually won the wrestling bout.

Martof (1953) and Wells (1977) both studied territoriality and spatial relationships in green frogs. Wells, however, took Martof's ideas of spatial relationships and territoriality and tested them with more accurate, detailed methods. Wells found that most defendeded territories were located along the shoreline near artificial shelters and clumps of vegetation. Likewise, our study showed a high association between calling frogs and habitat with emergent vegetation. Additionally, the negative association with open water and calling males suggests that areas absent of cover, such as open water, are less likely to have calling frogs inhabiting them. Interestingly, Wells (1977) did not find territories established in the center of his study pond where cover was absent. In contrast, the Hyla house pond has considerable amounts of emergent vegetation across the center of the pond as well as along the shoreline. Not surprisingly, calling males were distributed across the entire wetland (including the center and deeper areas).

To assess whether calling habitat was potentially selected because it represented better habitat for egg-deposition sites, we used an ANOVA to compare those variables identified in the logistic regression as associated with calling. We expected that the percentage of each habitat variable would not be significantly different in calling and egg-laying locations. We did expect significant difference between egg-laying and non-calling locations. Our results, however, were more ambiguous as none of the variables were significantly different between egg-laying and calling or non-calling locations. There were significant differences in the amount of emergent (medium or total) vegetation between calling and non-calling locations however. Unfortunately, these results may be a result of small sample size for egg-laying locations. When conducting VES throughout the pond, egg masses were difficult to locate, especially in areas of thick vegetation. We believe that there more egg masses laid over the breeding season, but were not found.

With the exception of open water (which showed no significant results in the ANOVA), the percentage of habitat in egg-laying locations were intermediate between calling and non-calling locations. This could suggest that calling males may be selecting habitat that may have more protection from predators. With egg-laying locations having less emergent vegetation, males could still be within close proximity to sites in which females will move to deposit eggs.

Wells (1977) found that most males occupied more than one site during the breeding season. Our results from the recapture data also suggested that males moved to different locations throughout the breeding period. In this study, it was found that there were some males recaptured in the same area on different nights throughout the sampling period, but it was also found that some individuals traveled quite a distance from the location where they were first captured. This movement may have been attributed to changes in territory quality as some areas become more overgrown and others areas that were more open increased in the amount of emergent vegetation. In his two year study, Wells (1977) found similar results when habitats that he had ranked as low quality in the first year were used occupied in the second year. These changes were attributed to the changing habitat (water levels) over both seasons.

An interesting finding of this study occurred when we mapped locations of individuals using GPS. We found that calling male frogs were rare in areas with very dense, tall vegetation (Figure 6). Some areas of the pond, for example, were overgrown with purple loosestrife and reed canary grass at the beginning of the sampling season and we rarely found or heard males in these areas. Additionally, vegetation in other areas of the pond grew thicker and taller as the sampling period progressed. A general trend noticed among the population of male green frogs was less individuals in areas of thickening vegetation. We hypothesize that there may be a balance between the how much emergent vegetation would be suitable for calling with too much vegetation making it difficult to encounter a mate and too little vegetation increasing exposure to predation. Considering the long breeding season and potentially long lifespan of green frogs, a male is more likely to maximize his lifetime fitness by reducing his risk of predation, even if that means occupying a mediocre oviposition site (Wells, 1977).

For future studies, it would be helpful to find out if there is a significant effect of the height and thickness of emergent vegetation on green frog breeding sites. If this is the case, invasive species such as those found in our pond may cause problems for breeding sites in the future.

The evidence presented in this paper suggests that calling males may select calling habitat that has moderate amounts of emergent vegetation. The amount of emergent vegetation is greater than the amount found at egg-mass locations. Although these results are not statistically significant (potentially because of too few egg-mass locations), it suggests that calling males may be selecting habitat with more emergent vegetation because it provides more protection from predators.

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Table 1. Ecological Variables thought to influence territoriality (reviewed by Maher and Lott 2002).

Ecological Variables	
Food	Resources
Population Density	Habitat Features
Mates	Space
Refuges/Spawning/Home sites	Predation Pressure
Host Nests	Energy Availability

Table 2. Habitat variables recognized for microhabitat sampling.

Aquatic	**Bottom**
Open Water (OW)	Bare Soil (BS)
Debris (D)	Rock (R)
Vegetative Litter (VL)	Woody Debris (WD)
Floating Aquatic Vegetation (AQF)	Vegetative Litter (VL)
Submerged Aquatic Vegetation (AQS)	Submerged Aquatic Vegetation(AQS)
Aquatic Rooted Vegetation (AQR)	
Short, Emergent Vegetation (SEV)	
Medium, Emergent Vegetation (MEV)	
Tall, Emergent Vegetation (TEV)	
Scrub/Shrub (SS)	

Table 3. The number of times males were captured over the sampling period as well as what percentage of the total captures they represent. Most males were captured 1 or 2 times.

Number of times captured	**Number of individuals**	**Percentage of total captures**
1	54	60.7%
2	20	22.5%
3	7	7.9%
4	6	6.7%
5	2	2.2%

Table 4. The size of males captured that were found to have called at some time during the survey period compared to those males captured that were never known to call using. There is no statistical difference between weight (p=0.512, t-Test) or snout-vent length (p=0.081, t-Test).

Type	N	Mean	Std. Deviation	Std. Error
		Weight		
Non-calling	54	45.0 g	7.6	1.0
Calling	41	46.1 g	8.2	1.2
		Snout-Vent Length		
Non-calling	54	77.1 mm	3.9	0.5
Calling	41	78.1 mm	5.2	0.8

Table 5. A comparison of average macrohabitat percentages where egg masses were found, at calling locations, and at non-calling locations. Eggs were more likely to be found in areas with more vegetation than either calling and non-calling sites, however, this was not significant (p=0.095, ANOVA). In contrast, ANOVA analysis did show that there were differences among calling, non-calling, and egg-laying locations for medium emergent vegetation (p=0.041), and combined emergent vegetation (p=0.015). Post-hoc analysis found that the calling and non-calling sites were significantly different with both medium emergent vegetation (p=0.017) and combined emergent vegetation (p=0.005).

Type	N	Mean	Std. Deviation	Std. Error
		OPEN WATER		
Non-calling	54	8.1%	12.1	1.6
Calling	41	4.1%	5.9	0.9
Eggs	7	2.9%	5.0	1.9
		MEDIUM EMERGENT VEGETATION		
Non-calling	54	15.1%	16.1	2.1
Calling	41	26.0%	24.7	3.2
Eggs	7	22.2%	25.6	9.7
		ALL EMERGENT VEGETATION		
Non-calling	54	31.1%	18.5	2.5
Calling	41	42.7%	20.5	3.2
Eggs	7	32.6%	18.4	7.0

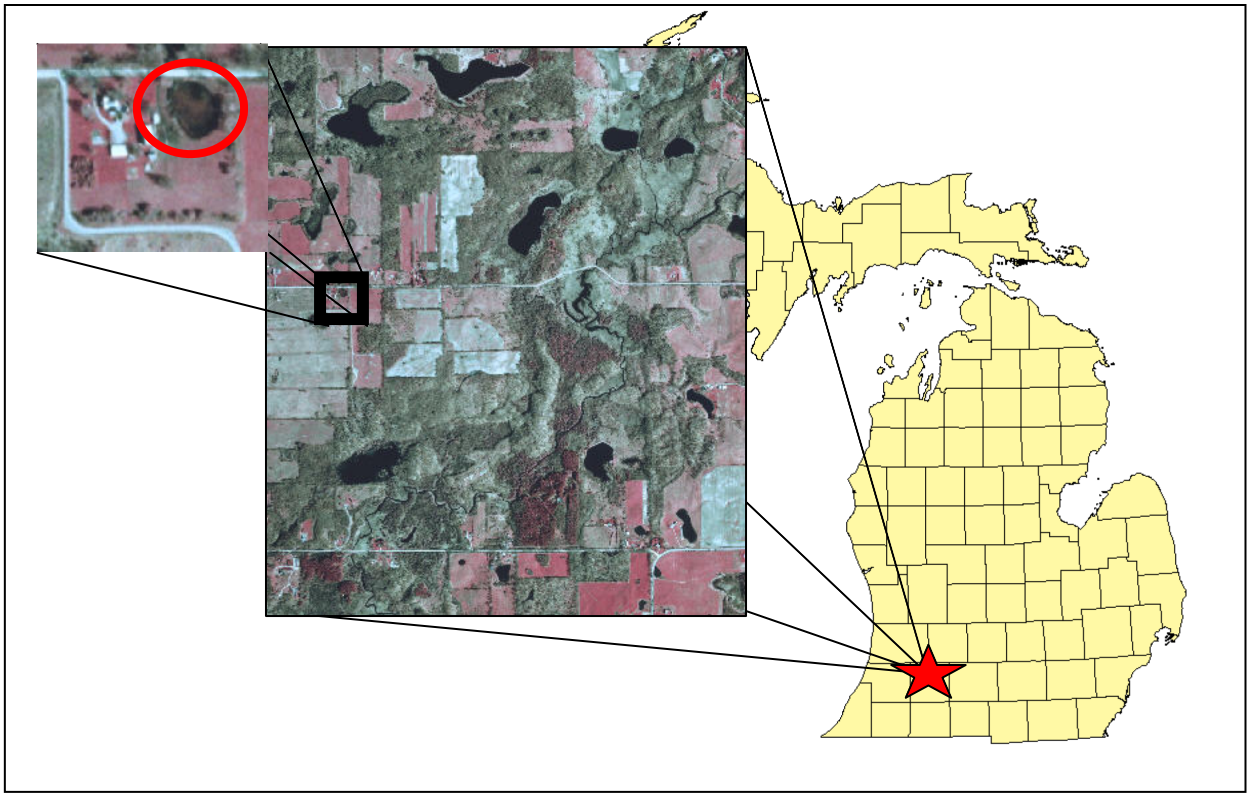

Figure 1. Location of Hyla House pond, Pierce Cedar Creek Institute, Hastings, Barry County Michigan.

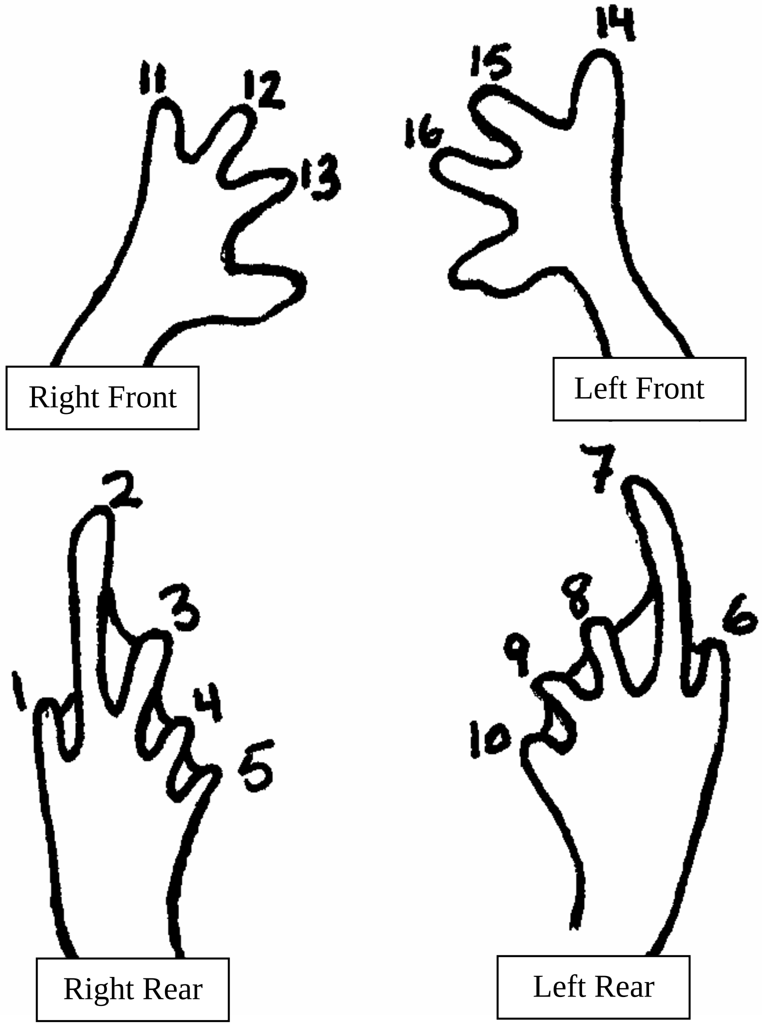

Figure 2. Diagram showing toe numbers used for toe-clipping. View is the ventral side of the feet. We uniquely marked each male by removing one to two toes using a marking system similar to one identified by Martof (1953). For instance frog 0-5 would have had only one toe removed (toe 5 on the right rear foot) and frog 8-16 would have had two toes removed (toe 8 on the left rear foot and toe 16 on the left front foot). No more than two toes were ever removed from an individual and never more than one toe per foot.

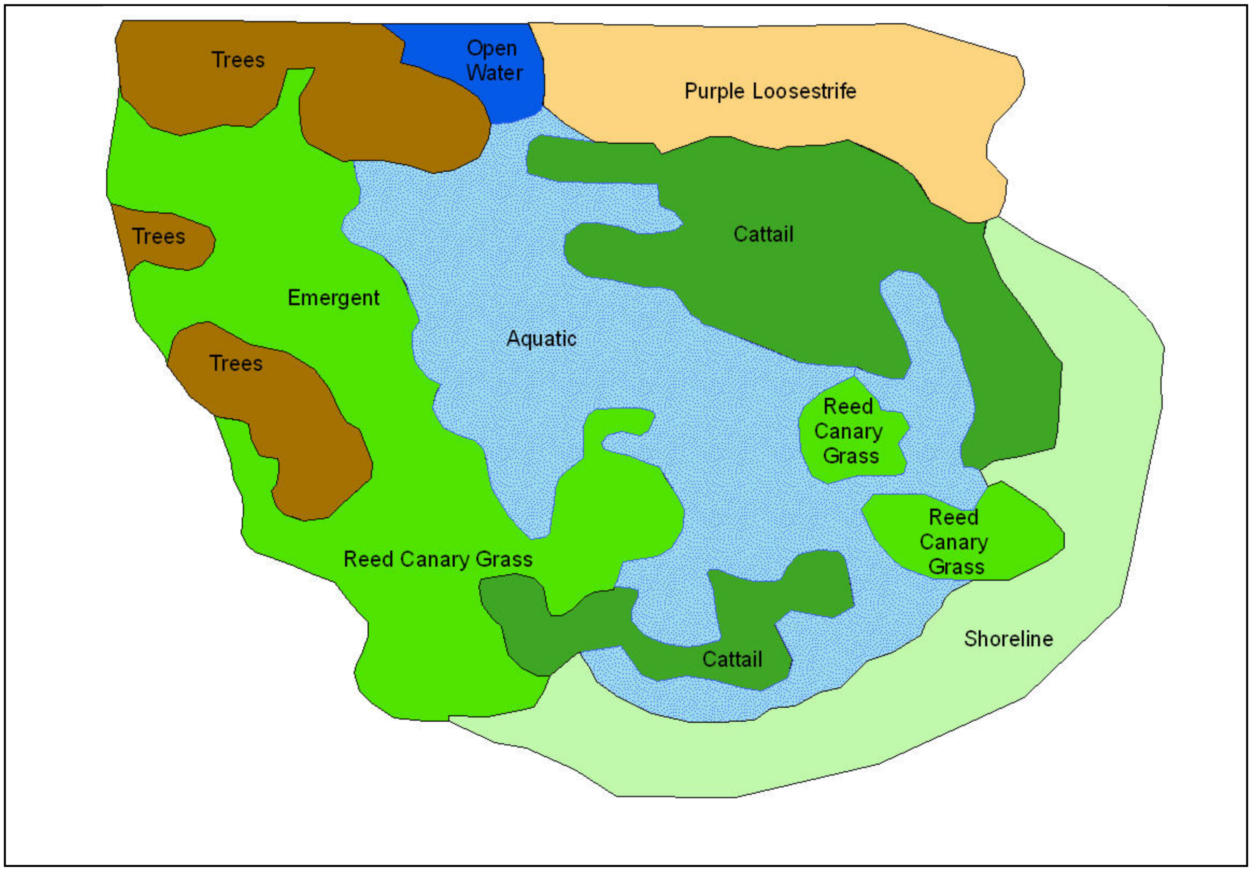

Figure 3. Distribution of macrohabitat in Hyla House Pond. Reed canary grass was considered emergent vegetation in microhabitat data collection. Purple loosestrife were considered scrub/shrub in microhabitat sampling.

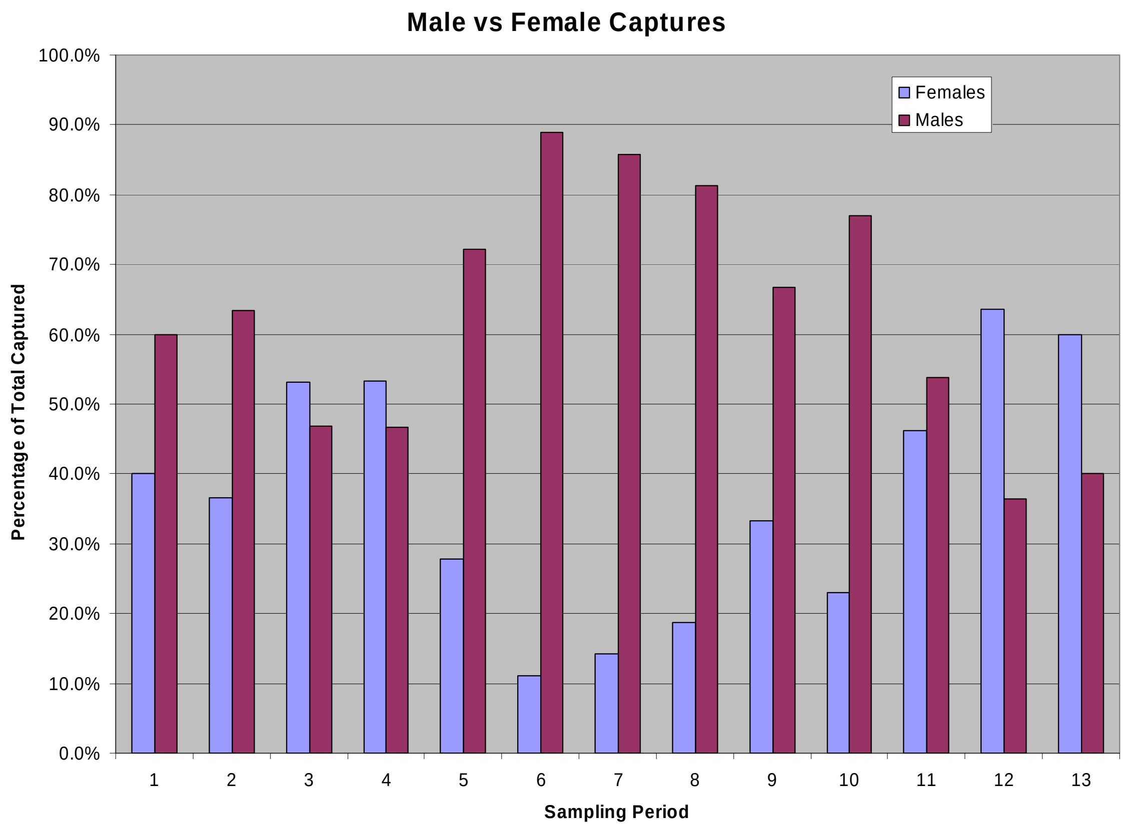

Figure 4. A comparison of the ratio of males to females across the sampling period. Results show that males outnumbered females for a majority of our sampling periods. During the middle sampling period the ratio was heavily skewed toward males.

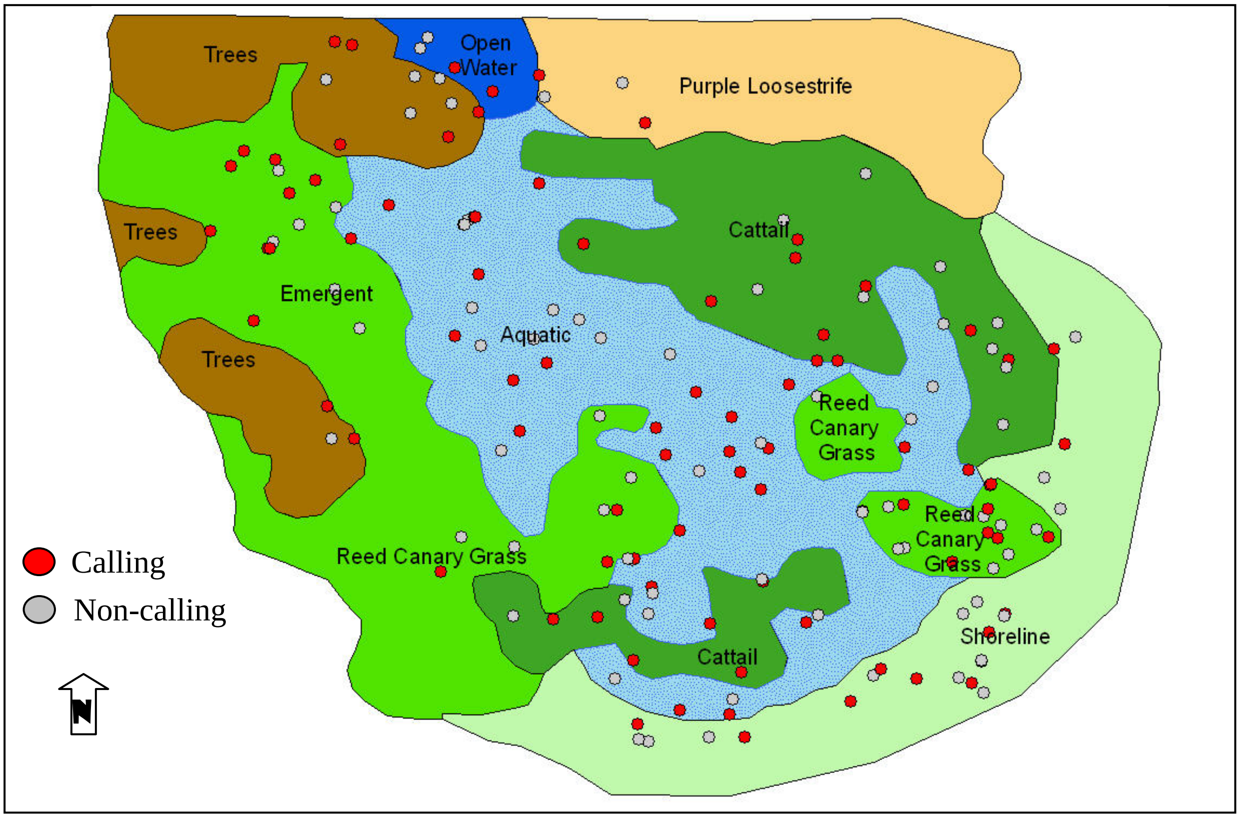

Figure 5. Distribution of capture locations in Hyla House Pond across the sampling period. Frogs were found distributed across the pond, however, the northeast and southwest, and west of the pond had much lower activity.

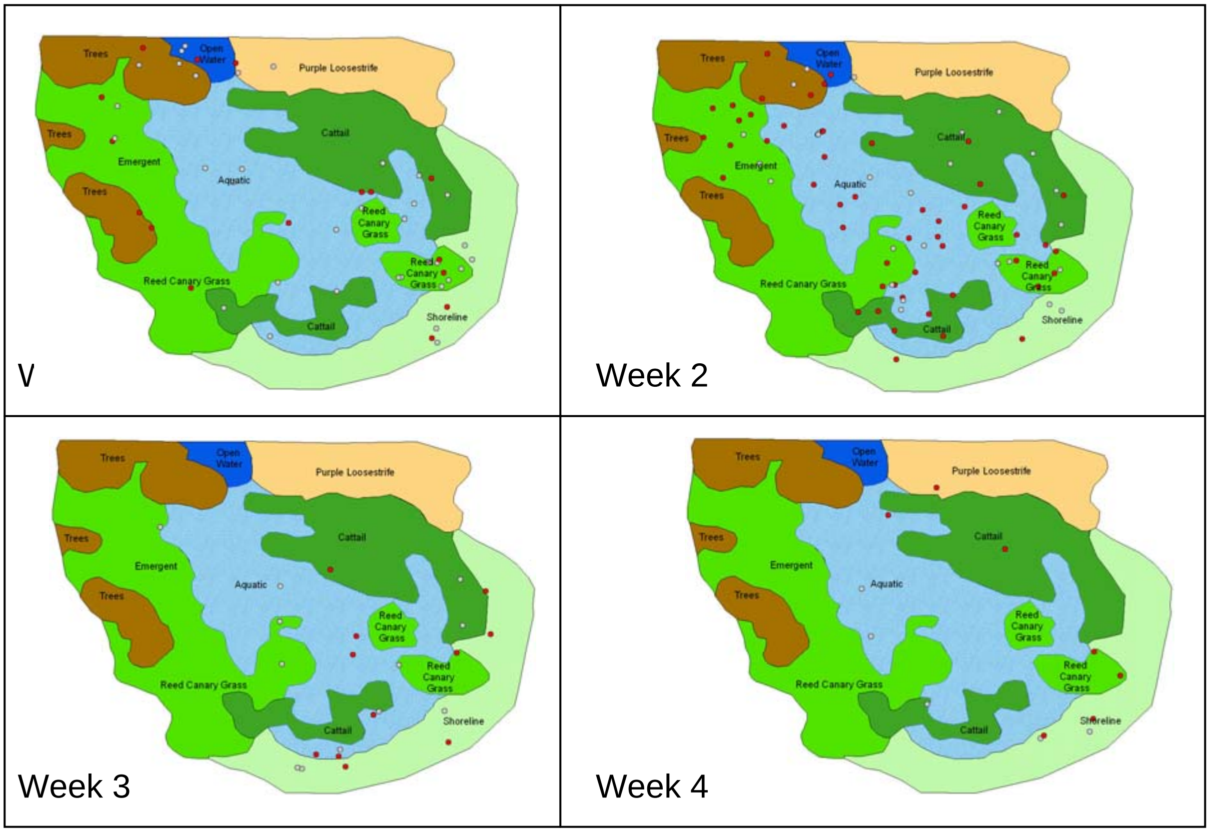

Figure 6. Weekly distribution of capture locations in Hyla House Pond across the sampling period. Early in the sampling period frogs were found in the northwest and southeast corners. By week 2, frogs had distributed across the pond with large concentrations in aquatic vegetation and emergent vegetation. By week 3 and 4, fewer frogs were found and mostly in the southeast corner.