EFFECTS OF WATER CHEMISTRY ON ANURAN SPECIES DIVERSITY WITHIN HUNTINGDON COUNTY

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ABSTRACT

Conductivity, pH and dissolved oxygen were tested at different sites within Huntingdon County to see if these chemical properties affect the species diversity of anurans. These factors are believed to have an influence on species diversity as amphibians have very permeable skin and are considered to be bio-indicators that can be used to help determine the health of an ecosystem. The species diversity of anurans was determined at each site by identifying distinct mating calls. Some of the species that were used to determine diversity were the Pickerel Frog, Southern Leopard Frog, American Bullfrog, Spring Peeper, and Green Frog. Spring is the mating season for anurans and an ideal time to determine their species diversity via mating calls.

Keywords: Anurans, conductivity, dissolved oxygen, frogs, pH, species diversity, water chemistry

INTRODUCTION

Monitoring anurans is important as they are excellent bio-indicators of water quality. This is due to their thin, permeable skin which is sensitive to environmental changes and fluctuations. Often, they are the first species affected when these changes occur (Hardin, 2009). Embryonic and larval anurans have been found to be sensitive to a variety of biotic and abiotic factors (Skelly, 2001). The factors that affect immature anurans may also affect mature anurans.

Local weather patterns are of great importance when it comes to reproductive events for anuran species (Conant and Collins, 1998) as well as other abiotic factors. Anurans begin their lives in an aquatic environment as tadpoles with gills, later metamorphosing into terrestrial-dwelling adults with lungs (Carroll, 1988). They lay their eggs in water after which the developing larvae hatch into free-living embryos. As the larvae complete their embryonic development, they attain an adult body that allows them to leave aquatic habitats and wander to terrestrial ones. Anurans that live in temperate zones, like the US, breed during spring and summer. Breeding seasons for anurans vary between species. For instance: pickerel frogs (Rana palustris) breed from February to late May (Dundee and Rossman, 1989; Babbitt, 1937), American bullfrogs (Rana catesbeiana) call from April to July, southern leopard frogs (Rana utricularia) located in Northern areas of their range call in the early spring, green frogs (Rana clamitans melanota) call from April to August, and spring peepers (Pseudacris crucifer) call from March till
June (Conant and Collins, 1998). These species are the ones that were most likely to be encountered during this observational study due to habitat selection, which is similar to the habitats found at the study sites, as well as the time of year.

Through this study we will be looking at different environmental factors, mainly water chemistry, such as acidity (pH), dissolved oxygen (DO), and conductivity. In this observational study, we focused on the effects of water chemistry on those anuran species that emerged earliest in the season. The null hypothesis is that these factors will not affect the diversity of species, and that all anuran species should be found with similar diversity at all sites. Our working hypothesis states that species diversity is dependent on water chemistry. Anuran mating calls will be used to measure species diversity at thirteen different sites within Huntingdon County. The species diversity will be compared to the abiotic factors at each site, such as pH, DO, and conductivity.

FIELD SITES

The sites studied consisted of vernal pools, wetlands, ditches, ponds, and streams. There were a total of fourteen sites that we used in our observational study. These sites were located at the following places. Fouse’s Crossing Wetland is located off of Fouse’s Crossing Rd on the left hand side when heading towards James Creek Rd. Fouse’s Crossing Rd is located off of Route 26, also known as Bedford Pike, after passing through the town of Marklesburg. A site was visited on Cold Springs Rd. It was found when headed from Huntingdon to Route 26. The site is located on the right hand side of the road and is identified by crosses which have a pull off area in front of them. The water where frogs were located is down the bank from the road. Old Crow Wetland area is situated on William Penn Hwy headed west out of Huntingdon located on the left hand side of the road. Once you enter the parking area there is a trail that heads down into the wetland area and the site we used is located on the left hand side of the trail. There is another pond located farther to the right that we used from this location as well. Another site is positioned in Muddy Run on the Juniata College campus behind the baseball field. We also visited pond at Grove Farm. To get there when headed out to the field station driving down Juniata College Field Station road turn off at signs for Grove Farm. A pond is located approximately 100 feet from the storage shed down the dirt road on the left. Almost directly after the turnoff (300ft) from Fairgrounds Rd onto Station Rd, on the right hand side, there is a ditch in which observations were taken. Approximately 275ft after passing Station Rd, while heading towards William Penn Hwy on Fairgrounds Rd there is another ditch that runs perpendicular to Fairgrounds Rd. Yet another site is found on Fairgrounds Rd towards William Penn Hwy, approximately 1700ft, from Beaver Lane directly off the left hand side of the road is a ditch where water accumulates. A site that is comprised of a pond with a small wetland area off to the side is located on Pond Rd. To reach this site make a left on Newtown Road when heading south on Route 26. Take Newtown Rd all the way to the end and make a right onto Pond Rd. The pond and wetland area we observed is located on the right hand side of this road. Another spot is positioned on Petersburg Pike. Turn onto Petersburg Pike from Cold Springs Rd and drive about 1 mile north. There is a game lands turnoff to the right. A pond is located on the right about 50 feet from the gate. Trough Creek State Park was visited and two of the study sites are located in the park. One location was on a utility road off of Trough Creek Drive where a bridge went over a tributary of Trough Creek. The other site within the State Park was a vernal pool which was located on the right hand side of a utility road. The utility road where the bridge is located forked further down and the road to the right is the one taken to access the vernal pool.

METHODS AND MATERIALS

We collected water chemistry data and frog analysis data from a variety of sites in Huntingdon County, Pennsylvania during the months of March and April, 2009. Water chemistry data were collected using a Markson Digital pH Meter Model 88, YSI Incorporated Dissolved Oxygen Meter Model 55/12 FT, and Oakton Conductivity Meter CON5 Acorn Series. Water and air temperature were also recorded using the aforementioned YSI Dissolved Oxygen Meter. At each site, we collected frog species data by listening to and identifying species calls. To do so,
we waited several minutes after arriving at each site to ensure that our presence did not interfere with the frogs’ calls. We collected data between eighteen hundred hours and twenty one hundred hours, the time at which the sun was almost completely behind the horizon and there was increased anuran activity.

Binary Logistic Regressions and One-way ANOVAs were used to analyze water chemistry data collected between locations where anurans were present versus absent. A probability plot was used to assess the normality of the data used in the One-way ANOVA tests. Because these data were not normal, the statistical analysis was repeated using a Kruskal-Wallis test.

RESULTS

Thirteen sites were visited during this study. Out of these thirteen sites Spring Peepers were the only anuran species found. Spring Peepers were found at the following nine locations: Fouse’s Crossing Wetland, Cold Springs Road, both Old Crow Wetland sites, Muddy Run, Station Road, both sites on Fairgrounds Road and Petersburg Pike. Four of the thirteen sites did not contain any anuran species. These locations were the pond at Grove Farm, Trough Creek Vernal Pool, Trough Creek Bridge and Pond Road.

When looking at the table containing the average values for the abiotic factors it would seem that there is a slight difference in pH value. At sites where frogs were found the pH appears to be lower than the site without frogs. Also the DO at sites containing frogs is lower than the DO at sites where frogs were absent. Conductivity also appears to be higher at sites with versus without frogs. The air and water temperatures also show a difference, but one that does not appear to be large enough to affect whether frogs would be present or not. The humidity at the sites also differs, but it is unlikely that this would affect whether or not the frogs were present. However, it may affect the frogs calling, meaning if there was a lower humidity the frogs may be less inclined to call. Statistical tests were run to see if there were significant similarities between water chemistry composition of the sites with and without frogs.

One-way ANOVAs were used to analyze water chemistry versus sites visited. After doing so, a probability plot was used to determine if the One-way ANOVA met the assumptions of normality. The probability plot showed that the data did not meet the assumption of normality (Fig. 9). To correct this and receive accurate statistical information the data were then analyzed using the Binary Logistic Regression. Binary Logistic Regressions were utilized in this study to analyze the different values of acidity (pH), dissolved oxygen (DO), and conductivity. When observing the acidity we found that the pH was not significantly different, with a p-value of 0.180, in terms of all the observations that were taken (Fig. 1).

In terms of the DO there was a significant difference observed using binary logistical regressions as a p value of 0.023 was observed (Figure 4). This p value, being lower than 0.05, made it significant, hence an integral part of our observations and measurements. With conductivity there was also a significant difference observed as the binary logistical regression test gave us a p value of 0.031, less than the significant p value of 0.05 and hence being significant (Figure 5).

Next we took our pH and DO readings and ran a BLR test using both sets of data simultaneously. From this we noticed that the results were not significantly different when combined. Even individually we did not observe any significance, as we obtained high p values (Figure 2). When the pH and DO values were combined we obtained a p value of 0.061 for both. Individually the pH gave a p value of 0.507 and the DO p value came out to be 0.137, higher than the significant p value of 0.05 and hence not significant.

<table>
<thead>
<tr>
<th>Abiotic Factor</th>
<th>With Frogs</th>
<th>Frogs Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.924</td>
<td>7.328</td>
</tr>
<tr>
<td>DO</td>
<td>6.687 ppm</td>
<td>9.702 ppm</td>
</tr>
<tr>
<td>Conductivity</td>
<td>247.600 µΩ/cm</td>
<td>105.950 µΩ/cm</td>
</tr>
<tr>
<td>Air Temperature</td>
<td>15.183 °C</td>
<td>14.433 °C</td>
</tr>
<tr>
<td>Water Temperature</td>
<td>13.950 °C</td>
<td>12.667 °C</td>
</tr>
<tr>
<td>Humidity</td>
<td>63.17%</td>
<td>53.17%</td>
</tr>
</tbody>
</table>

Table 1. Average value for each abiotic factor observed in comparison to the presence or absence of frogs.
A binary logistical regression test was run looking at pH, DO, and conductivity they were found to be significantly different as a whole (Figure 3). Yet, individually they were not as they gave p values for pH to be 0.687, DO the p value came out to be 0.178, and conductivity came out to be 0.125. However when the three factors were combined they gave an overall p value of 0.015 which is significant as it is less than that significant p value of 0.05.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-9.27899</td>
<td>7.08950</td>
<td>-1.31</td>
<td>0.191</td>
<td>0.46</td>
<td>21.19</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>1.14322</td>
<td>0.974620</td>
<td>1.17</td>
<td>0.241</td>
<td>3.14</td>
<td>0.46</td>
<td>21.19</td>
</tr>
</tbody>
</table>

Log-Likelihood = -12.598
Test that all slopes are zero: G = 1.796, DF = 1, P-Value = 0.180

Figure 1. This Binary Logistic Regression shows a comparison of the site number, which correlates with frog presence or absence, versus the pH.

A probability plot was used to determine if the One-way ANOVA met the assumptions of normalcy. However, it did not and hence it failed to provide normality assumptions. This led us to use the Kruskal-Wallas as it does not make use of assumptions (Figure 10). As part of the Kruskal-Wallas test we looked at the pH and compared it to the sites in which anurans were observed versus sites in which anurans were absent. This found the pH insignificant as it gave us a p value of 0.251 (Figure 6). When comparing and analyzing the DO at the sites that had frogs and those that did not we found that data to be significant as it gave us a p value of 0.042 (Figure 7). Conductivity was found to be insignificant as the p value was 0.092 (Figure 8) and when the Kruskal-Wallas test was run combining all our data we obtained a p value of 0.046 hence making our data significant. To check the One-way ANOVA we ran a normality test. This is what led us to realize that we needed to make use of a Kruskal-Wallas test instead of using the one way ANOVA test as it gave up better, reliable, and significant data (Figure 9).

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>Z</th>
<th>P</th>
<th>Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.535095</td>
<td>8.24988</td>
<td>0.06</td>
<td>0.948</td>
<td>0.81</td>
<td>4.80</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-1.03170</td>
<td>1.55335</td>
<td>-0.66</td>
<td>0.507</td>
<td>0.36</td>
<td>7.48</td>
<td></td>
</tr>
<tr>
<td>DO (ppm)</td>
<td>0.675980</td>
<td>0.455099</td>
<td>1.49</td>
<td>0.137</td>
<td>1.97</td>
<td>4.80</td>
<td></td>
</tr>
</tbody>
</table>

Log-Likelihood = -10.698
Test that all slopes are zero: G = 5.597, DF = 2, P-Value = 0.061

Figure 2. This Binary Logistic Regression shows a comparison of the site number, which correlates with frog presence or absence, versus the pH and DO levels.
Binary Logistic Regression: Site Number versus pH, DO (ppm), Conductivity
Logistic Regression Table

Odds  95% CI
Predictor  Coef  SE Coef  Z  P  Ratio  Lower  Upper
Constant  0.115817  8.13717  0.01  0.989
pH  -0.654465  1.62155 -0.40  0.687  0.52  0.02  12.48
DO (ppm)  0.635024  0.471105  1.35  0.178  1.89  0.75  4.75
Conductivity  -0.0112543  0.0073385 -1.53  0.125  0.99  0.97  1.00

Log-Likelihood = -8.254
Test that all slopes are zero: G = 10.484, DF = 3, P-Value = 0.015

Figure 3. This Binary Logistic Regression shows a comparison of the site number, which correlates with frog presence or absence, versus the pH, DO, and conductivity levels.

Binary Logistic Regression: Site Number versus DO (ppm)
Logistic Regression Table

Odds  95% CI
Predictor  Coef  SE Coef  Z  P  Ratio  Lower  Upper
Constant  -4.99006  2.43727 -2.05  0.041
DO (ppm)  0.461499  0.259641  1.78  0.075  1.59  0.95  2.64

Log-Likelihood = -10.924
Test that all slopes are zero: G = 5.145, DF = 1, P-Value = 0.023

Figure 4. This Binary Logistic Regression shows a comparison of the site number, which correlates with frog presence or absence, versus DO levels.

Binary Logistic Regression: Site Number versus Conductivity
Logistic Regression Table

Odds  95% CI
Predictor  Coef  SE Coef  Z  P  Ratio  Lower  Upper
Constant  0.423543  0.860923  0.49  0.623
Conductivity  -0.0094094  0.0052726 -1.78  0.074  0.99  0.98  1.00

Log-Likelihood = -11.175
Test that all slopes are zero: G = 4.643, DF = 1, P-Value = 0.031

Figure 5. This Binary Logistic Regression shows a comparison of the site number, which correlates with frog presence or absence, versus conductivity levels.

One-way ANOVA: pH versus Site Number

Source  DF  SS  MS  F  P
Site Number  1  0.734  0.734  1.39  0.251
Error  22  11.605  0.528
Total  23  12.339

Figure. This One-way ANOVA shows a comparison of the pH versus the site number, which correlates with frog presence or absence.
One-way ANOVA: DO (ppm) versus Site Number

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site Number</td>
<td>1</td>
<td>40.91</td>
<td>40.91</td>
<td>4.68</td>
<td>0.042</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>192.38</td>
<td>8.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>233.29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. This One-way ANOVA shows a comparison of the DO levels versus the site number, which correlates with frog presence or absence.

One-way ANOVA: Conductivity versus Site Number

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site Number</td>
<td>1</td>
<td>90291</td>
<td>90291</td>
<td>3.10</td>
<td>0.092</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>640459</td>
<td>29112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>730750</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. This One-way ANOVA shows a comparison of the conductivity levels versus the site number, which correlates with frog presence or absence.

Kruskal-Wallis Test: DO (ppm) versus Site Number

Kruskal-Wallis Test on DO (ppm)

<table>
<thead>
<tr>
<th>Site Number</th>
<th>N</th>
<th>Median</th>
<th>Ave Rank</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>7.415</td>
<td>10.8</td>
<td>-2.00</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>9.550</td>
<td>17.5</td>
<td>2.00</td>
</tr>
<tr>
<td>Overall</td>
<td>24</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

H = 4.00  DF = 1  P = 0.046

Figure 8. This Kruskal-Wallis test shows a comparison of the DO levels versus the site number, which correlates with frog presence or absence.

Probability Plot of RESI1

Figure 10. This is the normality test of the One-way ANOVA.
DISCUSSION

The Binary Logistic Regression results show that pH, DO and conductivity are significantly different as a whole at sites where anurans are present versus sites where anurans are absent. This was demonstrated by the Binary Logistic Regression tests that were conducted and produced p-values of 0.015, 0.023 and 0.031 for pH, DO and conductivity respectively. When these were looked at individually through the Binary Logistic Regression they were not found to be significant. Water chemistry viewed as a whole has more of an impact on anurans as opposed to individual chemical factors. As expected, our results show that anuran activity at the locations varied based on water chemistry. However, unexpected results were obtained from the pH data. After statistical analysis, it was found that pH value between sites where anurans are present versus sites where anurans are not absent, was not significantly different, shown by a p value of 0.507. This may because the range of pH values that were observed was relatively narrow.

Anurans were not observed at all sites, and the only species observed was the Spring Peeper. Spring Peepers live in wetlands, marshes, and pond or swamp regions in order to provide the aquatic environment the larvae need, and most of the sites that we visited were similar to the aforementioned habitats. The Spring Peeper breeds between the months of March to June, and deposits around 900 eggs that are hidden under vegetation or debris at the water base. After hatching, they transform into frogs and are ready to leave the water in about eight weeks. Hence another reason why Spring peepers were so prevalent in our observations as we collected our data during the peak of their reproductive season between March and April. Moreover Spring Peepers are very sensitive to temperature and hence they spend the winter burrowed into soil or under logs and leaves and when spring comes around they emerge out of hibernation due to the warm temperatures associated with the coming of spring, yet another explanation of why we observed so many Spring Peepers. Also much of our observations were conducted late in the day and mostly at night, which is when Spring Peepers are most active.

Certain factors directly influenced our collection of data. One factor was weather fluctuations. Some days would be ideally warm and moist, and some were too cold for accurate data as anurans are biologically unable to withstand cold weather. Lastly, barometric pressure has an effect on the calls of anurans. As barometric pressure increases anuran calling also increases, thus on nights leading up to rain events anuran calling was more prevalent. Originally, each site was supposed to be visited three times. However, some sites were only visited once due to weather limitations, as well as time limitations. Also, the dissolved oxygen meter/thermometer that was used in this study was inconsistent in recording air and temperature readings. Often, the meter would report that the water temperature was warmer than the air temperature. However, when we tested the water using our hands, water temperature seemed to vary from what the meter reported. In addition to this equipment error, we also experienced several issues regarding the conductivity meter. As our observational study progressed, the conductivity meter began to give inaccurate readings. This problem was exacerbated when the probe was placed in shallow water.

If this observational study were to continue, a few suggestions can be made to future researchers. This observational study was conducted early in the season, when many anurans had not yet emerged from hibernation. If the observational study continues, it should start later when greater species diversity is present. Only then will the most accurate survey data be obtained. In addition, a suggestion could be made to add site locations known for their acid mine drainage (AMD). This would be an excellent study site to add to the existing study as these sites are known for their poor water quality.

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LITERATURE CITED


