# FACTORS INFLUENCING CALLING MALE GREEN FROGS AT PIERCE CEDAR CREEK INSTITUTE

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## Introduction

Organisms that have prominent breeding displays, such as vocalizations, face significant challenges for survival and reproduction (Ryan et al., 1981). Calling displays are often energetically costly (Pough et al., 2004; Schwartz et al., 1995) making males more conspicuous to other males and females, but also more obvious to predators influencing survival (Ryan et al., 1982). As a result, individuals must choose both the right time and location to display in order to be evolutionarily successful.

For amphibians, "the right time" may largely be influenced by their ecothermic metabolism. For amphibians, environmental temperature can influence the onset, number and duration of calling displays (Navas and Bevier, 2001; Navas, 1996; C et al., 1997). For instance, the occurrence of calling in green frogs and bullfrogs was significantly associated with water temperature (Oseen and Wassersug, 2002) . Other ranids have also shown significant relationships with calling activity and air temperature (Obert, 1975) suggesting that temperature may be a significant factor in determining calling activity.

While temperature may have an important influence, choosing appropriate calling habitat could also influence breeding success. Frogs choosing sites that have habitat conducive to maintaining warmer body temperatures (insulated habitat) may result in greater likelihood of obtaining a mate as they are able to maintain calling for longer periods. Alternatively, choosing a location with habitat in which females are more likely to be encountered or they may lay their eggs may also be critical. For instance, Martof (1953) and Wells (1977) found that egg deposition occurred in the vicinity calling green frog males. At the same time Wells (1977) described habitat of the territories defended by calling male green frogs centered on artificial shelters, clumps of bulrushes and sedges, and occasionally an abandoned muskrat tunnel. This suggested that males are defending calling territories might provide better protection. Thus, calling amphibians must select habitat that maximizes reproductive success while minimizing predation risk.

Strategies to address challenges in selecting appropriate breeding habitats include focusing on one wetland type with its specific selective pressures. For instance, spadefoot toads are more likely to use temporary wetlands and face pressure of undergoing metamorphosis before the ephemeral pools disappears (Denver et al., 1998; Newman, 1989). At the other extreme, Bullfrogs are only reproductively successful in deeper permanent ponds as their larvae often take up to 2 years to develop (Harding, 1997). A second strategy would be to show greater plasticity in traits associated with the range of selective pressures encountered (Van Buskirk, 2002; Carey, 1978; Bider, 1981). This would allow a species to occupy a variety of wetland habitats.

Green frogs are common and widespread species found throughout the great lakes (and much of Eastern U.S.). As habitat generalists, known to breed in numerous wetland habitats (Harding, 1997), it would appear that they have adopted a strategy of greater plasticity in traits. This ability to occupy multiple habitats may explain their relative success in establishing and maintaining populations while other conspecific amphibian populations have shown significant declines (Lannoo, 1998). However, each of these various habitats the species can occupy still has the potential to have unique selective pressures to which a specific population of green frogs must adapt (Berven et al., 1979). Pierce Cedar Creek Institute (PCCI) contains a variety of wetland habitat types that provide opportunities to explore how temperature and habitat might influence calling in green frogs. For this project we focused on the following questions:

- Does calling vary at these three different wetlands located at Pierce Cedar Creek Institute?
- Are there relationships with temperature (both air and water) and onset and/or level of calling at these three different wetlands?
- Are locations chosen for calling more likely to have moderate levels of emergent vegetation?
- Are there differences in the type of habitat in which calling males are located compared to locations where females or egg masses are located?

## **Methods and Materials**

*Study Area*

This study was conducted in three separate wetlands at Pierce Cedar Creek Institute (PCCI) in Hastings, MI *(figure 1*). We collected both water and air temperature data at these wetlands using HoboTemp Data Loggers set to record air temperature (1m shaded) and water temperature (2-3 cm below the surface) every 15 minutes. Unfortunately, the data logger at Hyla House Pond stopped working a month into the study, limiting our information for this wetland. On amphibian sampling nights, a Kestrel 2500 Pocket Weather Meter was used to record the ambient temperature, wind speed, wind chill, and barometric pressure.

Hyla House Pond is a relatively shallow wetland located in an abandoned agricultural field. It can be considered a seasonal wetland; maintaining water throughout the spring and part of the summer during dry years while in wet years it may maintain water the entire year. This wetland is mostly unshaded with abundant aquatic vegetation. It is assumed that the high solar radiation and shallow depth may result in this pond reaching warmer temperatures early in the season. In 2007, we studied green frog calling and territorial behavior in the Hyla House Pond at PCCI (Weeks and Burton, 2007). We found that calling males were significantly associated with a moderate amount of emergent vegetation (vegetation extending above the water surface). Unfortunately, this association with vegetation above the water in moderate densities did not adequately support or refute the hypothesis that males are selecting habitat for egg laying habitat as we had too few egg-masses with which to make comparisons. Further, we did not examine how abiotic factors such as temperature impact calling activity.

Our second wetland (referred to as Wood Pond), is another relatively shallow wetland at PCCI. It is surrounded on all sides by deciduous forest and is classified as a permanent to semi-permanent pond. Previous researchers working at or near this pond have indicated that green frogs can be found consistently at this wetland, however, no calling has ever been heard (McCurdy; Burton, personal communication). As the work conducted at this location was limited in duration and not focused on calling, it is unclear if calling ever occurs at Wood Pond. The forest canopy in the area will possibly result in cooler temperatures throughout the season which might limit calling activity. Further, the habitat at this wetland is limited to downed woody debris with most emergent structure occurring in the center of wetland consisting of woody shrubs. The shoreline is largely soil and fallen leaves.

Our final wetland, Brewster Lake, is a large oligotrophic lake consisting primarily of deep open water. The shoreline is relatively wide and shallow, lined with deep muck and emergent vegetation. It is almost entirely surrounded by deciduous forest and is fed and drained by Cedar Creek. While this lake has similar levels of solar radiation compared to Hyla House Pond, the volume of water may slow warming through the season.

*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, 1997). From previous studies, this species is known to occur on the PCCI property (McCurdy and Krum, 2005; McCurdy and Lupek, 2006; Weeks and Burton, 2007).

*Amphibian Sampling*

We used nighttime calling surveys to estimate the number of calling males at each wetland. Calling surveys, consisting of 5 minute periods of listening for the number of calling males, were conducted at each site. Calling surveys were conducted between 2030 and 2200 hours with calling recorded on a scale from 1 to 5. A rating of one would indicate a single individual heard. A rating of two indicated that up to five frogs were calling, with every individual distinguished in the chorus. A call rating of three resulted when more than 5 frogs calling yet individuals could be distinguished. A rating of four would describe a small chorus where we were unable to distinguish individuals (the chorus was likely limited to specific region of the wetland). Finally, a five rating would indicate a large chorus where we were unable to hear individuals, with the calling widespread across the wetland.

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. 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. The VES were conducted after dusk between the hours of 2030 and 0200 from May 5 – June 29. We were able to survey a total of 35 sampling nights at Hyla House Pond, 14 at Wood Pond, and 13 at Brewster Lake. Because of the vast differences in habitat between the wetlands, sampling patterns varied to fit best each wetland.

At Hyla House Pond, observers surveyed the pond while focusing on 25 plots chosen for homogenous habitat type. The observers searched the area between plots and diligently within plots, listening and watching for frogs. At Wood pond, surveying was conducted by searching the shoreline slowly around the entire pond. Surveying the middle of the pond was never necessary because all frogs were within 3 meters of the shoreline. At Brewster Lake, sampling was limited to four 50 meter transects of shoreline because of the vast amount of shoreline present.

After capture, all individuals were placed in a plastic bag and weighed to the nearest gram using a spring scale. The snout-vent length was measured in millimeters using a plastic digital caliper. To measure SVL, the sacral joint was pressed down to flatten and elongate the body and a measurement was taken from tip of snout to the vent. If a frog was already marked (recaptured) then it was released in its capture location following measurements. New captures had to go through the tagging/clipping process before returning to the pond. Sex of each individual was determined and if a male, calling status at the time of capture was indicated (calling or non-calling). Males could be determined from a few secondary characteristics including a yellow throat or nuptial thumb pads. However, these characteristics can vary between individuals, so the key factor in identifying gender was the tympanum to eye size ratio. The females exhibit a 1:1 ratio, while the males have a much larger tympanum than eye.

We uniquely marked each individual *(figure 2)* by using Visible Implant Elastomer Tagging (VIE) produced by Northwest Marine Technology Inc. When placing tags between toes of rear feet, the tag was placed in the webbing. When using the front feet, the tag was placed between the toes. Toe 2 *(figure 2)* was the only toe collected and frozen for potential DNA analysis in future projects. Sharp surgical scissors were disinfected in a 70% ethanol solution and the toe was clipped at the first joint (just above the webbing). A bamboo stake with flagging tape was inserted at all locations where an individual was captured to allow us to return the next day and record geographic locations for each point using a Magellen global positioning system. Many of the methods used in the project were developed by making adjustments to methods previously used by Shepard (2002).

*Egg Sampling*

For Hyla House Pond, egg mass surveys were conducted during the daytime hours on Monday, Wednesday, and Friday of each week. During these surveys, the entire pond was searched. For each egg mass identified, the location was flagged, recorded in the GPS unit, and a habitat assessment (described below) was completed. At Wood Pond and Brewster Lake, the egg mass surveys were conducted the day following the night VES. Using this methodology, Brewster Lake and Wood Pond were surveyed four times each week for eggs (twice during the day and twice during nighttime VES).

*Habitat Sampling*

To gather available habitat information from Hyla House Pond, microhabitat was sampled within 25 separate plots *(figure 3)* with relatively homogenous habitat each week. *Table 1* lists the indicated habitat at the time plots were established. Each of the 25 plots was $3m^2$ in size and divided into 9 mini plots (each $1m^2$ in size) for sampling. Sampling was done using a 1 $m^2$ point frame to estimate the percent cover for floating debris (anything the frog can rest on at the surface) and emergent vegetation. This was accomplished by collecting data from 25 points inside the point frame at 20cm intervals *(figure 4)*. At each of the 25 points, a meter stick was vertically inserted to assess the immediate habitat type touching the stick. At 4 inner corners and the center we measured water depth and height of vegetation to calculate an average for that plot. During the initial sampling week (5/5/08) a comprehensive sampling was conducted of all available habitats (225 mini plots). For each following week, 3 mini plots were chosen at random to use in sampling 33% of the weekly habitat changes. The methods for collecting habitat information at miniplots were also applied for each egg mass location.

*Comparing Habitats*

We used the 25 sampling plots described previously to determine if there were habitat differences where calling males, non-calling males, females, and egg masses were found. A plot was considered a calling plot if at least one male occupying the plot was calling during a sampling night. If males were found but not calling, it was considered a non-calling site. Calling and non-calling sites were independent on one another. We also indicated whether a site might have an occurrence of a calling male showing site fidelity (recaptured in the same plot multiple times). Sites containing females or egg masses were also indicated. It was possible for a particular calling site to also be considered a female and/or egg-mass site if these were also found (these would be non-independent).

*Statistical Analyses*

We compared water and air temperature among wetlands using an independent samples t-Test. We used linear regression analysis to evaluate the relationship between air and water temperature and calling level. We used ANOVA to compare variables among plots that contained calling males, non-calling males, and egg-masses. We used Levines Homogeneity of Variance to determine if the habitat variables showed similar variances. All tests were conducted using SPSS 16.0.

## **Results**

*Water and Air Temperature Comparisons:*

All three habitats showed similar cooling and warming trends during sampling the period with air temperature was much cooler at the beginning of May and peaking around mid-June and early July *(figure 5)*. The limited Hyla House Pond data show similar temperatures of those at Brewster Lake. Air temperatures at Wood Pond were significantly cooler than Brewster Lake ($p<0.001$).

Wood Pond water temperatures were significantly cooler throughout sampling compared to Brewster Lake ($p<0.001$), and consistently cooler than Hyla House Pond; rarely reaching over 20 ºC *(figure 6)*. Brewster Lake's water temperatures usually were between 20-30 ºC. Data from early May shows Hyla House Pond water temperatures were warmer than the other two wetlands.

*Weekly Calling Activity*

Calling levels for Brewster Lake and Hyla House Pond both show similar patterns, starting out low in May *(figure 7)* and peaking around the beginning of June. Calling levels were higher than Brewster Lake at their peak. Immediately following the highest peak in calling activity, we began to find egg masses. Fewer egg masses were found at Brewster Lake compared to Hyla House Pond. Calling was never heard at Wood pond and subsequently there were no egg masses.

*Calling Level vs. Physical Parameters*

Calling rates at Hyla House Pond show a significant, positive relationship ($p<0.001$, $r^2=0.439$) with air temperature determined from Kestrel data *(figure 8)*. The correlation between air temperature and calling frequency at Brewster Lake is not as evident with all of the calling data ($p=0.913$, $r^2=0.027$). However, two data points which are both distinguished by high temperature but very low calling occurred on 6/10/2008 and 6/12/2008. Just prior to these dates, there was a period of heavy rainfall (6/6 – 6/9) that resulted in flooding of areas where sampling transects were located. The shallow marshy terrain previously utilized by the green frogs for calling became completely submerged. If these two potential outliers are removed, there is a non-significant but positive relationship between calling level and air temperature ($p=0.11$, $r^2=0.258$).

Water temperature and calling activity also appear to be correlated at Brewster Lake when the two are overlaid *(figure 9)*. It is unfortunate that water temperature data is unavailable for Hyla House Pond making it difficult to evaluate any relationships. A regression analysis of the water temperature and average number of males calling per plot for Brewster lake shows a weak, positive relationship (p=0.38, $r^2$=0.071). However, if the same outliers removed in the air temperature analysis are also removed, the relationship becomes significantly positive (p=0.001, $r^2$=0.705).

*Habitat Associations*

ANOVA analysis did not include habitat of plots having males showing site fidelity as the sample size was too small (n=5). The analysis of calling, noncalling, female, and egg habitat indicates that only emergent structure height was not different among the different groups (*table 2).* Pairwise analysis shows differences between pairs (indicated by matching number for each variable). Water depth for plots containing females was significantly less than plots with calling males, non-calling males, and eggs. Percent floating debris was significantly less in plots with eggs compared to the others. Further, plots with non-calling males had significantly less floating debris than plots with females. Plots with females or eggs had significantly higher percent emergent structure compared to plots with non-calling males. Percent emergent structure in plots with eggs was significantly greater than plots with eggs.

## Discussion

*Calling and Temperature:*

Calling activity levels varied throughout the project and this variation appears to be tied to both air and water temperature. There is a strong relationship between air temperature and calling at Hyla House Pond, and there is a very strong relationship between water temperature and calling at Brewster Lake. As green frogs are ectothermic and spend the majority of their time in and around water, water temperature inherently has a major impact on their internal temperature. As the water warms up, the green frog body temperatures will also increase. It is known that ectotherms become more active as their body temperatures increase. Therefore, it can be expected that breeding would not occur until their body temperatures increased to a certain point. Green Frogs are considered to be summer breeders. Their activity appears to be more closely associated with warmer temperatures, suggesting they are not physiologically adapted to cooler water temperatures (Oseen and Wassersug, 2002). Our results from Brewster Lake and limited results from Hyla House Pond appear to reaffirm that these frogs will not begin calling until they have reached an appropriate temperature threshold. Wright (1914) indicated that 25$^{o}$C might be the calling temperature threshold for green frogs, however, calling had already started at Hyla House Pond and Brewster Lake when we initiated the project. Water temperature results show that these areas were not at 25$^{o}$C. Instead, our results suggest that the threshold may be closer to 20$^{o}$C.

The water and air temperatures at Wood Pond were significantly cooler than those at Brewster Lake and may provide an explanation for why calling did not occur during the project. Since water temperatures at Wood Pond *(figure 6*) rarely reached 20$^{o}$C, we should not be surprised that calling does not occur. However, the number of males and females captured at this location throughout the project would suggest that it is somehow important for the population. For instance, Wood Pond may, in warmer springs, reach the appropriate temperature threshold resulting in males calling and breeding occurring. If that were the case, the males and females found around the wetland may be a recently successful cohort that returned to their natal pond to breed. With longevity of green frogs unknown, but unlikely to be more than 10 years, we would expect that green frogs may become extinct at this site and have to recolonize from surrounding areas frequently. Wood Pond might also serve as an overwintering site. In this case, the males and females we found would have recently emerged and would then travel to breeding sites. However, we recaptured a number of individuals in this area suggesting that some individuals may have never left the wetland during the project. Further, we would expect to have seen a pulse of frogs early in the season with most frogs leaving by the end of the project. Instead, population sizes appear to be relatively stable. Alternatively, these frogs may be from nearby Cedar Creek and are using this wetland as a foraging location. Clearly, this site presents some interesting questions about the population that warrant further study.

Interestingly, calling had already started at both Hyla House Pond and Brewster Lake when we initiated this project. This is antithetical to what was expected, as Brewster Lake should have been much cooler later into the project. However, Brewster Lake has a wide expanse of shallows, lined with emergent vegetation that may buffer lake water and shallow water. In turn, this could allow the shallows to heat quicker than the rest of the lake in the early part of the season resulting in the appropriate temperature threshold occurring early. It is clear, however, that Brewster Lake water temperatures were slightly cooler than at Hyla House Pond (at least for the data available from Hyla House Pond). This might explain why the calling indices were lower at Brewster Lake compared to Hyla House Pond. However, it is critical to note the onset of calling may also have a social control (Wells, 1988). Males that hear nearby calling males may be more likely to initiate calling. The size of Hyla House Pond results in a concentration of green frog males that could perpetuate an intensive calling effort when they arrive. In contrast, green frogs appear to be much more widely dispersed at Brewster Lake, reducing possible social cues that might have increased the calling index.

### *Calling and Habitat*

Our study appears to confirm that calling male green frogs at the Hyla House Pond are using habitats with moderate amounts of emergent vegetation much like our previous work (Weeks and Burton, 2007). However, our comparisons suggest that these locations are significantly different than habitat in which egg-masses are found. Habitat in which females were found was significantly shallower than calling site locations suggesting that males are not selecting habitats in which females are likely to be found. However, they may be selecting habitat that does maximize the likelihood of a female locating them while reducing the probability of discovery by a predator.

Given the relationship between temperature and onset/level of calling, these habitats may actually represent sites that provide the appropriate microhabitat for maintaining calling for longer periods while still allowing opportunities to encounter females. Emergent vegetation likely restricts air movement, maintaining warmer water and surface air temperatures longer in the night than open water.

Of the 13 calling frogs that we captured, 5 of them were recaptured multiple times in the same location. This observation suggests that site fidelity does exist and can last for a significant length of time (up to a month in this study). Past studies have examined site fidelity behavior in male frogs of the genus *Rana*. In one particular study on closely related mink frogs, it was found that males do not maintain long-term specific territories but may return to the same general area on consecutive nights (Bevier et al., 2006). By temporarily maintaining an individual calling area, males may increase their chances of finding a mate. The study also suggested that these calling areas may contain preferred features for calling such as emergent vegetation. When we compared the habitat differences of our 5 individuals showing site fidelity to all other males, there was no real difference to help in determining what factors influence individuals to stay for any length of time (Table 3). Wells (1977) found that most males occupied multiple sites during the breeding season, but some sites were occupied by one individual for up to 7 weeks. He also found that larger males spent more time in individual territories and smaller males usually took over territories only after larger males had abandoned them. Previous studies have shown evidence that smaller males may lower the pitch of acoustic signals to defend territories (Bee et al., 2000). Small males produced lower frequency calls in response to a large-male stimulus. From this we can infer that calling status can play an important role in the ability to defend a territory. To further investigate, we looked at size differences between the 5 individuals that we observed showing site fidelity and all the other males. There appeared to be no difference in size for individuals practicing site fidelity. This suggests that site fidelity may have less to do with size at Hyla House Pond.

Habitat information for egg masses suggests that eggs are laid in habitats with large amounts of tall emergent vegetation and lower amounts of floating debris. The differences are quite large in comparison to all males and females. This suggests that males are choosing calling sites to find mates and the pair moves to a new location to lay eggs. The type of habitat most largely used appears to be one that provides the greatest amount of protection from outside factors such as predators and weather changes.

*Implications for Pierce Cedar Creek Institute*

Our results support the need to maintain a wide range of wetland habitat in order to maintain robust green frog populations. Each of the wetlands in this study may contribute differently to the overall population in different years. For instance, the cooler spring during this study resulted in an apparent delay in calling compared to 2007 (Weeks and Burton, 2007). In drier years, this could have resulted in green frogs breeding successfully only at Brewster Lake. Unfortunately, frogs at Brewster Lake must also contend with large vertebrate predators. With cooler and drier springs, Hyla House Pond would likely dry before any larvae successfully metamorphose. This year appeared to have more precipitation, potentially resulting in Hyla House Pond maintaining water long enough for successful metamorphosis with later breeding. Further, years with warmer temperatures may allow Wood Pond to become a breeding site if the water temperatures at this site are impacted by air temperatures. However, the lack of emergent structure along the shoreline may reduce the likelihood that this site would ever become a breeding site. Clearly, each of these wetlands present very different selective pressures for green frog populations and maintenance of this variety of habitat may provide for a greater variety of genetic traits. In the long run, this type of genetic diversity could allow the general population be more successful.

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Table 1. The original habitat types used to choose each plot. The habitat type listed may not reflect all of the habitat within the plot or how it changed during the remainder of the project, but reflects overall observation of the dominant habitat type during the establishment and 5/5/08 sampling period.

| Plot | Type | Plot | Type |
|---|---|---|---|
| 1 | Tree + Emergent | 14 | Cattail |
| 2 | Reed Canary | 15 | Open Water |
| 3 | Cattail | 16 | Reed Canary |
| 4 | Tree | 17 | Open Water |
| 5 | Lily Pads | 18 | Cattail |
| 6 | Open Water | 19 | Emergent + Open Water |
| 7 | Emergent+Debris | 20 | Open Water+ Debris |
| 8 | Brush+Debris | 21 | Emergent+ Debris |
| 9 | Cattail | 22 | Reed Canary + Debris |
| 10 | Open Water | 23 | Emergent |
| 11 | Reed Canary | 24 | Open Water |
| 12 | Cattail | 25 | Cattail |
| 13 | Brush | | |

Table 2. A comparison of average habitat measurements/percentages in relation to calling status, calling frogs that exhibited site fidelity (SF males), gender, and egg mass locations. ANOVA analysis (did not include SF males as sample size was too small) indicates that only emergent height were not different among the different groups. Pairwise analysis shows differences between pairs (indicated by matching number for each variable). Water depth for plots containing females was significantly less than plots with calling males, non-calling males, and eggs. Percent floating debris was significantly less in plots with eggs compared to the others. Further, plots with non-calling males had significantly less floating debris than plots with females. Plots with females or eggs had significantly higher percent emergent structure compared to plots with non-calling males. Percent emergent structure in plots with eggs was significantly greater than plots with eggs.

| Type | N | Mean | Std. Deviation | Std. Error |
|---|---|---|---|---|
| WATER DEPTH ($p<0.001$) | | | | |
| Calling[1] | 16 | 30.23 | 8.69 | 1.34 |
| Non-calling[2] | 42 | 29.54 | 11.50 | 2.88 |
| SF Males | 5 | 27.65 | 6.21 | 2.35 |
| Females[1,2,3] | 15 | 19.40 | 7.69 | 1.99 |
| Eggs[3] | 12 | 33.43 | 7.20 | 2.08 |
| % FLOATING DEBRIS ($p=0.025$) | | | | |
| Calling[1] | 16 | 60.96% | 23.93% | 3.69% |
| Non-calling[2,3] | 42 | 57.17% | 25.92% | 6.48% |
| SF Males | 5 | 64.86% | 22.65% | 8.56% |
| Females[2,4] | 15 | 71.38% | 23.31% | 6.02% |
| Eggs[1,3,4] | 12 | 34.33% | 30.48% | 8.80% |
| EMERGENT HEIGHT ($p=0.210$) | | | | |
| Calling | 16 | 62.87 | 20.98 | 3.24 |
| Non-calling | 42 | 55.66 | 24.00 | 6.00 |
| SF Males | 5 | 65.11 | 18.07 | 6.83 |
| Females | 15 | 55.08 | 13.38 | 3.45 |
| Eggs | 12 | 68.48 | 23.44 | 6.77 |
| % EMERGENT STRUCTURE ($p=0.001$) | | | | |
| Calling[1] | 16 | 44.08% | 15.77% | 2.43% |
| Non-calling[2,3] | 42 | 37.09% | 17.50% | 4.38% |
| SF Males | 5 | 47.43% | 16.18% | 6.11% |
| Females[2] | 15 | 44.98% | 18.02% | 4.65% |
| Eggs[1,3] | 12 | 59.00% | 8.72% | 2.52% |

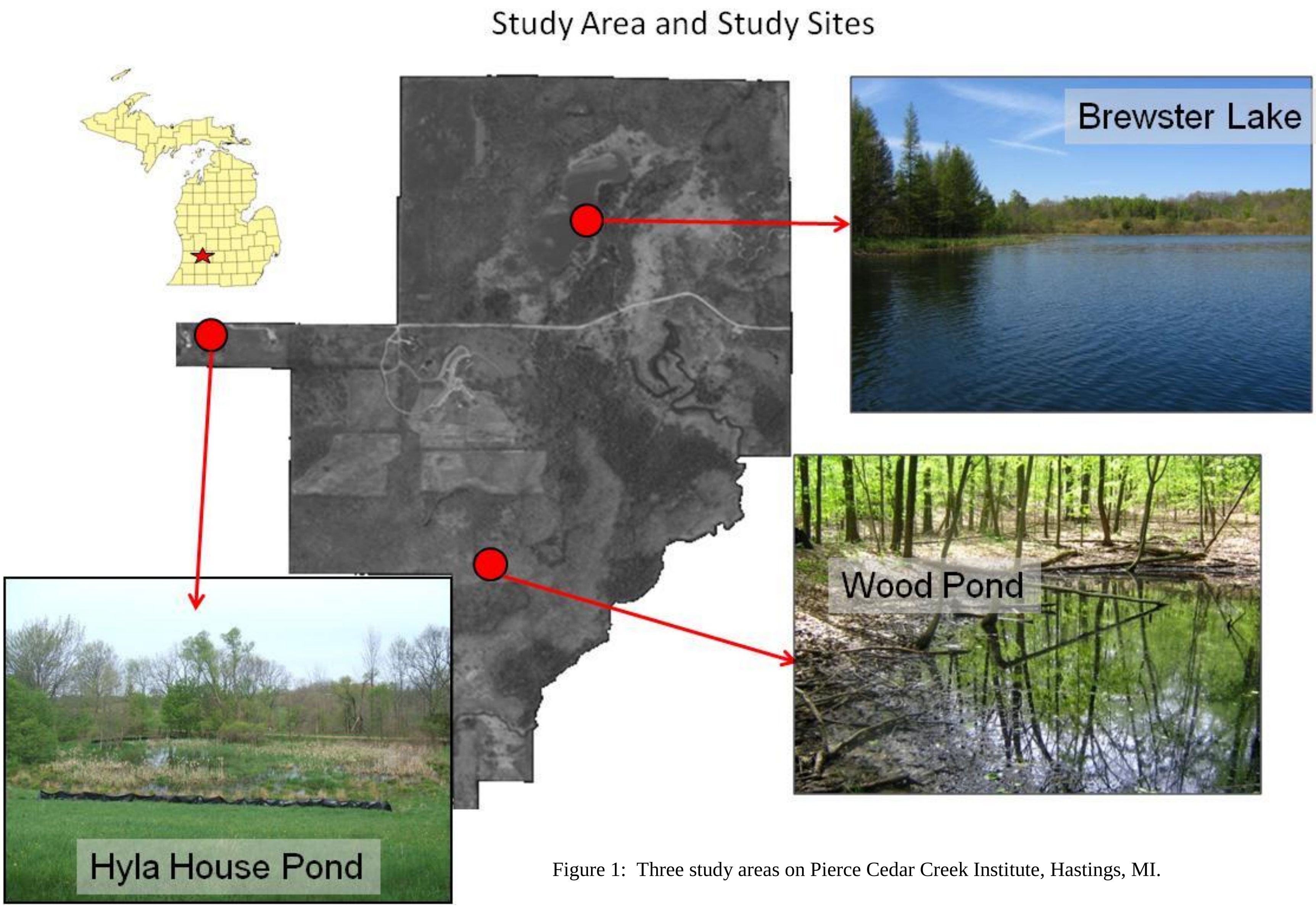


Figure 1: Three study areas on Pierce Cedar Creek Institute, Hastings, MI.

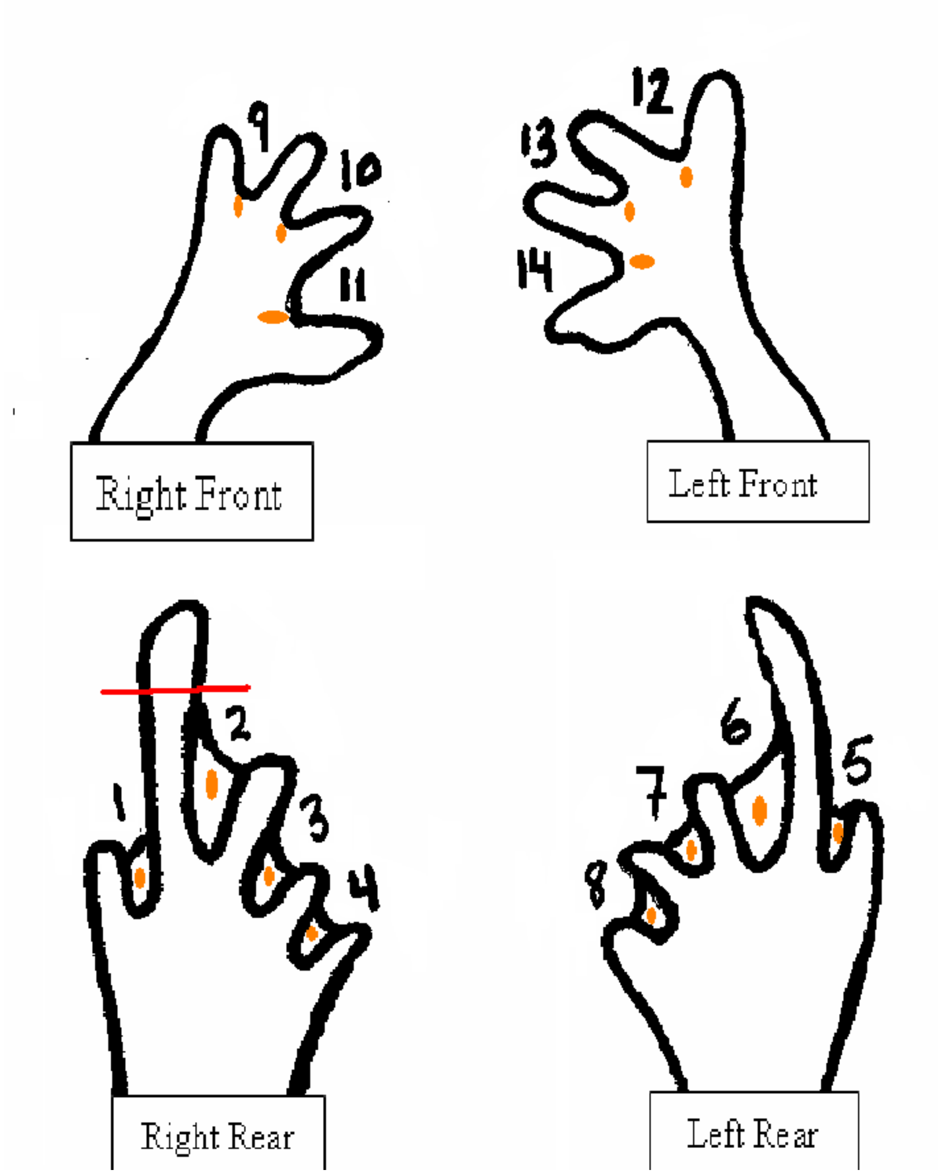


Figure 2: Diagram showing toe numbers used for tagging. View is the ventral side of the feet. We uniquely marked each individual by using Visible Implant Elastomer Tagging (VIE). Tags were placed within the webbing of the rear feet and between the toes of the front feet. The designated numbering system allowed for each individual to have a unique code. At most, three toes were injected with elastomer. Toe 2 (indicated with red line) was collected and frozen for potential DNA analysis in future projects.

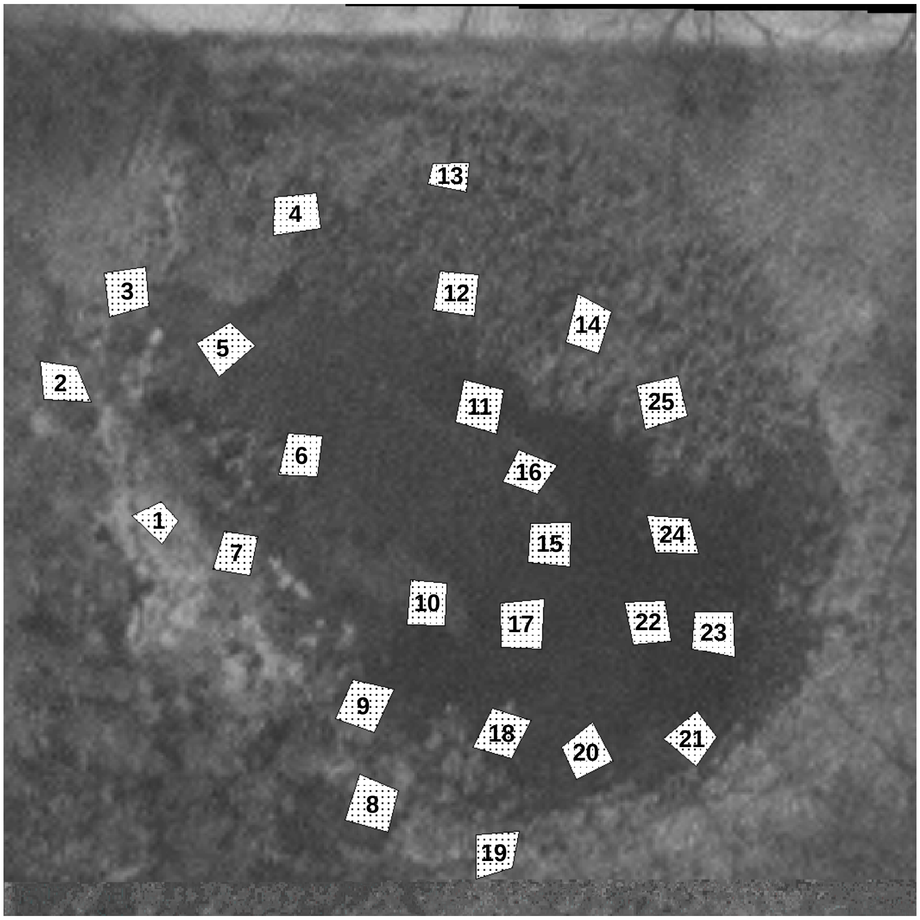


Figure 3: Overhead map using ArcGIS and aerial photography showing the locations of the 25 plots used for sampling in Hyla House pond. Plots numbering begin on the west side of the wetland and follow a north-to-south, south-to-north pattern until ending on the east shoreline.

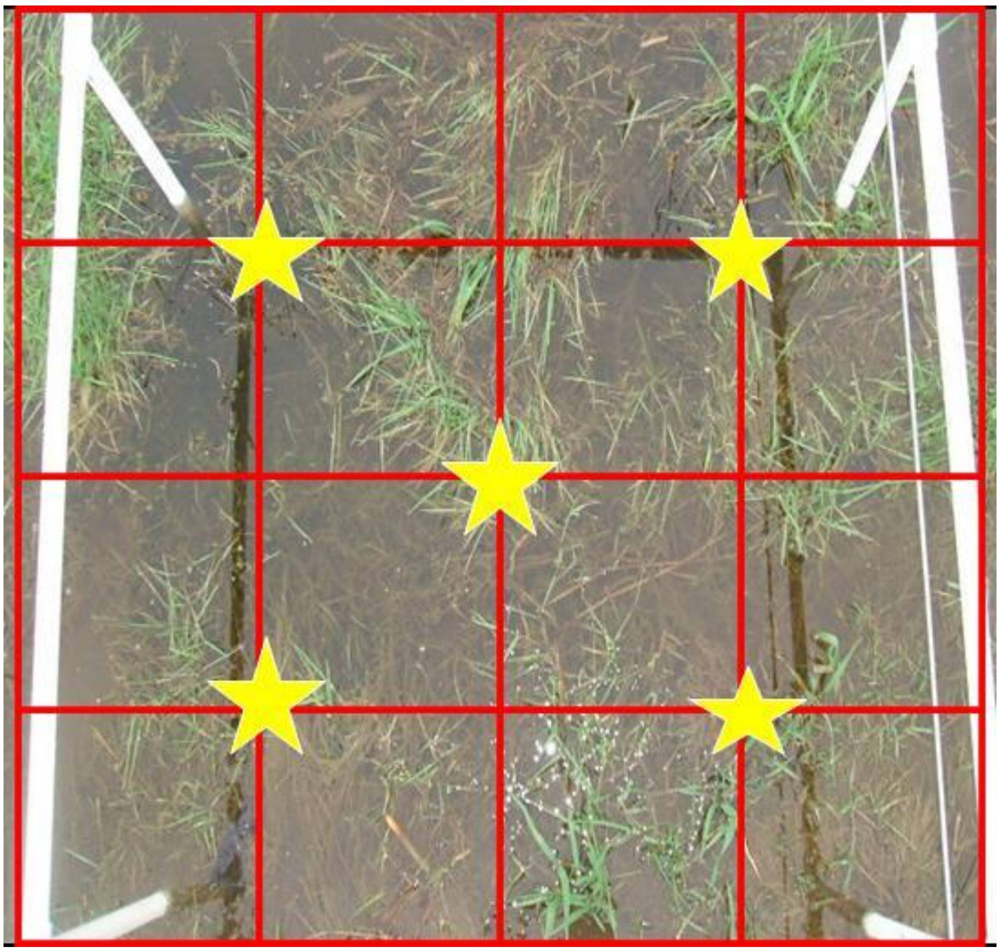

Figure 4: Photograph and overlaid diagram of the $1m^2$ point frame to demonstrate where measurements were taken. Floating debris and emergent structure density information was collected at each location at each right angle for a total of the 25 points. At the 5 star locations, water depth and emergent structure height information was also collected.

# Air Temperatures

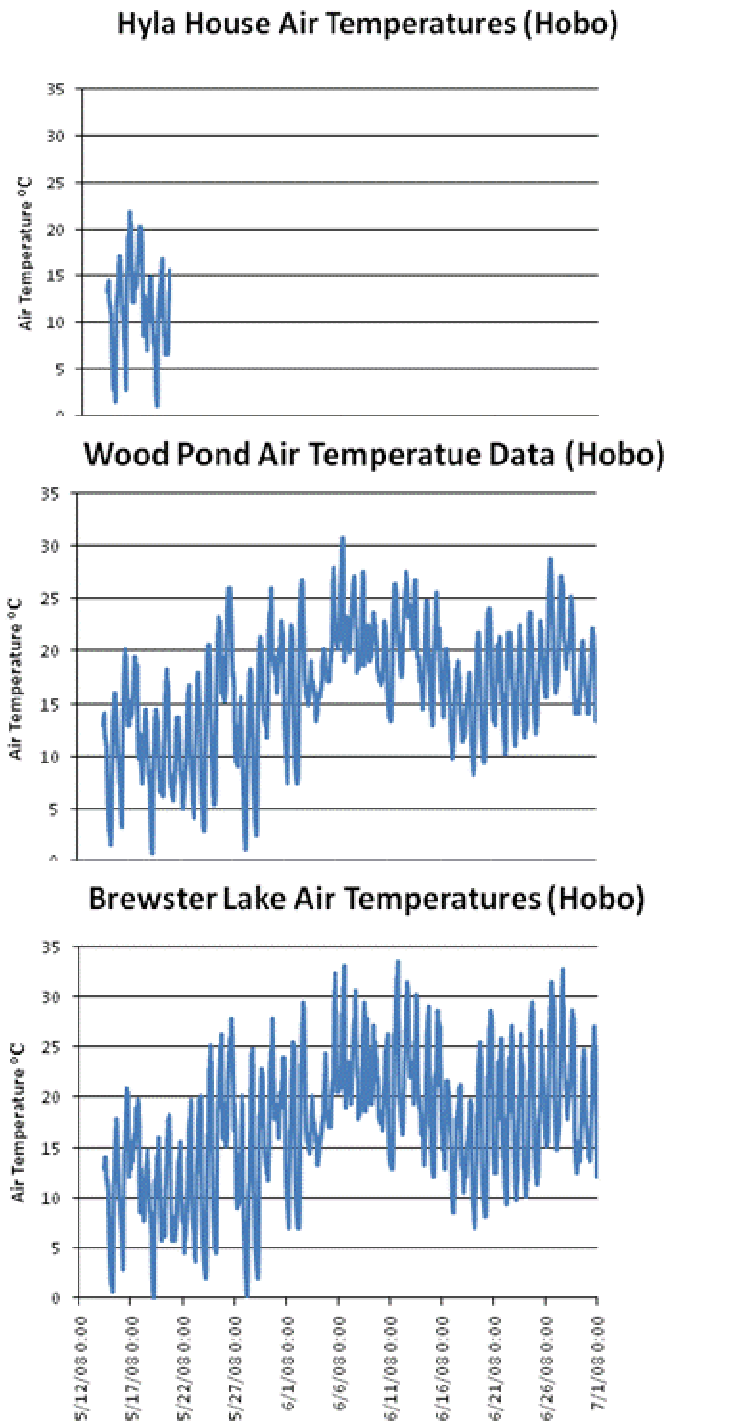


Figure 5: A comparison of air temperatures at the three sampling habitats. The temperature readings were recorded using a HoboTemp Data Logger monitoring system (probe was at 1m height and shaded). Both Brewster Lake and Wood Pond show a similar temperature pattern, with the temperatures at Wood Pond relatively cooler. Wood Pond air temperatures rarely exceeded 20$^{o}$C during the sampling period. Brewster Lake temperatures exceeded 20$^{o}$C much more frequently, beginning at the end of June. Data loggers at Hyla House Pond only recorded temperatures for a short period, but the pattern follows that of Brewster Lake and Wood Pond during the early part of the sampling period.

# Water Temperatures

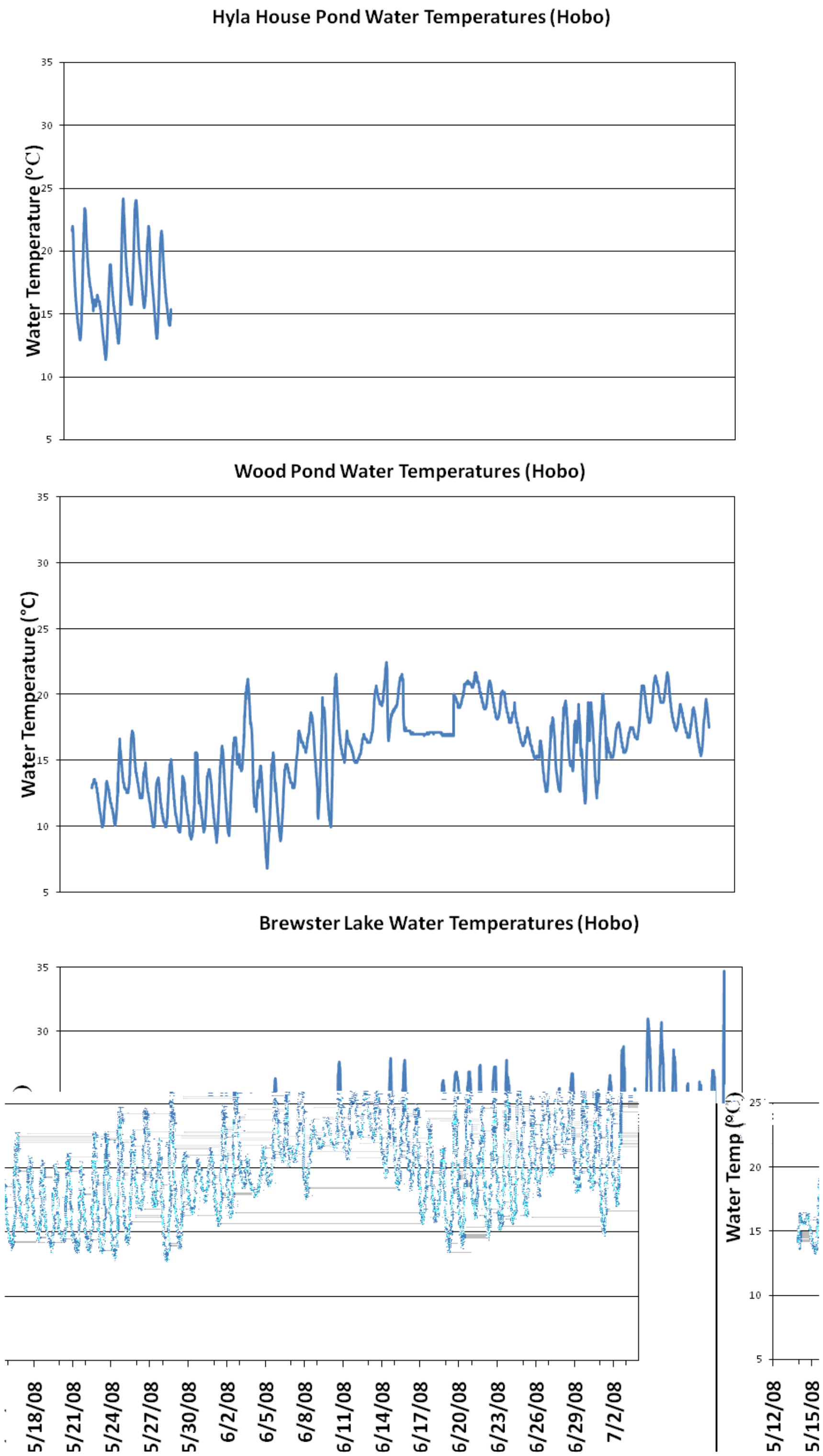


Figure 6: A comparison of water temperatures of the three sampling habitats. The temperature readings were recorded using a HoboTemp Data Loggers monitoring system. Both Brewster Lake and Wood Pond show a similar temperature pattern, with the temperatures at Wood Pond much cooler overall. Wood Pond water temperatures rarely exceeded 20°C over the sampling period. Brewster Lake water temperatures exceeded 25°C much more frequently, beginning at the end of June. Data loggers at Hyla House Pond only recorded temperatures for a short period, but the pattern follows that of Brewster Lake and Wood Pond.

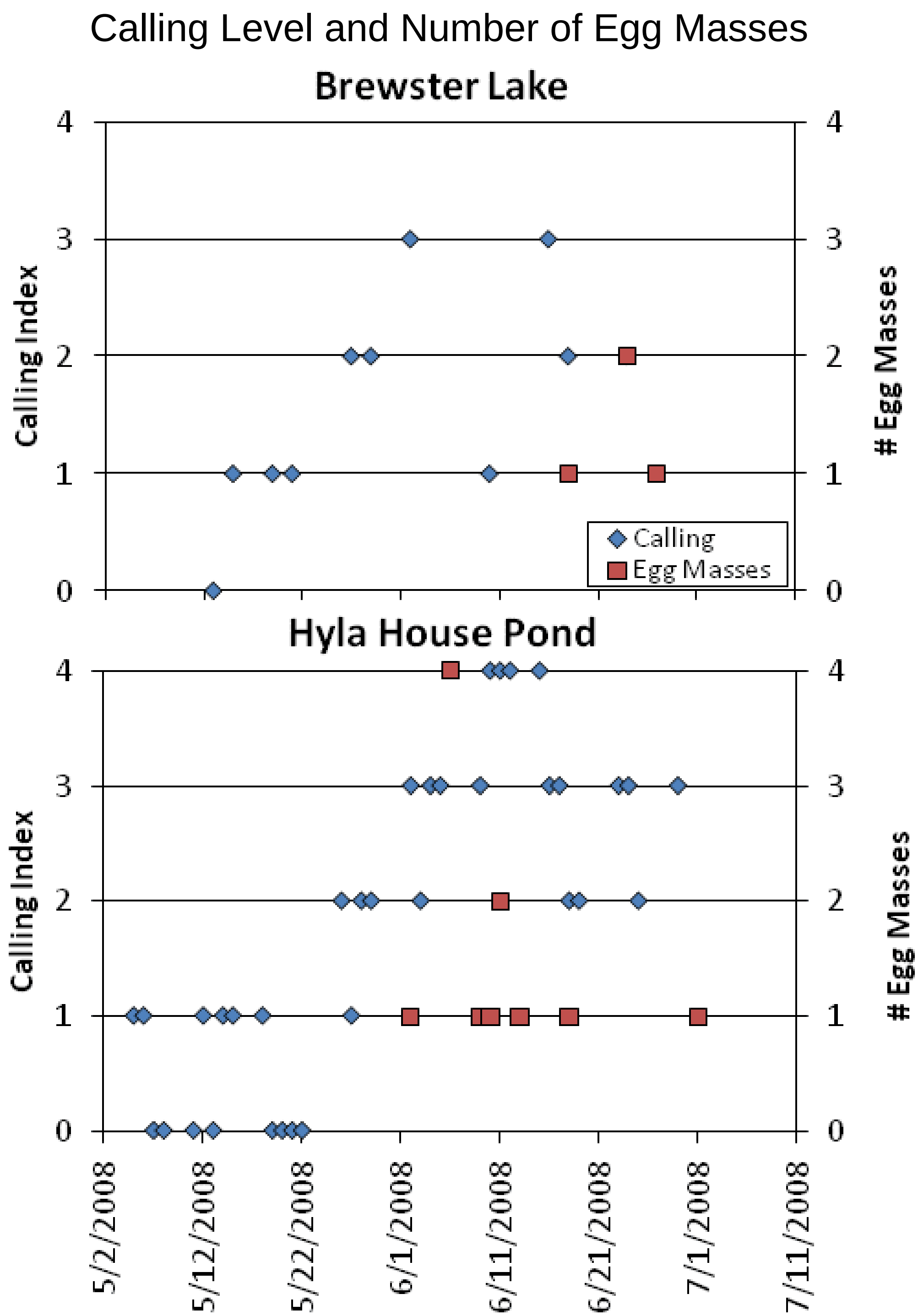


Figure 7: Calling index and number of egg masses by week. Calling indices represent the amount of calling that was heard during call surveys. Calling had already started in a limited way before the study began; however, no egg masses were found in early surveys. Calling was lower at Brewster Lake compared to Hyla House Pond. First egg masses at Hyla House Pond were found almost a month before egg masses were found at Brewster Lake. Overall, more egg masses were found at Hyla House Pond than at Brewster Lake.

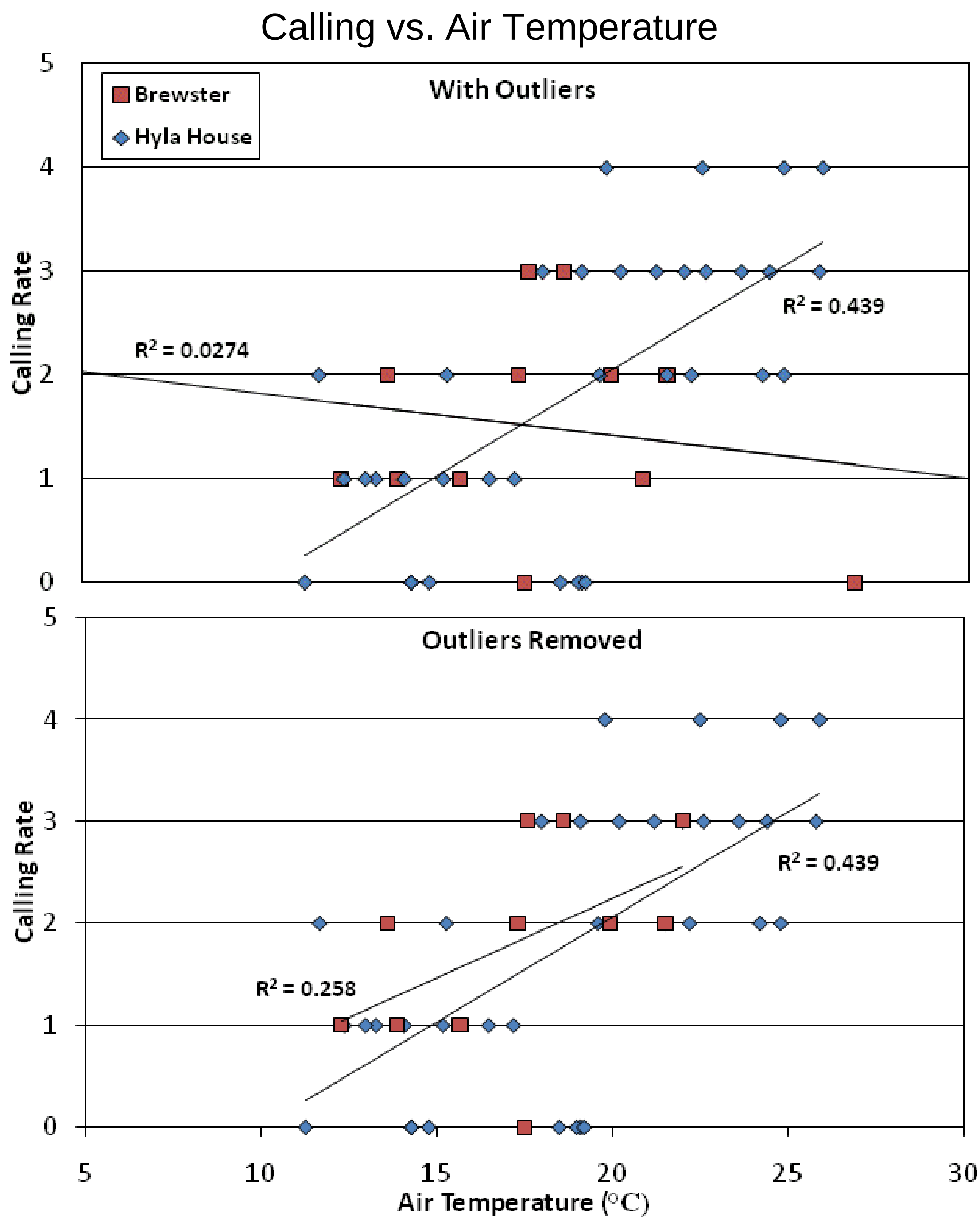


Figure 8: Calling rate versus air temperature at Hyla House Pond and Brewster Lake. Air temperatures were measured at the time of the calling survey at each wetland. Calling rate at Hyla House Pond shows a significant positive relationship with air temperature (regression $p<0.001$). Brewster Lake shows a negative but non-significant relationship ($p=0.913$). We re-ran the Brewster Lake analysis removing two data points considered outliers as they occurred after a rainstorm that raised water levels and flooded previous calling sites. The results show a stronger and positive relationship; however, it was not a significant relationship ($p=0.11$).

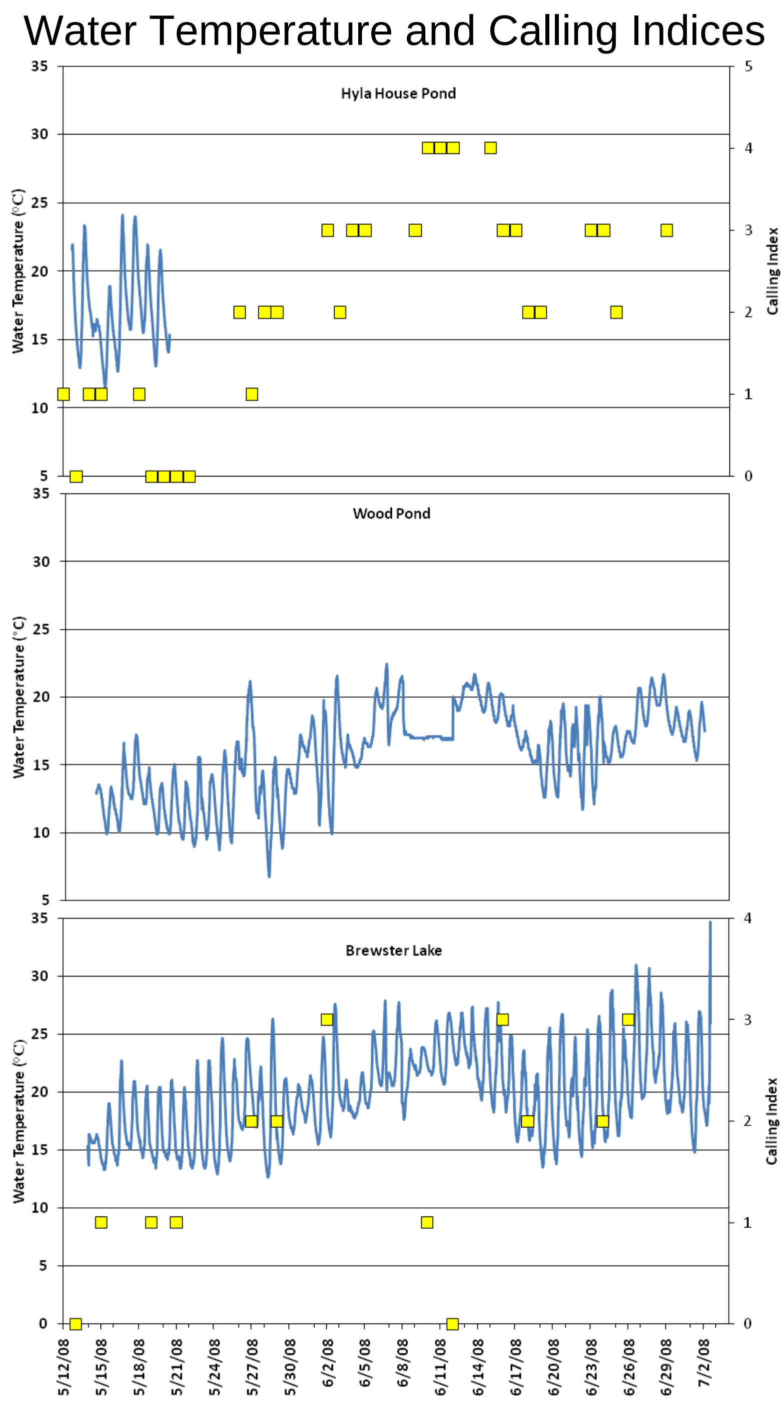


Figure 9: A comparison in water temperatures and calling index of the three sampling habitats.

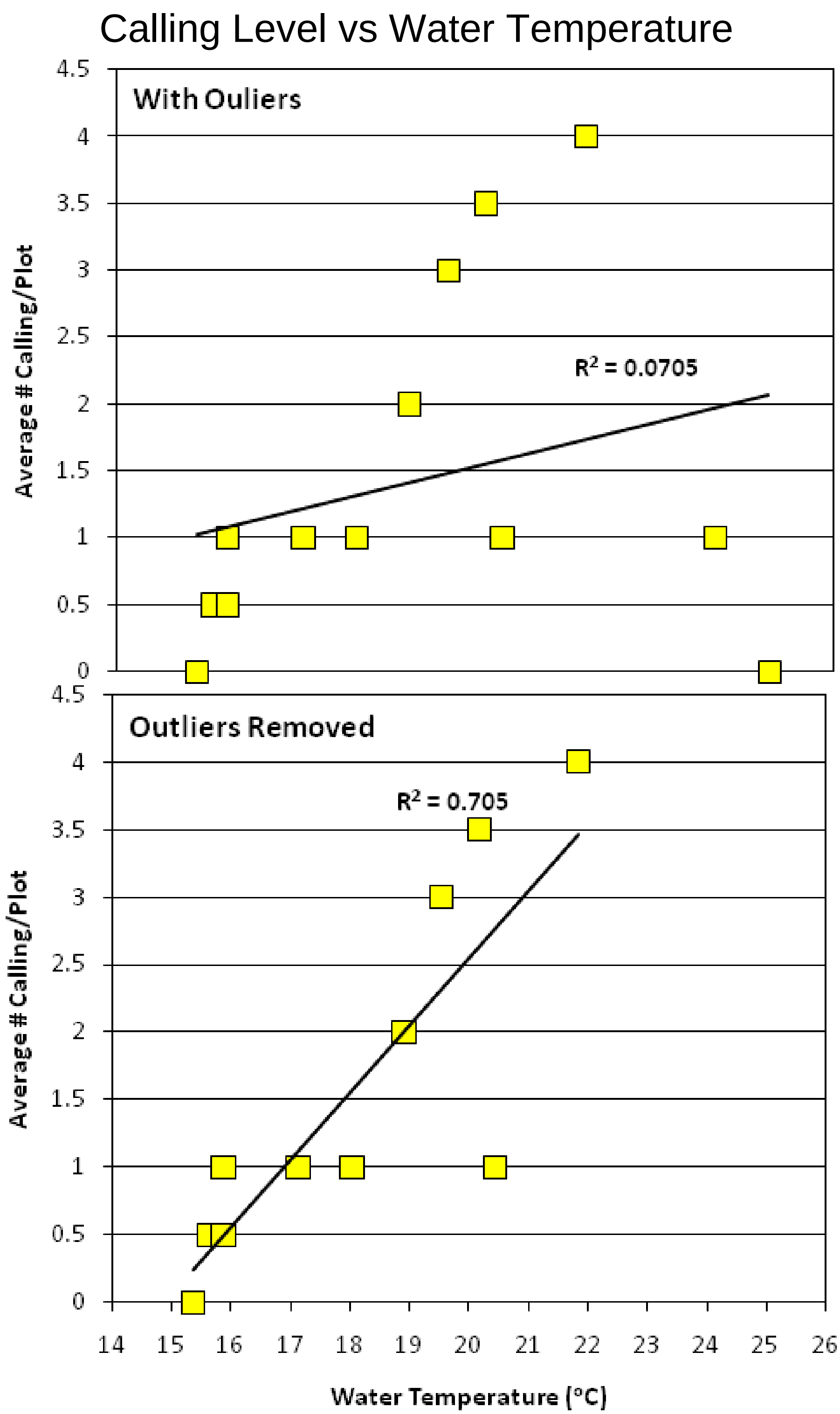


Figure 10: Average number of calling males per plot versus water temperature at Brewster Lake shows a weak and non-significant relationship (p=0.38). We re-ran the analysis removing two data points considered outliers as they occurred after a rainstorm that raised water levels and flooded previous calling sites. The results show a significant positive relationship (p=0.001).