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# Amphibian community composition and its relationship to salinity along the Salt Creek in Wilderness Park, Lancaster County, Nebraska

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**Amphibian community composition and its relationship to salinity along the Salt Creek in Wilderness Park, Lancaster County, Nebraska**

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**Abstract:**

Amphibians serve as an indication of proper ecosystem health. Due to their permeable skin and cutaneous respiration, amphibians are prone to be adversely affected by intensified water quality parameters (Welsh and Olliver 1998) . Salinity (the measure of dissolved salts) can have a serious effect on amphibian reproduction and recruitment (Smith et al 2006). A research project was enacted during the 2012 summer field season in Wilderness Park located in Lancaster County, Nebraska to determine if a relationship exists between amphibian species richness and relative abundance with measures of salinity (ppm). Breeding sites were determined and placed in three relative salinity regimes (Low, Moderate and High). Frog call surveys in addition to tadpole dip netting surveys were administered at each site once each month (April-July). Shannon Diversity Indices (H') were calculated for each regime to measure species richness levels (Figure 1). Relative abundances were measured for each regime by dividing the total number of individuals encountered by the total measured area ($m^2$) of the sites within each regime (Formula 4). No significant relationship was found across the salinity regimes for either species richness or relative abundance. Tadpoles were not documented in the Moderate and High salinity regimes. The absence of tadpoles was most likely a result of lack of ideal microhabitat for reproducing frogs as well as insufficient cover from potential predation.

**Introduction:**

This research project entails measuring amphibian species diversity and relative abundance along Salt Creek to see if there is a correlation between these two metrics and salinity concentration. Amphibians (frogs, toads and salamanders) native to Lancaster County, Nebraska will be the focus of the investigation (Table I). Amphibians are good indicators of ecosystem health since they are relatively long lived and occur in stable populations in undisturbed habitats (Welsh and Olliver, 1998). Amphibians have permeable skin causing them to be more susceptible to damage as a result of changes in environmental factors. Previous studies in coastal regions have determined that high salinity levels have an adverse effect on amphibian reproduction success and breeding site selection (Smith et al 2006). However, few studies have been done in determining this relationship in freshwater ecosystems. It is important to determine if there is a relationship between amphibian community composition and salinity concentrations in order for proper management of the watershed. Species diversity refers to the number of different species present in a study area. Relative abundance refers to the amount of each species determined at each area. Salinity refers to the amount of dissolved salt present in the water (mg/L or ppm).

The Salt Creek watershed differs from others in Nebraska due to its higher concentration of salinity. These higher concentrations of salinity result from the shallow ground water, containing large amounts of dissolved salts, that feeds the creek (Hulvershorn, 1999). Although the salinity concentration in Salt Creek is higher than other freshwater sources there are still problems with secondary salinization due to human disturbance. A correlation between amphibian community composition and salinity is sought so the community and businesses surrounding the park, understand the implications of pollution prevention in this natural area. In addition, it is important to get a general idea of what amphibians are present in Wilderness Park so that those interested can help join in support for protecting the park and the native flora and fauna that it promotes. The encroaching development surrounding the park could have adverse effects on the quality of water in the Salt Creek, and therefore, could have a negative effect on amphibian habitat. Construction, agricultural practices and road salting can all attribute to increased salinity concentrations and, in turn, decreased water quality (Smith et al., 2006).

There were several assumptions made in this study:

1. Besides salinity, all other water quality factors are constant throughout the study area, and are thus negligible in determining a correlation. Ideally all the water quality parameters would be tested but given the scope of the study and the limited time and resources available to the researcher, they will be ignored.

2. Any variability in amphibian community composition will be attributed to varying salinity concentrations.

3. All species of amphibians expected to be found in this study have equal chances of being caught.

Overall, the goal of this study is to determine if there is a relationship between salinity concentrations and amphibian species diversity and relative abundance. Also, it should provide information that helps Lincoln Parks Department in managing this natural area for native biodiversity.

**Methods and Materials:**

To measure amphibian species diversity and relative abundance in Wilderness Park, several surveys were conducted. Two common survey techniques for amphibian breeding sites are frog call surveys and a tadpole dip netting surveys. An initial outing took place in April 2012 to get an idea of the breeding habitats of Wilderness Park. During this excursion the monthly survey sites were identified (Table II). Each site was marked on GPS and given a title, starting with “WP1” and onward to “WP11”. Initial parameter readings were recorded at each site. These are given in detail below. Sample sites were found using information provided in the literature about preferred breeding areas of amphibians

(Fogell, 2010, Welsh and Olliver, 1998). The sites range from small vernal pools to shallow, slow moving stretches of the creek as well as smaller puddles formed in ditches.

In order to determine if there is a relationship between species richness and salinity levels, the level of electro-conductivity (mS/cm) of each site was recorded using an ExStik© measurement device. For the purpose of this study, electro-conductivity (EC) will serve as a measure of salinity. The initial EC measured for each site was converted to a reference level EC standard for 25°C (Formula 1).

Formula 1: $\mathbf{EC_t = EC_{25} [ 1 + a * (t-25^oC)]}$

When analyzing the data, EC was converted to salinity using a formula that corrects for temperature's effect on the EC-salinity relationship (Formula 2).

Formula 2: **Salinity (ppm) = EC (mS/cm) * 640**

This formula is derived from the EC-Salinity relationship in which: 1 mS/cm = 640 mg/L (or ppm)

The sites were grouped into three salinity regimes: Low, Moderate and High (Table III). The labels given to each regime are relative for Wilderness Park and are solely for the purpose of this study. They are not intended to portray low, moderate or high levels of salinity in general terms.

Once the eleven potential breeding habitats for the study were established, initial parameters were taken. There are several parameters detailed in Scott's (1994) chapter on inventorying and monitoring amphibians that need to be taken at each outing in order to account for covariance (Scott, 1994). These include the current moon phase as well as the amount of cloud cover (%). Moon phase for this study will be determined from an online weather database (wunderground.com). Cloud cover will be determined on site with a best guess estimate of total percentage. Average wind speed (mph), relative humidity (%), and air temperature (°C), can all be taken by a handheld Kestrel© device. When taking these measurements it is crucial to allow the average wind speed to stabilize, so that accurate data is retrieved. Water temperature (°C), pH and EC (mS/cm) can all be measured using an ExStik© conductivity meter. It is integral to get a quality, standardized reading. In order to do this, the ExStik©

should be allowed to stabilize before a reading is taken and is then washed with distilled water after each use. Based off of standard conditions found in the literature, no sampling would occur if: winds are greater than 15mph on average, air temperature is less than 5$^{o}$C or thunderstorms are predicted (Scott, 1994).

Each month from April through July, a frog call survey and tadpole dip netting survey at each breeding site were conducted. Each survey method was performed once during each month at all eleven sites. The tadpole dip netting was done within ten days of the frog call survey in order to catch new hatchlings before they metamorphose (Ferraro, personal communication).

Frog call surveys were performed no earlier than thirty minutes after sunset. Most frogs are nocturnal and engage in reproductive behavior at night in order to evade predation (Scott, 1994). As discussed earlier, each survey started with recording the parameters. To successfully identify species, a handheld digital recorder which was used to double-check the calls identified by ear (Scott, 1994). The following details standard frog call survey procedure. Once the information is recorded, all headlamps are extinguished and a two minute acclamation period begins. This acclamation period allows time for the frogs and toads to return to a state of normal activity. After two minutes, the recording begins and continues for five minutes, totaling seven minutes of undisturbed silence (Scott, 1994). At the end of the recording, the observers recorded the species believed to have been calling with any additional notes. To aid in estimating abundance, a chorus-size ranking scale of 1, 2, 3, 4, 5 and 6 was used for each species heard (Stevens and Paszkowski, 2004). Chorus-size ranks and their descriptions are shown below (Table IV). A 6 on the chorus ranking scale was given when enough individuals of one species were calling that it appeared to be a full chorus. This number serves as a threshold value in which no accurate number of calling individuals can be assigned.

Tadpole dip netting surveys began like a frog call survey by recording the environmental parameters. This survey however, was conducted during the day. The main caveat to doing both of these sampling methods is that a dip netting survey cannot be done before a frog call survey in the same day. Dip netting disturbs the natural behavior of the frogs and they do not behave characteristically as a result (Scott, 1994). A dip net with a small enough sein to capture the smallest tadpole species was used. Each survey consisted of 10 one-meter sweeps of the net. A starting point for each was established upon discovery of the sites and the sweeps started from there. Each sweep was five meters apart with five sweeps each to the left and the right of the starting point. A sterilized bucket was used as a depository for the tadpoles (Scott, 1994). In addition a visual encounter survey was conducted during the tadpole survey to help identify species present at each breeding site. Adults seen upon each survey were recorded along with the tadpole data. A larger number of sweeps would have been better for a more accurate sample. However, given the time and labor constraints of the researchers, this method was preferred. The surveys were conducted and each sweep's tadpole species and relative abundances were recorded.

To analyze the relationship between species richness and relative abundance with salinity levels, data from the monthly surveys was compiled. Data retrieved from the frog call surveys and tadpole surveys was used to determine species richness of each site within each salinity regime. The Shannon Diversity Index (H') was used to compare the species richness of each regime (Formula 3).

Formula 3: $H' = -\sum p(i) \ln p(i)$

Where p(i) is the proportion of individuals found in the -ith species. P(i) is estimated as n(i)/N, where N is the total number of individuals observed.

The Shannon Diversity Index ranks species richness by giving each species a value that usually falls between 1.5 and 3.5. A Shannon Index was calculated for each salinity regime using frog call data, tadpole data and the combination of both methods. Relative abundance of each species at each sample site was determined through chorus size rankings and totaling tadpole numbers. Area ($m^2$) of each breeding site was measured using GoogleEarth which helped determine relative abundances based on area of each habitat (Formula 4).

Formula 4: **Rel. Abundance = [# of individuals / area (m2)]**

The measures of species richness and relative abundances were observed in each salinity regime in order to make observations and conclusions on any apparent relationship.

**Results:**

Due to drought conditions present in the Midwest during the summer of 2012 two breeding sites were lost in the month of April. Those two sites (Wp 6 and Wp7) were replaced by two sites containing water located nearby (Wp8 and Wp11). No species were identified or recorded at wp6 and wp7 in the month of April which allowed for replacement of the two wetlands without serious effect on species richness and abundance results.

According to known and projected distributions there are expected to be 10 amphibian species present in Wilderness Park (Fogell 2010). A list of these species is provided in Table I. Of these 10 possible species, 5 were documented in surveys throughout the course of this project. The following species were documented: Cricket Frog (*Acris blanchardi*), Cope's Gray Treefrog (*Hyla chrysoscelis*), Chorus Frog (*Pseudacris maculata*), Bullfrog *(Lithobates catesbeianus*) and Plains Leopard Frog (*Lithobates blairi*). Additionally, for record purposes only, Woodhouse's Toad *(Anaxyrus woodhousii*) was heard calling at a breeding site during a tadpole dip netting survey. In this study all five of the species documented were heard calling at sites being surveyed. Only four of the species were found in tadpole or metamorphose stages (no Bullfrog larvae were documented). Of the 11 total potential breeding sites included, only two sites were documented to have had tadpoles present (Wp4 and Wp8). In all three salinity regimes the Cricket Frog and Cope's Gray Tree frog were heard calling. The Chorus Frog was heard calling in both the Low and Moderate salinity regimes. The Bullfrog was heard calling in both the Low and High salinity regimes and the Plains Leopard Frog was only heard calling in the Moderate salinity regime. The Cricket Frog was the most commonly found species with 43 individuals documented and the Bullfrog was most rare species found with only four individuals heard calling (Table IV).

A Shannon Diversity Index was calculated for each salinity regime (Low, Moderate and High) using solely frog call data. The Low salinity regime resulted in the highest observed diversity index with a value (H' =1.01). The High salinity regime resulted in the lowest observed diversity index with a value (H'= 0.6). The Moderate salinity regime had a medium value (H' = 0.955) (Figure I).

A second Shannon Diversity Index was calculated for each salinity regime using the data collected in the tadpole dip netting surveys. In this study no tadpoles were documented in either the Moderate or High salinity regimes. This resulted in a value (H'= 0.0) for both Moderate and High regimes (Figure I). In the Low salinity regime tadpoles of several species were identified and the diversity index value (H'=0.99) was determined (Figure I).

A third Shannon Diversity Index was calculated to estimate the species richness of each salinity regime using both frog call data and tadpole seining data. Since the Moderate and High salinity regimes lacked any tadpole presence their indices remained the same. However, the Low salinity regime contained both data of calling frogs and tadpoles present and the diversity index calculated resulted in a higher value (H'=1.34)(Figure I).

Relative abundances were determined for each site using the GoogleEarth© software. Each site's area was measured using this software and abundances were calculated using Formula 4. The highest observed abundance occurred in the Low salinity regime with a value of 0.3545 individuals/$m^2$. The lowest observed abundance occurred in the Moderate salinity regime with a value of 0.0461 individuals/$m^2$. The High salinity regime resulted in the medium value of 0.0646 individuals/$m^2$ (Table VI).

**Discussion:**

This data indicates that species richness is highest in the Low salinity regime and lowest in the High salinity regime. However, the difference between the two H' values is not significant since it equals only 0.06. This difference is not significant enough to draw a strong correlation between species richness and relative salinity measures. The Low salinity regime resulted in a much higher Shannon Diversity Index (H'=1.34) than the Moderate and High salinity regimes (H'=0.955, 0.60 respectively) only when tadpole data was included (Figure I). Without tadpole data included the Shannon Diversity Index value for the Low salinity regime was H'=1.01. A study by Smith et al (2006) shows that anuran use of breeding habitat and larvae production and survival should not be negatively affected by waters with an EC below 3 mS/cm. All three salinity regimes present in Wilderness Park are well below the suggested value of 3 mS/cm with the highest value of EC being 1.31 mS/cm (Table II). The relative abundances of each salinity regime have no clear correlation with salinity measures. This can be observed in Table VI which indicates the relative abundances of each salinity regime. The Low salinity regime has the highest relative abundance of 0.3545 ind./$m^2$ as a result of tadpoles only being documented in this regime. The lowest relative abundance is in the Moderate salinity regime with 0.0461 ind./$m^2$. The results of the abundance calculations indicate that relative abundance and salinity measures do not have a clear correlation since the moderate values of salinity result in the lowest relative abundance (Table VI).

Since no tadpoles were documented in the Moderate and High salinity regimes there may be other factors must have contributed to their lack of presence and subsequent lack of abundances. Examination of other parameters documented throughout the study (pH, temperature and relative humidity) and determined that due to their variability and inherent lack of correlation with any observed species richness and abundance they too are not the cause of the trend observed. This points to another explanation. The reason species richness and abundance were lower in the Moderate and High than in the Low salinity regime could be microhabitat selection and cover difference. The Moderate and High Salinity Regimes have less cover making it harder to evade predators at those breeding sites.

Although the potential breeding sites chosen for this study represented a variety of microhabitats they were not represented equally in each salinity regime. This would be related to the relative levels of salinity measured in each survey site. The High and Moderate salinity regimes tended to be both slow and fast moving stretches of the Salt Creek and smaller tributaries connected to the creek. Since the Salt Creek is known for its higher salinity concentrations it would make sense that these sites would be generally higher in salinity since they are often recharged by the saline groundwater. The sites within the Low salinity regime tended to be both small vernal pools and larger pools which could have been a result of pooled rainfall or drainage from the surrounding area. Oftentimes, these sites were in lowland areas that would collect water and were not as regularly recharged by the saline groundwater.  The location of the Moderate and High sites along the Salt Creek would also allow for increased predation. Predators of adult amphibians and also their larvae and eggs (raccoons, opossums, fish and insects) were more commonly encountered along the stream banks in which several of these sites were located.  The larger predators such as raccoons commonly travel along stream banks in the night when searching for food (Gibbs 1997). Fish are more readily available as predators in the stream since they can avidly move throughout the stream searching for food. The sites in the Low salinity regime often contained more vegetation for frogs to cling on to when administering their frog calls than the stream bank sites which often were shallow and rocky with little to no vegetation.

**Conclusion:**

Overall, this study served as a great learning experience for the student as a researcher. Future research would benefit from increased preparation and better time management.  Frog Call surveys administered earlier in April would produce more pronounced species richness' and relative abundances data. Additionally, a future project including a more comprehensive look at the Salt Creek Watershed could lead to a better understanding of the relationship between amphibian species richness and relative abundance with levels of salinity. Including the Salt marshes northeast of Lincoln as well as additional wetlands throughout the watershed could result in a more pronounced correlation between these two parameters and the varying levels of salinity. This investigation was beneficial for both Wilderness Park and its recreational users as it serves as a documentation of the amphibian species present in this wild area close to Lincoln, Nebraska. It is integral to promote the wildlife of the park in order to help the conservation efforts of organizations such as the Friends of Wilderness Park and the City of Lincoln Parks Department.  If nothing else, this investigation aids the park in serving its purpose, since no research documenting amphibian species had been done for this study area before.

**Tables and Figures:**

## Table I. Species Native to Lancaster Co., Nebraska

| Species Native to Lancaster County, Nebraska | |
|---|---|
| **Amphibian Species** | **Common Name** |
| *Anaxyrus cognatus* | Great Plains Toad |
| *Anaxyrus woodhousii* | Woodhouse's Toad |
| *Acris blanchardi* | Blanchard's Cricket Frog |
| *Hyla chrysoscelis* | Cope's Gray Treefrog |
| *Pseudacris maculata* | Boreal Chorus Frog |
| *Lithobates blairi* | Plains Leopard Frog |
| *Lithobates catesbeianus* | American Bullfrog |
| *Lithobates pipiens* | Northern Leopard Frog |
| *Spea bombifrons* | Plain's Spadefoot |
| *Ambystoma mavortium* | Barred Tiger Salamander |

## Table II. Survey Sites

| Site | EC (mS/cm) | Intial Salinity (ppm) | Regime |
|---|---|---|---|
| Wp4 | 0.19 | 121.6 | Low |
| Wp6 | 0.09 | 57.6 | Low |
| Wp7 | 0.19 | 121.6 | Low |
| Wp8 | 0.28 | 179.2 | Low |
| Wp11 | 0.19 | 121.6 | Low |
| Wp3 | 0.78 | 499.2 | Moderate |
| Wp5 | 0.48 | 307.2 | Moderate |
| Wp10 | 0.58 | 371.2 | Moderate |
| Wp1 | 1.31 | 838.4 | High |
| Wp2 | 1.42 | 908.8 | High |
| Wp9 | 1.08 | 691.2 | High |

**Tables and Figures:**

## Table III. Salinity Regimes

| Relative Labels: | Salinity Concentrations (mg/L or ppm) |
|---|---|
| Low | 0-300 |
| Moderate | 301-601 |
| High | > 602 |

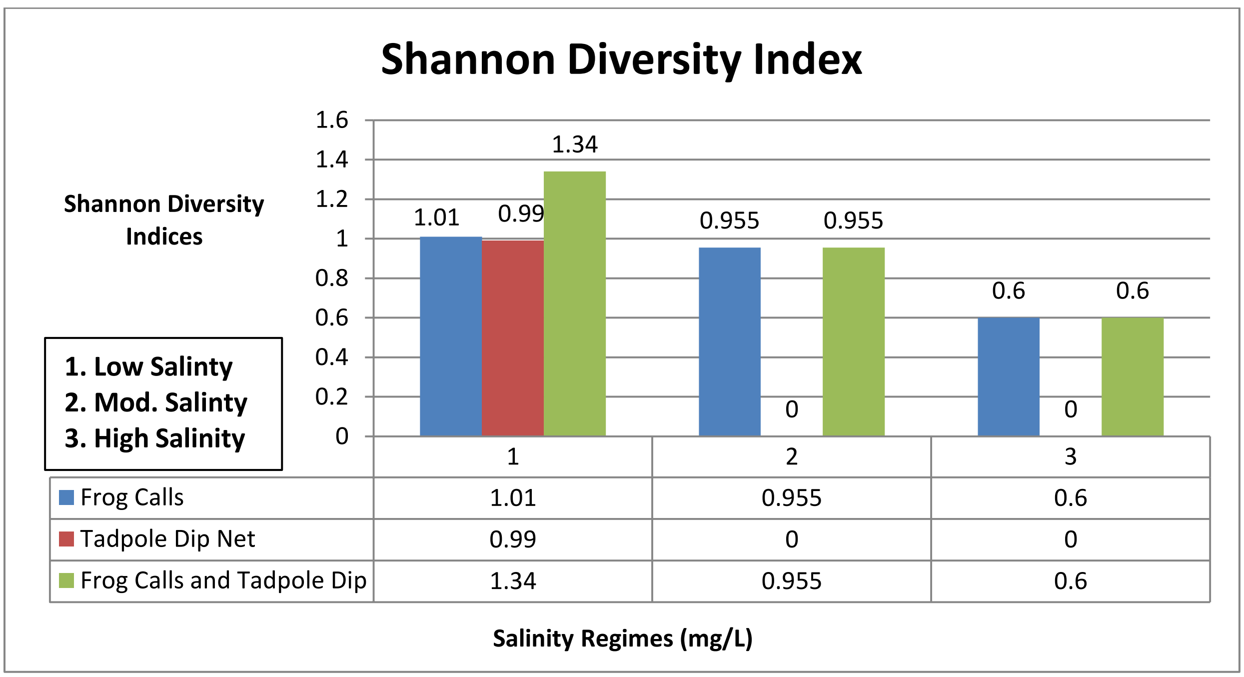
## Table IV. Chorus-size Ranks

| Chorus-size rank | Description |
|---|---|
| 1 | No overlap of calls, number of frogs can be reliably estimated. One individual calling |
| 2 | Number of frogs can be estimated accurately. Two individuals calling |
| 3 | Number of frogs can be estimated less accurately. Three individuals calling |
| 4 | Number of frogs can be estimated less accurately. Four individuals calling |
| 5 | Number of frogs can be estimated less accurately. Five individuals calling |
| 6 | Full chorus, no number of frogs can be estimated |

**Tables and Figures:**

## Table V. Total Individuals Encountered

| Survey: | | Low (0-300ppm) | | Moderate (301-601 ppm) | | High (602 + ppm) | | Total Study |
|---|---|---|---|---|---|---|---|---|
| **Frog Call** | **Species:** | Present (X) | # of Individuals | Present | # of Individuals | Present (X) | # of Individuals | # of Individuals |
| | A. blanchardi | X | 17 | X | 14 | X | 9 | 40 |
| | H. chrysoscelis | X | 5 | X | 4 | X | 1 | 10 |
| | P. maculata | X | 2 | X | 1 | 0 | 0 | 3 |
| | L. catesbeianus | X | 3 | 0 | 0 | X | 1 | 4 |
| | L. blairi | 0 | 0 | X | 2 | 0 | 0 | 2 |
| **Tadpoles** | A. blanchardi | X | 3 | 0 | 0 | 0 | 0 | 3 |
| | H. chrysoscelis | X | 2 | 0 | 0 | 0 | 0 | 2 |
| | P. maculata | X | 26 | 0 | 0 | 0 | 0 | 26 |
| | L. catesbeianus | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | L. blairi | X | 13 | 0 | 0 | 0 | 0 | 13 |




## Figure I. Shannon Diversity Index (H')

**Tables and Figures:**

## Table VI. Relative Abundance

| Site | Abundance (individual/m^2) |
|---|---|
| wp1 | 0.0076 |
| wp2 | 0.0088 |
| wp3 | 0.0126 |
| wp4 | 0.2931 |
| wp5 | 0.0249 |
| wp6 | 0 |
| wp7 | 0 |
| wp8 | 0.0309 |
| wp9 | 0.0482 |
| wp10 | 0.0086 |
| wp11 | 0.0305 |
| Regimes | --- |
| Low | 0.3545 |
| Moderate | 0.0461 |
| High | 0.0646 |

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