ASSOCIATIONS OF AN AQUATIC INVERTEBRATE COMMUNITY AT BITTER LAKE NATIONAL WILDLIFE REFUGE WITH EMPHASIS ON NOEL'S AMPHIPOD, ROSWELL SPRINGSNAIL, AND KOSTER'S SPRINGSNAIL

by

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ABSTRACT

Bitter Lake National Wildlife Refuge (NWR), Chaves County, New Mexico, harbors at least 19 different aquatic macroinvertebrates in spring systems, wetland units, and channels. Of these 19 invertebrate taxa, 3 are endemic and federally listed: Noel's amphipod, Koster's springsnail, and Roswell springsnail. These organisms are dependent upon several environmental factors which have not been well-studied, locally. Environmental factors such as water temperature, dissolved oxygen, specific conductance, pH, and depth are thought to influence macroinvertebrate communities. Furthermore, pollutants especially from oil and gas, stochastic events such as fire, and invasive species are known to effect water quality parameters and potentially aquatic macroinvertebrate taxa. Therefore, I investigated species-environmental community interactions for benthic macroinvertebrates at Bitter Lake NWR, and specific water quality parameters that could influence Noel's amphipod abundances and springsnail abundances. Twenty-four monitoring sites were sampled for springsnails between 2014-2017 with a benthic grab trap quarterly across 3 systems: Sago Springs, Snail Unit, and Bitter Creek. Noel's amphipod were sampled at 37 monitoring sites with the inclusion of 13 additional monitoring sites in the Rio Hondo. At each monitoring site, specific conductance, dissolved oxygen (mg/l), water temperature (ºC), depth (cm), and pH were measured to obtain associations of Noel's amphipods, and springsnails with each parameter utilizing Canonical Correspondence Analysis (CCA), Generalized Linear Mixed Effects Models (GLMMs), and Classification and Regression Trees. Species-environmental factors were teased out utilizing a CCA, with bloodworm (order: Diptera) having the strongest association with salinity on the environmental gradient. Caddisflies, mosquito larvae, physidae, beetles, orange amphipods, and copepods were associated with the site Bitter Creek. However, Noel's amphipod was negatively correlated with Bitter Creek and preferred sandy substrates with vents, and rushes and sedges. Springsnails were negatively correlated with all environmental gradients except for temperature and were associated with the season Spring and saltgrass. Based on GLMMs Noel's amphipod was significantly influenced by dissolved oxygen, depth, and water temperature (p-values <0.001) where pH and salinity were not statistically significant. Springsnails were influenced by all water quality parameters (p-values < 0.001). Based on Classification and Regression Trees, approximately 46% of Noel's amphipods were most abundant in cooler water, whereas springsnails preferred specific ranges of a water temperature less than 20.6 ºC, a depth greater than 7.92 (cm), and a pH less than 7.87. With this knowledge I plan to implement these results to enhance current monitoring efforts by selecting more sites with more variability of water quality parameters and less species occurrences (e.g. sites where endangered species are not found) to obtain more robust conclusions of habitat and endangered species habitat use. Furthermore, current monitoring efforts include two springsnail species as one. Therefore, I wanted to investigate the efficacy of an external morphological character to discern these two

species in the field utilizing a field dissecting microscope. I utilized the initial observations of Taylor (1987) that operculum color can differentiate Roswell springsnail and Koster's springsnail. Roswell springsnail is assumed to have an amber operculum color and Koster's springsnail is assumed to have an opaque operculum color. Therefore, I separated springsnails by operculum color and verified my observations with another individual. I then utilized genetic analyses via Sanger Sequencing to determine species identity. I found that we had a field assigned ratio of 59:41 (amber:opaque) and a genetic ratio of 99:1 (Koster's springsnail:Roswell Springsnail. Therefore, studies based on operculum color should be suspect, and a third party should replicate my work to verify if these species are in fact differentiated by operculum color if this trait is going to be utilized in monitoring efforts at Bitter Lake NWR.

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Aquatic benthic macroinvertebrates are key organisms in both aquatic and terrestrial trophic systems (Knight et al. 2005). Benthic macroinvertebrates are a central part of the food web, being prey for fishes, birds, invertebrates, and other terrestrial organisms once emerged from the water (Richardson 1991, Knight et al. 2005, Merritt et al. 2008). In addition, aquatic macroinvertebrates feed on algae, bacteria, other macroinvertebrates, and decaying organic matter (Taylor 1987, Pennak 1989). Benthic macroinvertebrates are good indicators of water quality as they are sensitive to environmental changes and disturbances (Lenat 1988, Merovich and Todd 2010).

Bitter Lake National Wildlife Refuge (NWR) is located 16.1 km northeast of Roswell, Chaves County, New Mexico, with the Chihuahuan Desert in the south portion of the refuge and Southern Plains in the north portion of the refuge (Macanowics et al. 2013). The Pecos River runs through the east portion of the refuge, allowing karst topography to form sinkholes, caverns, springs, wetlands, seepages, and underwater springs (USFWS 2009). The different habitat types allow many species to flourish in places they do not exist otherwise. Within these habitat types, approximately 12 different classes of benthic macroinvertebrates persist on the refuge (Macanowicz et al. 2013).

The diversity of benthic macroinvertebrates on Bitter Lake NWR provides a suitable framework to investigate community structures (e.g. community ecology) of natural populations in the arid southwest United States. Community structure at small scales can be informative to habitat associations for specific taxa. Furthermore, interactions between biotic and abiotic factors influence local community structure (Losos 1996). Understanding these relationships is crucial for management practices as it has the power to inform biologists about interactions among factors, both biotic and abiotic, and therefore allowing for more informed management decisions. Bitter Lake NWR, has a large diversity of endangered species, invasive species, and abiotic factors that can influence community structure across the refuge. With 12 classes of benthic macroinvertebrates on Bitter Lake NWR, 3 of which are federally listed I can investigate species patterns to environmental variables on a large scale and investigate correlations to determine associations of flora and fauna (Macanowicz et al. 2013). Additionally, I want to investigate specific environmental associations with the 3 federally listed species on the refuge: Roswell springsnail (*Pyrgulopsis roswellensis*), Koster's springsnail (*Juturnia kosteri*), and Noel's amphipod (*Gammarus desperatus*) (USFWS 2009).

Noel's amphipod

Noel's amphipod is in the family Gamaridae within the superorder Peracarida, characterized by the absence of a carapace and the presence of 7 pairs of pereopods (Cole

1981, Pennak 1989, Covich and Thorp 1991). The species is also characterized by having kidney-shaped eyes, first antennae longer than the second antennae, and a third uropod (Holsinger 1976). Noel's amphipod occurs only near Roswell, Chavez County, New Mexico, wherein they can occur in patches in high densities (Noel 1954, Holsinger 1976, Pennak 1989). Historic occurrences were documented at North Spring (Roswell Country Club), and Lander Springbrook in Roswell, New Mexico. These populations are now thought to be extirpated (Cole 1981).

Currently, known occurrences are only found on Bitter Lake NWR (Lang 2002). Noel's amphipods prefer shallow, cool, oxygenated water in streams, ponds, ditches, sloughs, and springs (Holsinger 1976, Pennak 1989). The species is extremely sensitive to light, is benthic dwelling, and is most active at night (Holsinger 1976, Pennak 1989). Known food sources include algae, submergent vegetation, and detritus (Holsinger 1976, Cole 1988, Pennak 1989). They are sexually dimorphic with males being larger than females, with adult of both sexes ranging from 8.5 – 14.8 mm (Pennak 1989). Noel's amphipods form breeding pairs where the pairs remain in copula for 1-7 days, while continuing normal activities (Bousfield 1989). They are iteroparous and ovoviviparous with young being carried in the marsupium, brood size ranges from 3-20 offspring, and breeding events taking place between February and October (Holsinger 1976, Pennak 1989, Covich and Thorp 1991). The typical lifespan of Noel's amphipod is approximately 1 year (Pennak 1989). This species was listed in 2005 because of endemism, aquifer depletion and water contamination (USFWS 2005).

Koster's springsnail

Koster's springsnail is a molluscan, prosobranch, gastropod in the family Hydrobiidae ranging from 4-4.5 mm. They possess internal gills for respiration and contain an operculum (Pennak 1989). They possess eyes on the antennae and are globose with a narrowly conical shell (Taylor 1987). They are sexually dimorphic with males being half the shell height of females (Hershler and Thompson 1987). This species' geographic range is restricted to Bitter Lake NWR, where it is found in sinkholes, seeps, wetlands, and springs (Lang 2002). Koster's springsnails are thought to be found in numerous sinkholes and springsystems across the refuge as well as marshes (Lang 2002).

The species has been documented at North Spring (Roswell County Club) but was presumed to be extirpated by Taylor (1987) and Cole (1988). However, Mehlhop (1993) suggested a population may persist at North Spring. Koster's springsnails are thought to occupy several habitats associated with fresh to slightly saline waters of spring systems, or groundwater upwellings (NMDGF 1988). They were also noted to be more abundant deeper in the substrate and less abundant in areas in gypsum substrate (Lang 2002). Koster's springsnails are ovoviviparous with multiple stages of development, causing continuous breeding throughout the period (Taylor 1987, Pennak 1989, Brown 1991). Koster's springsnails reproduce between March and September with an average lifespan of 9-15 months (Lang 2002, Taylor 1987). Primary diet of Koster's springsnail includes algae, bacteria, and decaying organic matter (Taylor 1987, Lang 2002). This species was listed in 2005 because of endemism, aquifer depletion, and water contamination (USFWS 2005)

Roswell springsnail

Roswell springsnails are molluscan prosobranch gastropods in the family Hydrobiidae. They possess internal gills, an operculum, and a globose to narrowly conical shell ranging from 3-3.5 mm (Taylor 1987, Pennak 1989). They have 5 whorls and are thought to have a pale amber operculum (Taylor 1987). Roswell springsnail is only known to occur on Bitter Lake NWR. A North Spring population at Roswell Country Club and a Lander Springbrook population are thought to be extirpated as well (Cole 1981, 1985; Taylor 1987).

Roswell springsnails are thought to occupy several habitats associated with fresh to slightly saline waters of spring systems, or groundwater upwellings (NMDGF 1988). They were also noted to be more abundant in areas within gypsum substrate (Lang 2002). Roswell springsnails are ovoviviparous with multiple stages of development, causing continuous breeding throughout the period (Taylor 1987, Pennak 1989, Brown 1991). Roswell springsnails reproduce between March and September with an average lifespan of 9-15 months (Lang 2002, Taylor 1987). Primary diet of Roswell springsnail includes algae, bacteria, and decaying organic matter (Taylor 1987, Lang 2002). Roswell springsnail was listed in 2005 due to endemism, aquifer depletion and water contamination (USFWS 2005).

Current threats

There are numerous threats to the 3 endangered invertebrates on Bitter Lake NWR. Non-native flora and fauna, such as the common carp (*Cyprinus carpio*), are amongst the most serious threats (Williams et al. 1989, Lodge et al. 2000). Other taxa such as crayfish, and invasive aquatic snails can also pose major threats to the 3 endangered invertebrates (Maffe et al. 1994) Western mosquitofish (*Gambusia affinis*) are thought to depredate on the 3 endangered invertebrates, but whether they are native or not is not confirmed (USFWS 2009). The greatest threat on Bitter Lake NWR is the common carp, as carp select for protein through foraging through vegetation (Garcia-Berthou 2001). Crayfish have been reported in the nearby Rio Hondo on the farm portion of the refuge, where populations of Noel's amphipods occur (Pers. Observation).

Native species such as damselflies (Zygoptera), dragonflies (Anisoptera), and Pecos pupfish (*Cyprinidon pecoensis*) depredate on the springsnails and amphipods (Kennedy 1977, Winemiller 1997). However, since these are native organisms, no management actions are currently being taken to reduce predation. Until further assessment of the impact of native species have on the 3 endangered invertebrates no management action will be taken. Non-native flora such as saltcedar (*Tamarix* spp.), common reed (*Phragmites australis*), and Russian thistle (*Salsola* spp.) alter the stream channel, reduces flow and can overload the system with organic material posing a potential threat to the 3 endangered species (USFWS 2009).

Parasites in snails on Bitter Lake NWR has been documented. Trematodes (Phyla: Platyhelminthes, digenera) were noted by Taylor (1987). These trematodes inhibit snail reproduction as they are the first intermediate host for the organism (Hickmann et al. 1974, Minchella 1985). However, the overall health of springsnail populations because of parasitic trematodes is unknown and until further assessment of identifying parasites in snails management action cannot be taken. Furthermore, detection of trematodes on Bitter Lake NWR is low, and they are rarely, if ever documented.

The most prominent threat to these endangered invertebrates is the loss of water and potential aquifer depletion. Because of scattered distribution, even on a fine scale, isolated desert springs make them susceptible to extinction (Hershler 1989, Hershler and Pratt 1990, Hershler 1994, Myers and Resh 1999, Lydeard et al. 2004). Evidence suggests that habitats for benthic macroinvertebrates have been eliminated by aquifer depletion in the arid-southwest (Hershler 1989, Hershler and Pratt 1990). Two historic sites have been depleted that are supported Noel's amphipod populations: South Spring, and Landerbrook Spring (Cole 1981, Jones and Balleau 1996). Climate change can also be contributed to loss of water. Although springs can be resilient to drought, the southwest United States is predicted to enter a long period of drought (McCabe et al. 2004, Seager et al. 2009). Furthermore, New Mexico Offices of State Engineer (2006) reported that warming trends in the American southwest exceed the global average by 50%, producing increased intensity, frequency, and duration of drought.

In addition to water loss, contamination is another threat on the refuge, particularly oil and gas operations. Balleau Groundwater, Inc. (1999) reported groundwater contamination coming from west of Roswell, and northeast of Salt Creek Wilderness that could potentially contaminate the refuge's spring fed system. In addition, there are 17 oil and gas leases within the critical habitat zone 2, including 2 active wells (BLM 2002). Contaminated soil and rock strata can decrease microbial growth and therefore deplete a food source for the 3 endangered invertebrates (Lang 2002).

Fire is thought to decrease dissolved oxygen levels post burn (Lang 2002). This is extremely prominent and potentially problematic near Bitter Creek where fuel loads are high because of the extensive strands of common reed (*Phragmites australis*). Lang (2002) noted there was a dramatic reduction in all aquatic macroinvertebrates after the Sandhill Fire on the western part of the refuge that occurred on 05 March 2000.

Based on these threats, the United States Fish and Wildlife Service began monitoring invertebrate populations in 2014. My intent was to evaluate abiotic and biotic correlates for this aquatic invertebrate community at Bitter Lake NWR. Additionally, I wanted to provide more detailed analyses of the influence of water quality parameters on the 3 protected aquatic invertebrate taxa: Noel's amphipod, Koster's springsnail, and Roswell springsnail.

METHODS

Study area

Bitter Lake NWR is located approximately 16.1 km northeast of Roswell, in Chavez County, New Mexico, residing in the Lower Pecos Valley (Figure 1.1). Bitter Lake NWR is 10,293 hectares alongside the Pecos River. Several sinkholes, natural wetlands, spring systems, and riparian areas occur on Bitter Lake NWR (Lang 1998, Lang 2002). In this study I focused analyses on streams fed by spring systems on the refuge: the restored portion of the Rio Hondo (Figure 1.2), Sago Springs, Bitter Creek (Figure 1.3) and Snail Unit (Figure 1.4)

Field Methods

Sago Springs, Bitter Creek, Snail Unit and the restored Rio Hondo were sampled on a quarterly basis from Summer 2014 to Summer 2016. Points were randomly selected using ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, California, USA), in each system. The restored Rio Hondo had 13 monitoring locations (Figure 1.2), Sago Springs had 6 monitoring locations, Bitter Creek 12 (Figure 1.3), and Snail Unit 6 (Figure 1.4). At each site 3 subsamples were taken from the center of the channel, the middle between the bank and center of the channel, and on the bank of the channel. I utilized a 0.003 m^2 benthic grab trap to capture aquatic invertebrates (Figure 1.5). Depth (cm) was taken with a standard ruler or tape measurer before the grab. Percent vegetation (0-25, 25-50, 50-75, 75-100) was noted in each trap and identified to closest genera. Overhanging vegetation was also noted such as Pecos Sunflower (*Helianthus pecoensis*) saltcedar, and saltgrass (*Distichlis* sp.). Percent substrate was noted (0-25, 25-50, 50-75, 75-100) in each trap and classified as sand, soil, sand and soil or rock. After assessment of environmental factors in the trap, invertebrates were placed in a white tray to identify each taxa. Because of the difficulty of identifying species of springsnails these were lumped as “springsnails,” rather than individual species for analysis (Taylor 1987). In addition, we noted presence/absence of each invertebrate taxa and enumerated Noel’s amphipod and springsnails. Invertebrates were subsequently relocated after the site was sampled. At each site a YSI Professional Plus (YSI, Yellow Springs, Ohio, USA) was utilized to collect water temperature (ºC), dissolved oxygen (mg/L), specific conductance (mS), and pH. All environmental factors collected are noted in Table 1.5.

Statistical analyses

I used Canonical Correspondence Analysis (CCA) as a direct gradient analysis to relate species patterns to environmental variables. I used presence/absence data collected from each monitoring site for all taxa, to define species space and environmental conditions such as water quality parameters to define environmental space in this ordination (Palmer 1993). Year, season, site, and sampling position (mid-channel, mid-point and bank) were utilized in my analysis. All taxa were scaled as present or absent, while water quality parameters were continuous. 1,000 Monte-Carlo permutation tests were utilized to determine which of the measured variables were most important in determining the best environmental-species space.

To clarify patterns for Noel’s amphipod, Koster’s springsnail, and Roswell Springsnail, abundances from each subsample were averaged as well as depth for each monitoring location. Data were diagnosed for normality, outliers of dependent and independent variables, and autocorrelated variables per methods of Zuur et al. (2010). After the data were diagnosed I utilized 6 generalized linear mixed effects models (one null model) in program R version 3.3.4 (The Comprehensive R Archive Network) and the package lme4 (Bolker 2018) to gain an understanding of abundance of springsnails and Noel’s amphipod compared to depth, dissolved oxygen, temperature, specific conductance, and pH. I then reported AIC, random effect intercept, R^2 for each variable, and the corresponding z value for each model to determine which model represented the data the best. Results were then plotted by site and water quality parameter to determine the predicted incidences of Noel's amphipod and springsnails. In addition, I utilized Classification and Regression Tree (CART) analysis to explore how much one variable contributed to abundances of the 3 federally listed invertebrates.

RESULTS

I detected 19 aquatic invertebrate taxa utilizing the benthic grab trap from a total of 1,135 grabs across 4 systems. Table 1.5 indicated all environmental variables utilized in the model and denotes which variables were dropped from the model. Specific conductance was the only continuous variable not selected in our model, while all other environmental factors were selected with the exception of percent substrate. The CCA suggested associations of water quality parameters with low scores. Caddisflies, mosquito larvae, physids, beetles, orange amphipods, and copepods were associated with the site Bitter Creek. However, Noel's amphipod was negatively correlated with Bitter Creek and preferred sandy substrates with vents, near 3-square bulrush (*Schoenoplectus americanus*), and spikerush (*Eleocharis palustris*), especially in Snail Unit. Flatworms and juvenile amphipods were also associated with this same habitat type. Springsnails were negatively correlated with all environmental gradients except for temperature and were associated with the season Spring and saltgrass. Water quality parameters did not show strong gradients for species except for bloodworm on the salinity gradient. All other community level interactions were weak or uninformative in the CCA. Total inertia for the model was 3.922 with a p-value of 0.001. The percentage of variance explained by the species-environmental relationship for the first axis was 30.7% and the second axis explained an additional 14.3% (Figure 1.6). The remaining axes provided minimal additional exploratory value.

Utilizing boxplots Noel's amphipod abundances were visualized by site, year, and season. Sago Springs harbored the most abundances, followed by Snail Unit, Hondo, and rare occurrences of Noel's amphipod in Bitter Creek (Figure 1.7). 2014, 2015, and 2016 were consistent in abundances while 2017 had the lowest abundances (Figure 1.8). Season was consistent throughout the Fall, Spring, and Summer, while Noel's amphipod abundances were less in the Winter (Figure 1.9). The selected model with an AIC of 788.3 suggests water temperature (ºC), dissolved oxygen (mg/l), and depth (cm) were significant with p-values < 0.001 and estimates of –0.10, -0.11, and -0.05, respectively (Table 1.1, 1.2). Specific conductance was not significant with a p-value > 0.05 and an estimate value of -0.04. Predicted incidents plots suggested more occurrences of Noel's amphipod in shallow water (Figure 1.10), in lower dissolved oxygen (Figure 1.11), with neutral pH (Figure 1.12), with lower specific conductance (Figure 1.13) and with cooler temperature (Figure 1.14). Following the CART analysis, a water temperature less than 13.6 ºC influenced abundances the most, while water temperatures between the range of 13.6 ºC and 19.9 ºC, followed by a specific conductance range of 8.52 mS, and 9.95 mS and dissolved oxygen between the range of 4.04 (mg/l) and 13.4 (mg/l) influenced abundances the second most (Figure 1.15).

Utilizing boxplots, I visualized springsnail abundance by site, year, and season. Sago Springs harbored the highest abundance, followed by Bitter Creek, and Snail Unit (Figure 1.16). 2014 and 2016 were similar in abundances while 2015 and 2017 had lower abundances (Figure 1.17). Abundance was consistent throughout the Fall, Spring, and Summer, but decreased in Winter (Figure 1.18). The selected model with an AIC of 13215.5 suggested specific conductance, water temperature, dissolved oxygen, depth and pH were significant with p-values < 0.001 and estimates of –0.06, -0.05, -0.02, -0.04, and -0.44, respectively (Tables 1.3; 1.4). Predicted incidents plots suggests more occurrences of springsnails in shallow water (Figure 1.19), lower dissolved oxygen (Figure 1.20) neutral pH (Figure 1.21), with lower specific conductance (Figure 1.22), and with warmer water temperatures (Figure 1.23). Following the CART analysis a water temperature less than 20.6 ºC, a depth greater than 7.92 (cm), and a pH less than 7.87 influenced springsnail abundances the most (Figure 1.24).

DISCUSSION

The community level analysis (CCA) did not produce a robust model. Out of 19 aquatic fauna, 6 species of flora, and 14 abiotic factors some species-environmental interactions were able to be clarified. In this model I was able to detect a handful of environmental-species interactions. Caddisflies, mosquito larvae, beetles, orange amphipods, and copepods were associated with the site Bitter Creek. Interestingly, caddisflies were associated with Bitter Creek, which has less flow than any other site we sampled (Pers. Observation). On the other hand, Georgian and Thorp (1992) suggest that caddisflies typically prefer fast flowing streams, allowing them to obtain more food sources, which contradicts my findings. Rejmankov et al. (1991) suggested that mosquito larvae prefer more stagnant waters, such as Bitter Creek, to obtain more food sources. This was also suggested with our CCA. Noel's amphipod is noted to be tolerant to salinity at 40 ppt before mortality, therefore, this species was negatively correlated with the salinity gradient (Siedal et al. 2010). However, we noticed that they were associated with vents and sandy substrates as well as the presence of spike rush and 3-sqaure bulrush. Springsnails were negatively correlated with all of our environmental gradients, with the exception of temperature. This correlation could be due to breeding season, since springsnails breed between March and October (Lang 2002, Taylor 1987).

This model was exploratory, since the data were already being collected with other goals in mind. In other words, this was a post-hoc analysis. In addition, at the beginning of this monitoring program in 2014, not all individuals had confidence that these 19 aquatic fauna were being identified correctly (e.g. dragonfly larvae vs. damselfly larvae). Therefore, this could have skewed the model from one direction to another. Furthermore, community ecology analyses are concerned with explaining the distribution, abundance and interaction of species and environmental variables (Palmer 1993). However, community theory focuses on the assumption that they are isolated systems (Liebold et al. 2004). With this monitoring protocol and analysis, another concern would not be taking into account dispersing species (e.g. Diptera, Anisoptera, Zygoptera) as they do not remain isolated in a system. The benthic grab trap was 0.003 m^2, therefore detecting invertebrates to analyze at a community level is not feasible as the focal species of the monitoring plan are low-mobile macroinvertebrates where others can easily escape the trap. Another approach a benthic invertebrate community analysis would be to enhance the trapping designs such as using more active methods such as epibenthic sled, Ponar grab, or pump sampler (Brown et al. 1987, Merritt et al. 2008). These more active approaches could be useful for obtaining larger surface areas and capturing the community in a more quantitative way.

This analysis provided management with some important management from the community level approach. Our environmental gradients are short, largely because of our sampling design. If management decides to investigate community level interactions further, assessing habitats where species do not occur and habitat conditions are unfavorable, we could detect more trends in our analysis. Furthermore, investigating the community of invertebrates can tell us what interactions are needed to enhance habitat for the 3 federally listed species. However, we were able to detect preferable habitat for Noel's amphipod in our model.

My results suggested that Noel's amphipod was most abundant in Sago Springs and Snail Unit. The abundance estimates could be influenced by the width of the stream channel and an increased likelihood to capture an amphipod with the benthic grab trap. However, Bitter Creek had a median of 1 individual throughout the monitoring throughout 2014-2017. With numerous volunteers, learning curves on identification, and the small size of Noel's amphipod, it is a possibility that this species does not exist in Bitter Creek and only *Hylella azteca* occurs in Bitter Creek. Furthermore, abundance by year was rather consistent, except for 2014, the first-year monitoring took place. 2014 had the highest median of all years, which could be caused by misidentification. Spring and summer had the highest abundances of Noel's amphipods, which aligns with Holsinger (1976) observations' that breeding takes place throughout February and October.

The best fit model for Noel's amphipod I utilized suggested that lower water temperatures, dissolved oxygen concentrations, and depth were significant factors, while specific conductance (mS) was not significant, and the model was more robust by removing pH. This outcome could suggest that Noel's amphipod persist in conditions where potential predators cannot exist. Furthermore, with significant low estimates could support Lenat (1988) and Merovich and Todd (2010) observations that macroinvertebrates are sensitive to change in water quality parameters. The predicted incidents plots align with the estimates suggesting more incidences of this species with lower abiotic factors. Lastly, the CART analysis that I utilized for data exploration suggested that this species is mostly influenced by water temperature, specific conductance and dissolved oxygen at particular ranges.

My results suggested springsnail abundance with boxplots, Sago Springs harbored the most springsnails compared to Bitter Creek, and Snail Unit with this highest median and fewer outliers than Bitter Creek. Bitter Creek had the most outliers than the other two systems. Bitter Creek is the longest system that was monitored with numerous bends, erratic flow, and large differences in depths at sampling sites caused by common reed and Russian thistle. This system had 14 more outliers than the other 2 systems, with the highest outlier being 536 springsnails at one site. I believe this is caused by patches of springsnails throughout Bitter Creek because of the threats Lang (2002) suggested. Springsnails were consistent throughout years with the median abundance. However, in the years 2015, and 2017 abundances appear to be lower. This could be an affect caused by lifespan and breeding as suggested by Taylor (1987) and Lang (2002) with the addition of stochasticity, however, I do not believe this suggests a declining population. Fall, Spring, and Summer have higher abundances than Winter which also supports the breeding observations of Taylor (1987) and Lang (2002).

The best fit model for springsnails I utilized suggested that specific conductance, water temperature, dissolved oxygen, depth, and pH were all important. This supports Lenat (1988) and Merovich and Todd (2010) observations' that macroinvertebrates are sensitive to change in water quality parameters. Furthermore, this supports the predicted incidences plots of each water quality parameter as I expect more occurrences with lower specific conductance, water temperature, dissolved oxygen and depth and higher occurrences with a higher pH. Interestingly, the model suggested that occurrences of springsnails in Bitter Creek were heavily influenced by water quality parameters. Bitter Creek experienced a fire in 2000, in which Lang (2002) reported a drastic decline in the invertebrate community. Subsequently, chemical control of common reed in 2014 could have altered the chemistry in Bitter Creek, thus, giving us the responses predicted incidences plots provided us at that site. Lastly, the CART analysis utilized for data exploration suggested specific ranges of lower water temperature, deeper water, and a neutral pH accounts for approximately half of springsnail occurrences. However, this analysis conflicts the selected model by suggesting nearly half of the occurrences of springsnails prefer deeper water. Lang (2002) observed Koster's springsnail preferring deeper water. Because of the inability to differentiate these 2 species in the field, the CART analysis could be biasing to a potentially larger population of Koster's springsnail and a smaller population of Roswell springsnail.

Many studies have tried to gain an understanding of preferred microhabitat of springsnails, especially *Pyrgulopsis* spp. (Malcom et al. 2005, Martinez and Thome 2006). Very few studies have been conducted on *Gammarus* spp. Most of these studies are uninformative, and rarely contribute knowledge to much needed facets to springsnail and *Gammarus* spp. such asdiet, habitat, and reproduction, although observations have been made (Taylor 1987, Pennak 1989, Lang 2002). Most studies include descriptions of species as well as genetics work (e.g. Morningstar et al. 2013, Liu et al. 2003, et al.)

Water chemistry parameters collected from the YSI Pro Series were consistent and dependent, therefore these data were heavily utilized. The YSI Pro Series only has the ability to detect 6-8 water chemistry parameters depending on the probes being used. Collecting additional water quality parameters such as minerals (nitrate, nitrite, potassium, and calcium carbonate), flow and total dissolved solids could add robustness to the models.

Concurring with current monitoring efforts, stratifying habitat types (e.g. Chara, Widgeon grass, Algal mats) and sampling abundances will allow us to obtain a finer resolution of preferred microhabitat. In addition to what is already collected, incorporating flow, total dissolved solids, nitrate, nitrite, potassium and calcium carbonate will give us a better understanding of water chemistry parameters required by these three endangered invertebrates. Springsnails, specifically, require calcium carbonate for shell formation (Eyster and Morse 1984). Nitrate, nitrite, potassium in systems is known to be a pollutant, causing algal blooms that can decrease dissolved oxygen levels (Stevenson et al. 2005). Total dissolved solids will allow us to look further into how much of an effect they have on springsnails and Noel's amphipods. Finding a definitive way to easily differentiate the 2 snails in the field with a simple hand lens or field dissecting scope could allow us to determine population estimates for each species, rather than lumping them into one.

Overall, the monitoring efforts did not have a specific question, the intent of the data being collected was to find a quantitative way to obtain accurate population estimates in known locations of Noel's amphipod, and springsnails. Fortunately, current monitoring efforts collected plenty of additional data that had the intent of being utilized in post-hoc analyses. Fortunately, this data was able to be analyzed in its entirety and produce a model to inform management at Bitter Lake NWR species-environmental interactions occurring at monitoring sites. Managers now have data in concert with population trends from this monitoring to think about habitats and water quality parameters that could help with translocation of species to new systems, such as springsnails into the restored Rio Hondo. Although the estimates were not high with the GLMM analyses, management can acknowledge the sensitivity of these three federally listed species and utilize monthly water quality estimates to make informed management decisions if abrupt change were to occur. Furthermore, if water contamination from oil and gas, fire, or other stochastic events occur Bitter Lake NWR is now informed on the water quality parameters these species are occupying and make quick management decisions.

Bitter Lake NWR harbors a unique environment for aquatic invertebrates with the refuge being on the cusp of the South Plains and the Chihuahuan Desert. This study should help future academics and managers address unique questions about benthic macroinvertebrates and their communities, globally. The results of this study should allow managers at Bitter Lake NWR to hon in more specific questions to tease out more habitat correlations, especially with the three federally listed invertebrates.

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Table 1.1: Generalized linear mixed effects models for Noel's amphipod at Bitter Lake National Wildlife Refuge concurring with quarterly monitoring efforts between years 2014-2017. The first model is a null model only using random effects. Water quality parameters were added and AIC was reported to obtain the best-fit model.

Model	Variables	AIC
5	Year + Site + Water Temperature + Dissolved Oxygen + Depth + Specific Conductance	788.3
4	Year + Site + Water Temperature + Dissolved Oxygen + Depth	788.8
6	Year + Site + Water Temperature + Dissolved Oxygen + Depth + Specific Conductance + pH	789.1
3	Year + Site + Water Temperature + Dissolved Oxygen	823.6
2	Year + Site + Water Temperature	869.5
1	Year + Site + 1 (=Null Model)	870.0

Table 1.2: Best-fit generalized linear mixed effects model for Noel's amphipod concurring with monitoring at Bitter Lake National Wildlife Refuge between the years 2014-2017. Variance of random effects are reported and estimate values for fixed effects are reported corresponding with their z value. * = p-value < 0.10, ** = p-value < 0.05, *** = p-value < 0.01

Variance of random effect intercepts	Estimates	z value
Year: 0.11	Water temperature: -0.10	Water temperature: -3.54***
Site: 2.32	Dissolved oxygen: -0.11	Dissolved oxygen: -7.482***
	Depth: -0.05	Depth: -5.66***
	Specific conductance: -0.04	Specific conductance: -1.62

Table 1.3: Generalized linear mixed effects models for springsnails at Bitter Lake National Wildlife Refuge concurring with quarterly monitoring efforts between years 2014-2017. The first model is a null model only using random effects. Water quality parameters were added and AIC was reported to obtain the best-fit model.

Model	Variables	AIC
6	Year + Site + Specific conductance + Water Temperature + Dissolved Oxygen + Depth + pH	13215.5
5	Year + Site + Specific conductance + Water Temperature + Dissolved Oxygen + Depth	13295.5
4	Year + Site + Specific conductance + Water Temperature + Dissolved Oxygen	13795.6
3	Year + Site + Specific conductance + water temperature	13996.0
2	Year + Site + Specific conductance	15805.0
1	Year + Site + 1 (=Null Model)	16201.1

Table 1.4: Best-fit generalized linear mixed effects model for springsnails concurring with monitoring at Bitter Lake National Wildlife Refuge between the years 2014-2017. Variance of random effects are reported and estimate values for fixed effects are reported corresponding with their z value. * = p-value < 0.10, ** = p-value < 0.05, *** = p-value < 0.01

Variance of random effect intercepts	Estimates	z-value
Year: 0.13 Site:1.06	Specific conductance: -0.06 Water Temperature: -0.05 Dissolved Oxygen: -0.02 Depth: -0.04 pH: -0.44	Specific conductance: -13.52*** Water temperature: 20.36*** Dissolved oxygen: -4.29*** Depth: -20.50*** pH: -8.40***

Table 1.5: List of environmental variables utilized in the Canonical Correspondence Analysis (CCA). Environmental variables with a Y indicate it was selected for the model and N indicates it was not. P-values for each environmental variable are reported where "-" represents a p-value not estimated due to collinearity.

Environmental variable	Included in model? (y/n)	P-value
2014	Y	0.001
2015	Y	0.001
2016	N	-
2017	Y	0.001
Bitter Creek	Y	0.001
Snail Unit	Y	0.001
Sago Springs	N	-
Winter	Y	0.001
Summer	Y	0.001
Fall	N	-
Spring	Y	0.001
Bank	N	0.0659
Mid Channel	N	0.9021
Mid Point	N	-
Soil	Y	0.026
Sand	Y	0.006
Rock	N	0.1409
Salt Grass	Y	0.001
Algae	Y	0.014
Phragmites	N	0.3347
Detritus	Y	0.001
Bulrush	Y	0.002
Kochia	N	0.8741
Thistle	N	0.8681
Chara	N	0.1828
None	N	0.9341
Spike Rush	Y	0.017
Widgeon Grass	N	0.0629
Sacaton	N	0.1598
Cattail	N	0.8382
Pecos Sunflower	Y	0.0529
Bacharis	N	0.6903
Scratchgrass	N	0.2717
Percent vegetation	Y	0.03

Table 1.5, con't: List of environmental variables utilized in the Canonical Correspondence Analysis (CCA). Environmental variables with a Y indicate it was selected for the model and N indicates it was not. P-values for each environmental variable are reported where "-" represents a p-value not estimated due to collinearity.

Environmental variable	Included in model? (y/n)	P-value
Depth (cm)	Y	0.001
Water temperature (ºC)	Y	0.001
Dissolved oxygen (%)	Y	0.1409
Dissolved oxygen (mg/l)	Y	0.002
Specific conductance (mS)	N	0.5205
Salinity (ppt)	Y	0.001
pH	Y	0.001

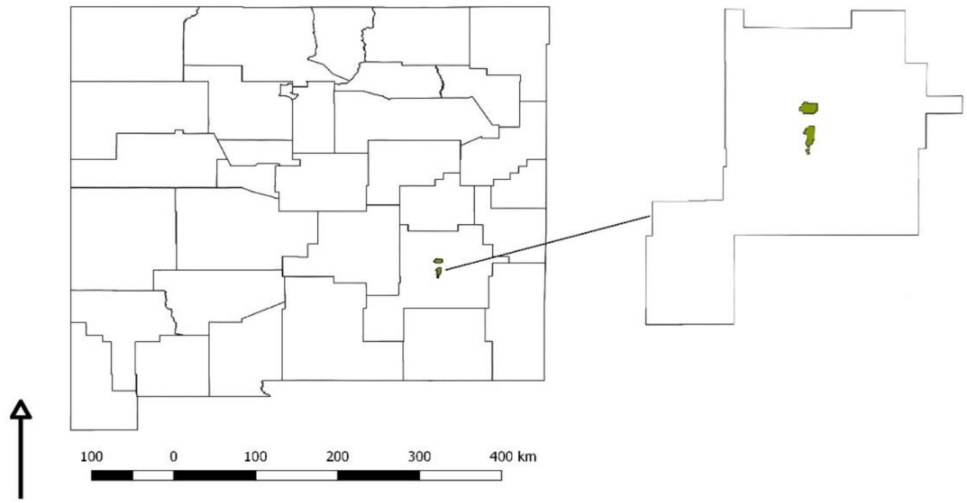

Figure 1.1: Location of Bitter Lake National Wildlife Refuge in relation to Chavez County and the state of New Mexico

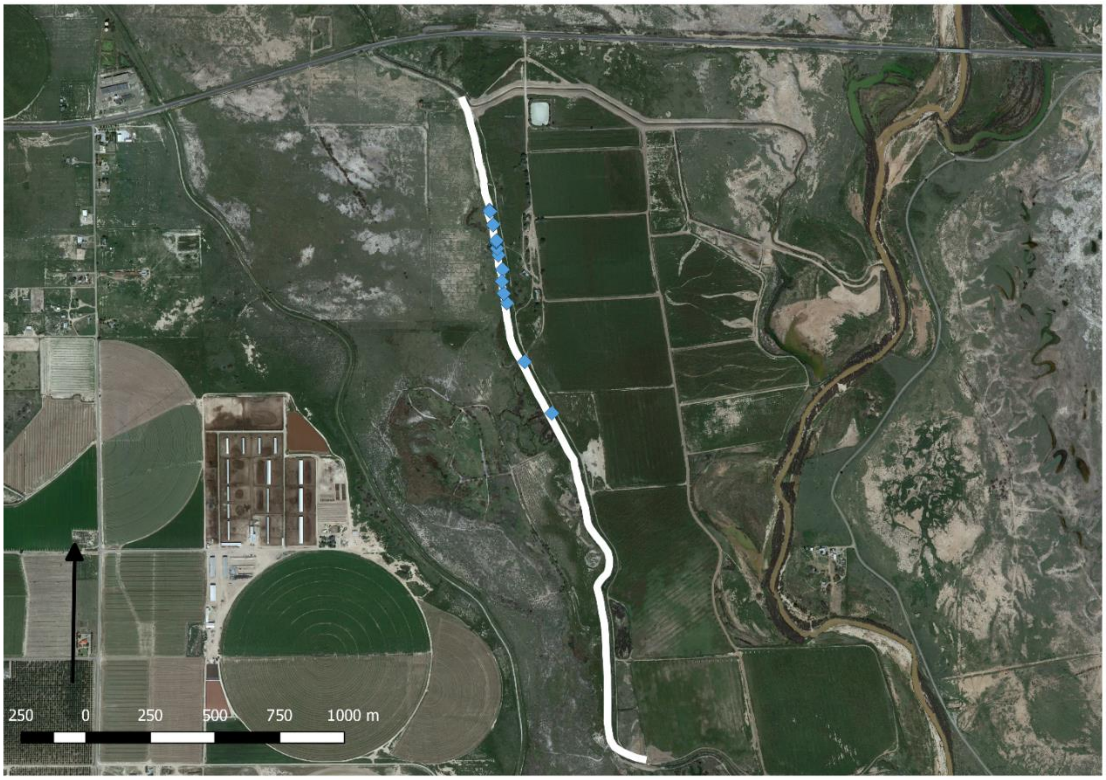

Figure 1.2: Stream channel of the restored Rio Hondo on Bitter Lake National Wildlife Refuge, Chaves County, New Mexico 2014-2017. 13 sampling locations for monitoring efforts are depicted by circles within the channel. The line represents the total length of the channel.

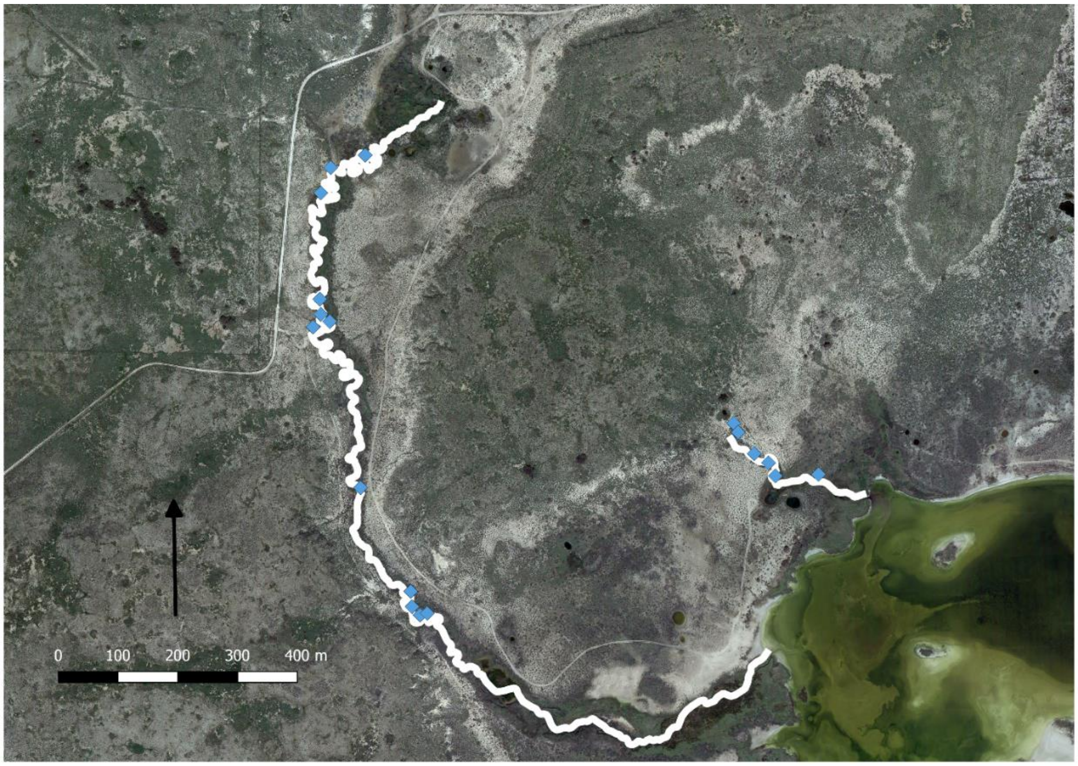

Figure 1.3: Stream channels of Bitter Creek (left) and Sago Springs (right) on Bitter Lake National Wildlife Refuge, Chaves County, New Mexico 2014-2017. Channels are depicted by lines, and monitoring sites for macroinvertebrates are depicted by circles. There are 12 sampling sites at Bitter Creek and 6 sampling sites at Sago Springs.

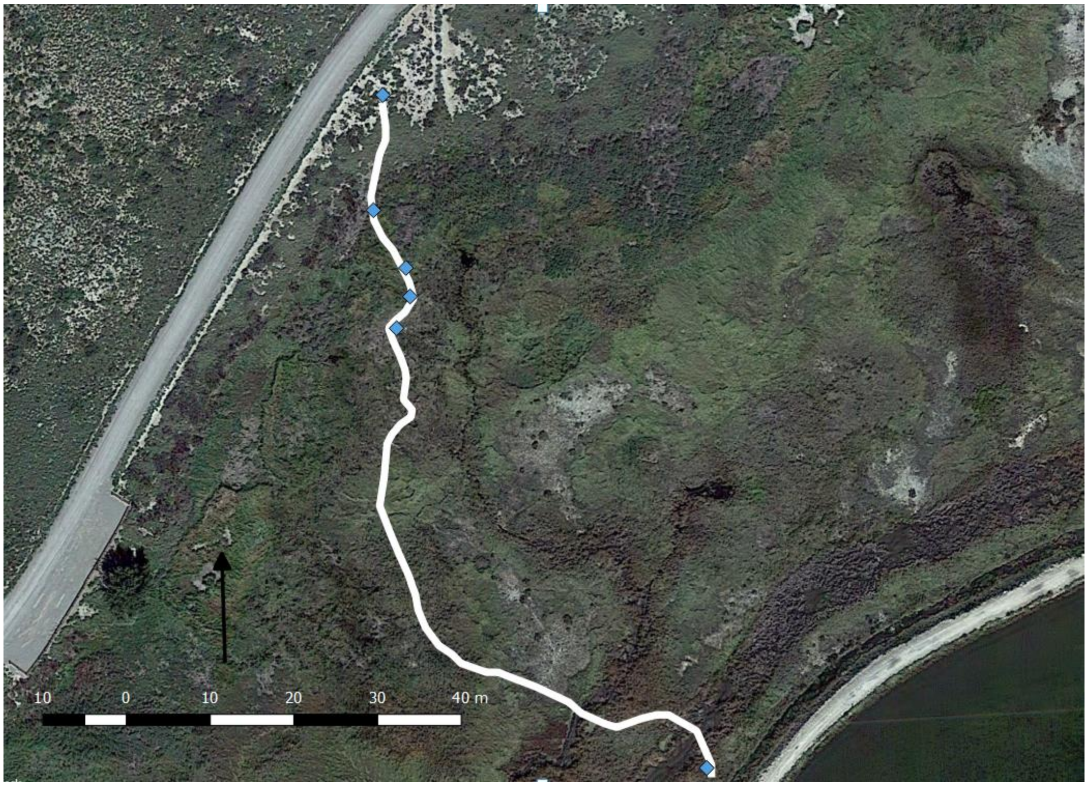

Figure 1.4: Stream channel of Snail Unit on Bitter Lake National Wildlife Refuge, Chaves County, New Mexico 2014-2017. Channels are depicted by lines, and monitoring sites for macroinvertebrates are depicted by circles. There are 6 sampling sites located in Snail Unit.

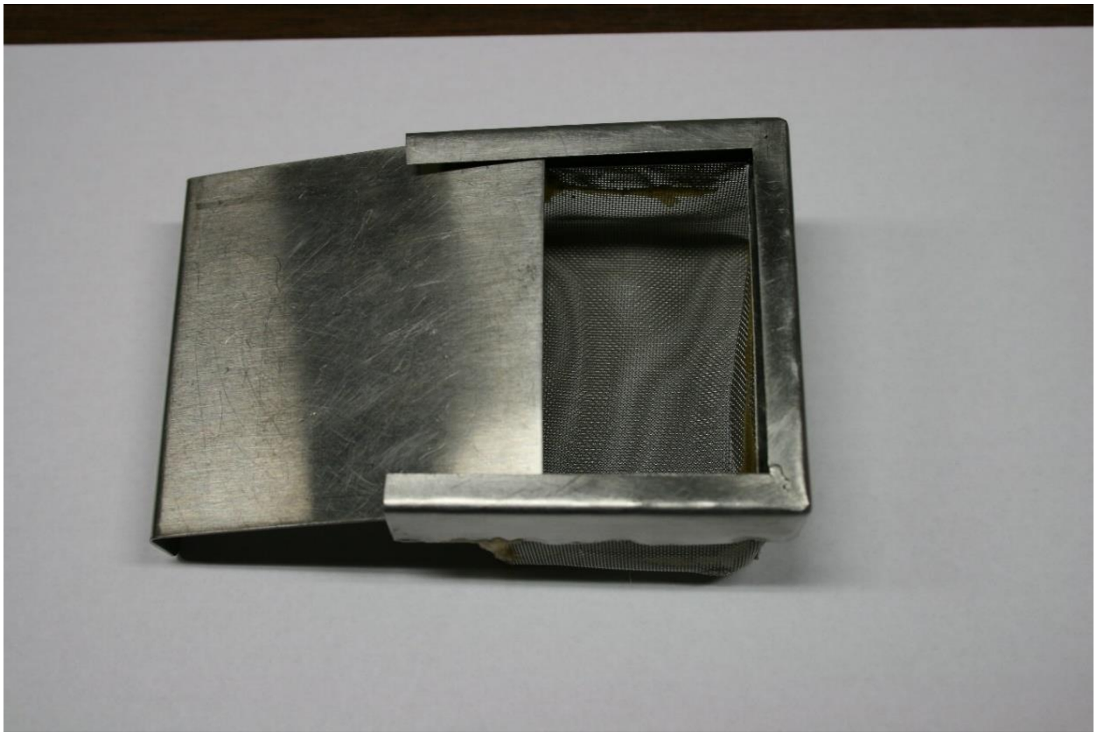

Figure 1.5: Benthic grab trap utilized to sample substrate at each monitoring site at Bitter Lake National Wildlife Refuge, Chaves County, New Mexico, 2014-2017. The grab trap depicted above is 0.003 m^2 with 300 µm mesh. This trap was utilized at each monitoring site to enumerate number of endangered invertebrates and presence/absence of non-listed invertebrates.

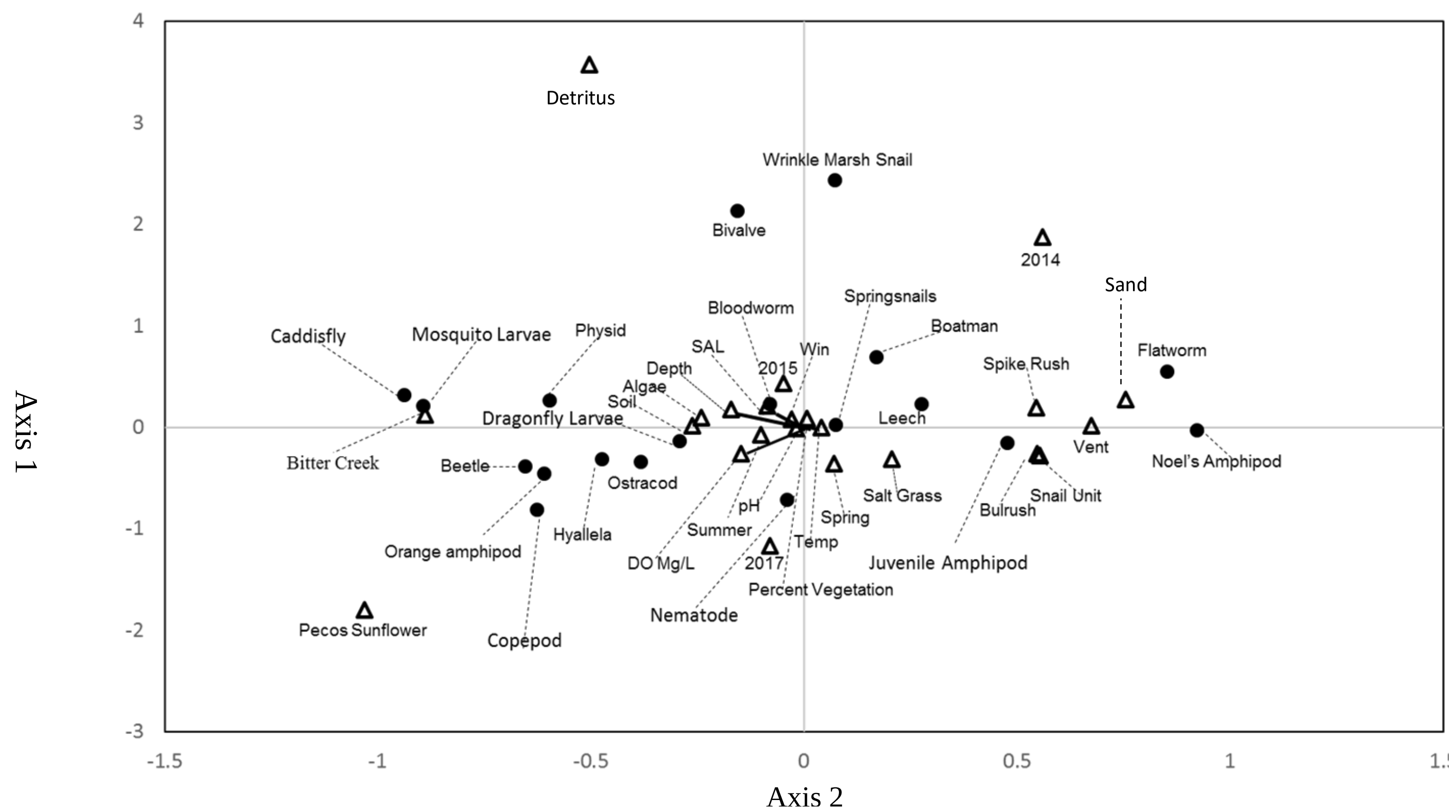

Figure 1.6: Canonical correspondence analysis depicting environmental variables with triangles and taxa with circles collected between 2014-2017 on Bitter Lake National Wildlife Refuge with invertebrate monitoring. Water quality parameters are depicted by solid lines on a gradient. Dashed lines depict the centroid and the variable name.

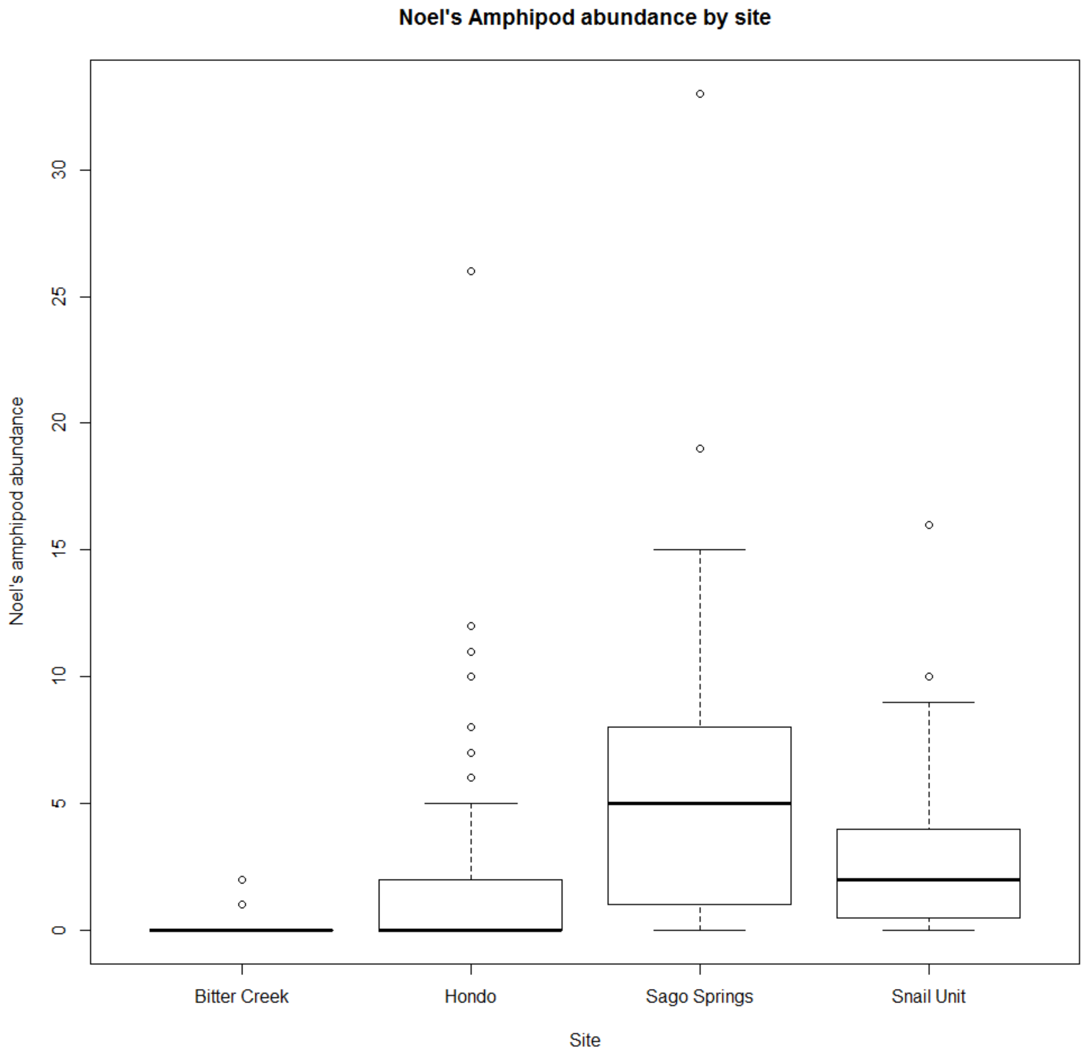

Figure 1.7: Box and whisker plot of Noel's abundances by site (=system) concurring with monitoring efforts on Bitter Lake National Wildlife Refuge from 2014-2017. Outer bars depict minimum and maximum, boxes depict interquartile range 1 and 3, and dark lines depict medians. Circles indicate outliers.

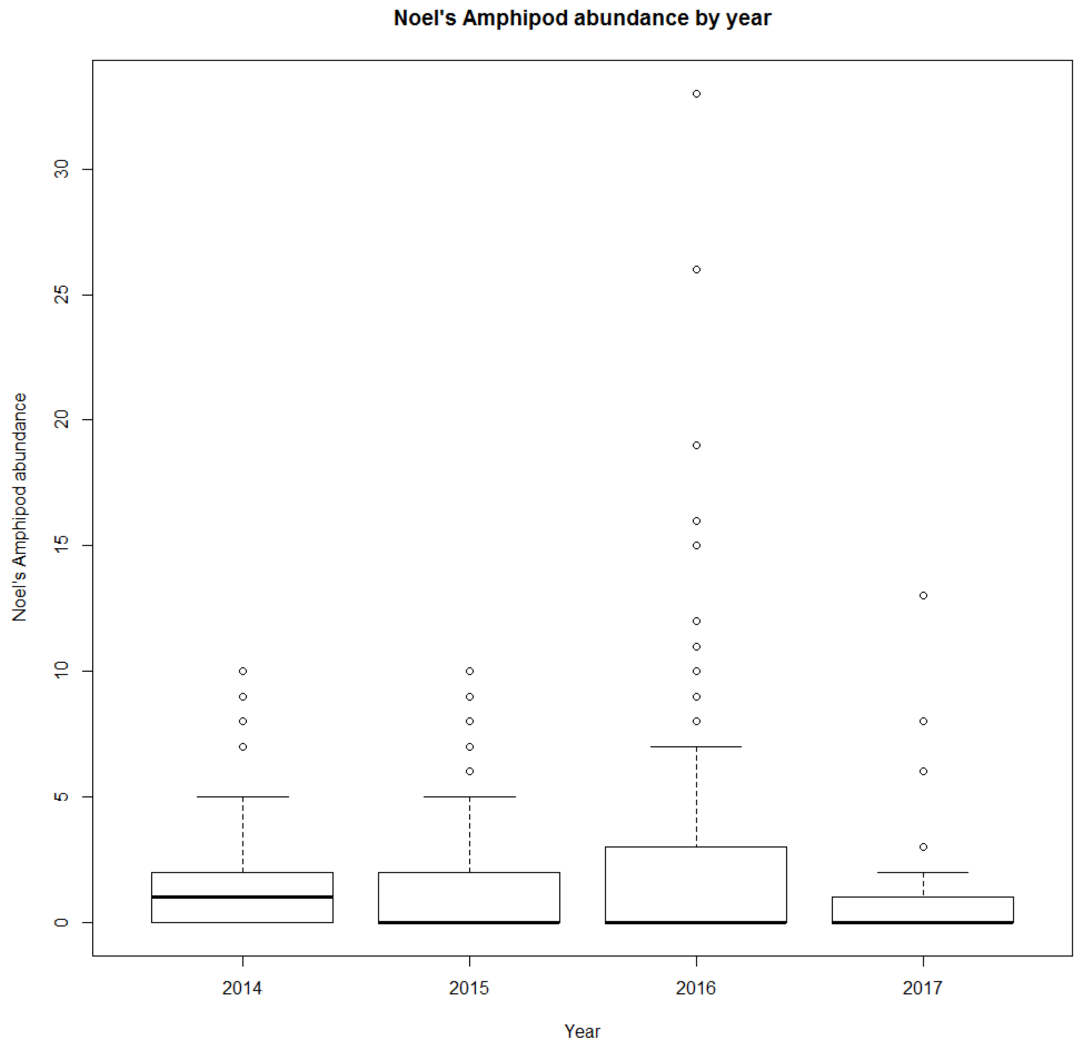

Figure 1.8: Box and whisker plot of Noel's abundances by year concurring with monitoring efforts on Bitter Lake National Wildlife Refuge from 2014-2017. Outer bars depict minimum and maximum, boxes depict interquartile range 1 and 3, and dark lines depict medians. Circles indicate outliers.

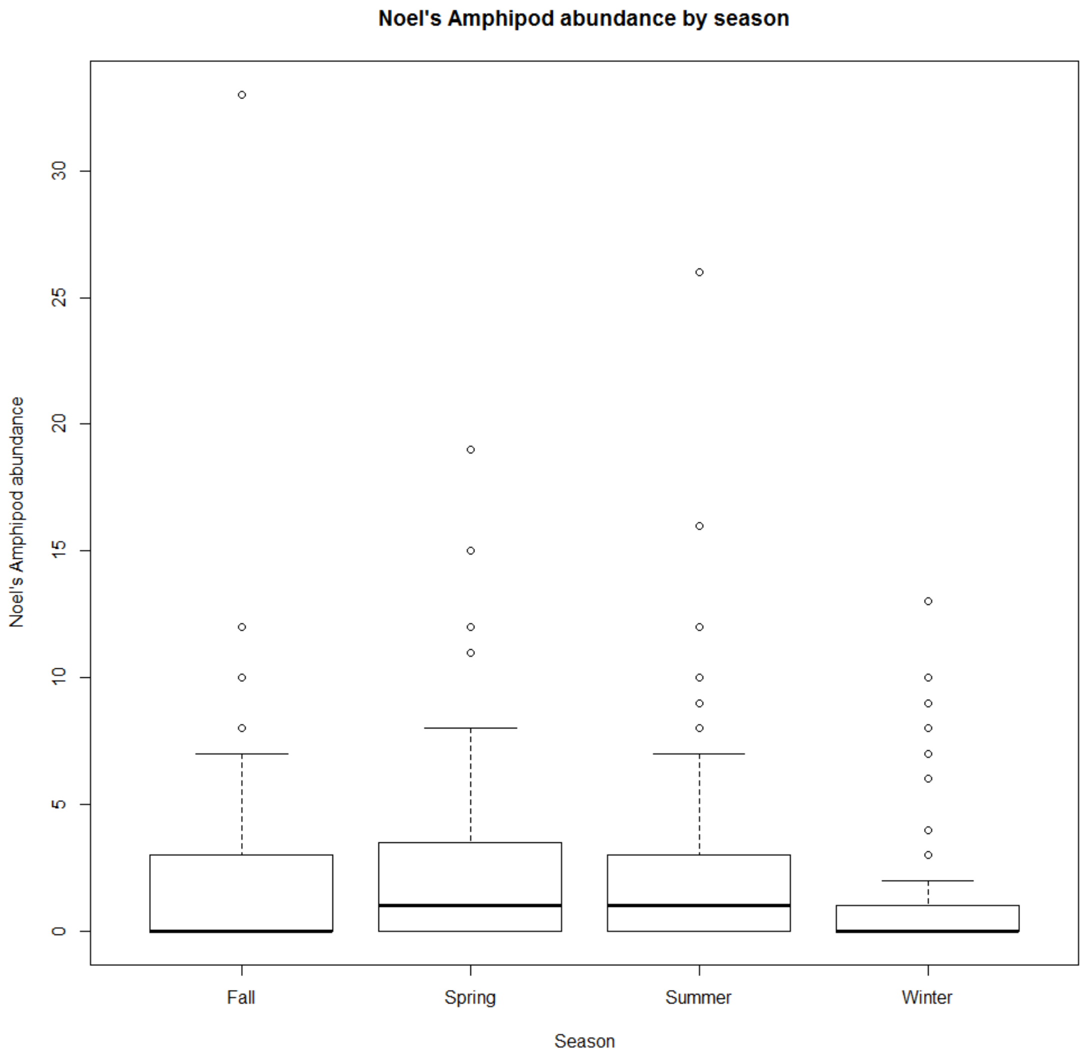

Figure 1.9: Box and whisker plot of Noel's abundances by seasons concurring with monitoring efforts on Bitter Lake National Wildlife Refuge from 2014-2017. Outer bars depict minimum and maximum, boxes depict interquartile range 1 and 3, and dark lines depict medians. Circles indicate outliers.

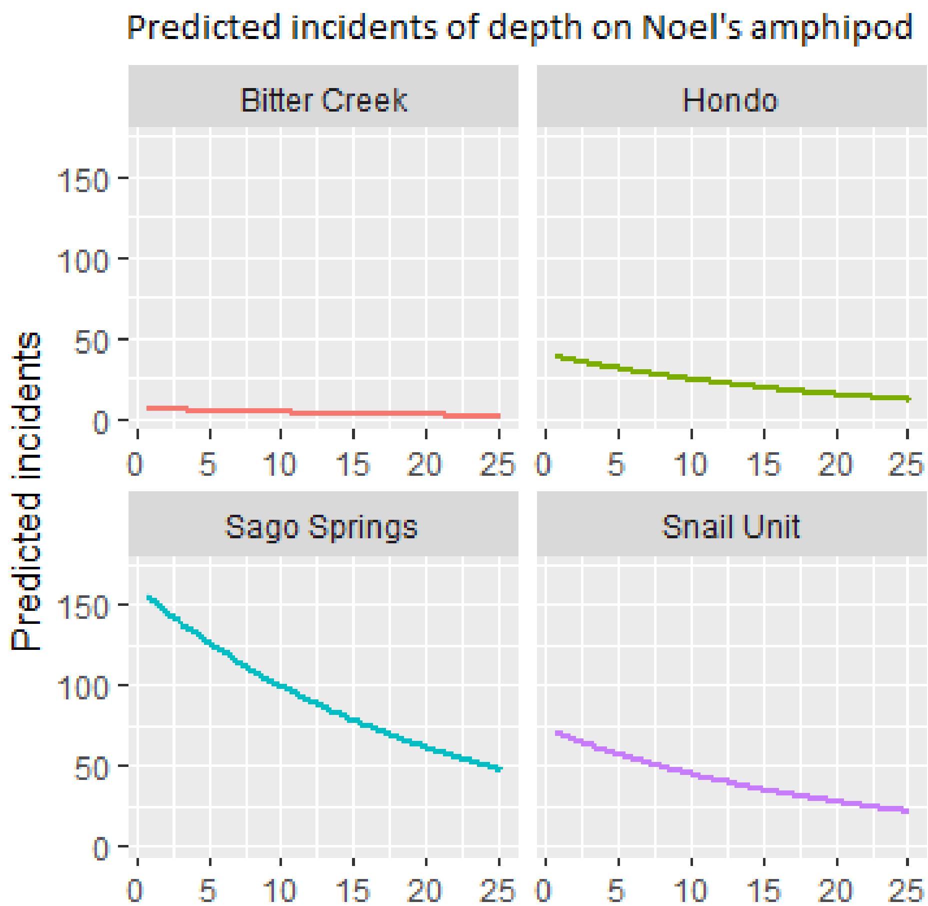

Figure 1.10: Predicted incidences of Noel's amphipod based on depth (cm) for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Depth is depicted in cm on the x-axis and number of predicted incidences on the y-axis.

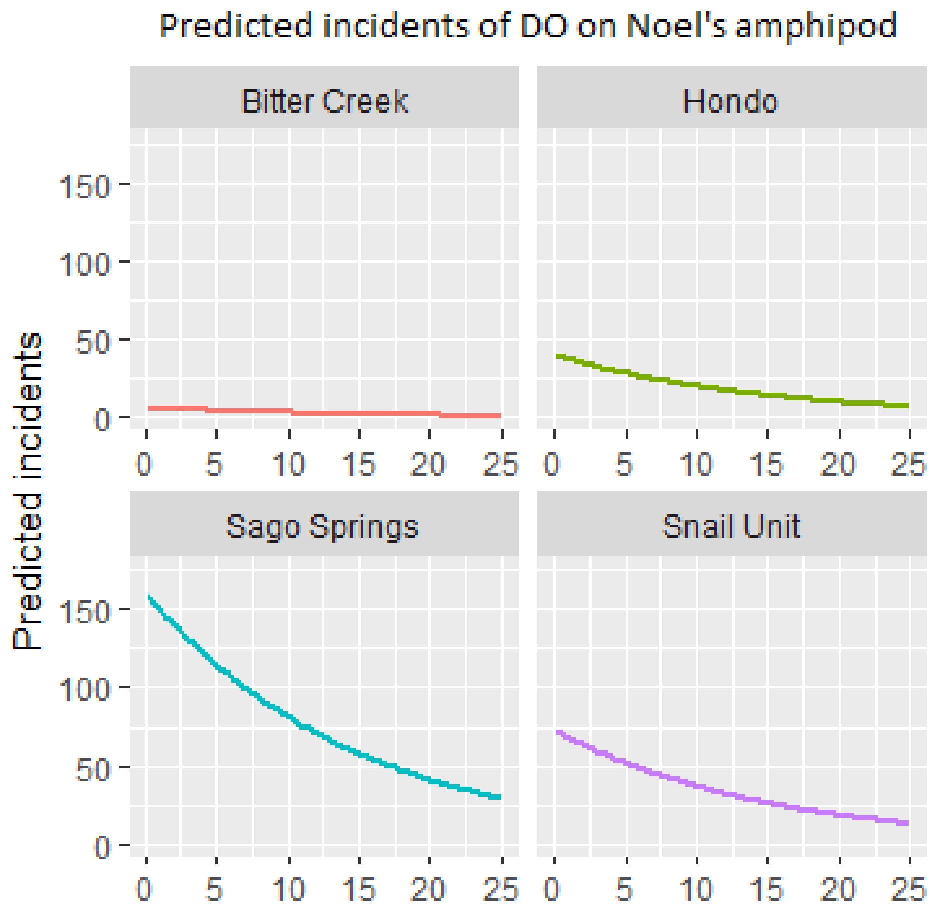

Figure 1.11: Predicted incidences of Noel's amphipod based on dissolved oxygen for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Dissolved oxygen (=DO; mg/l) is depicted in cm on the x-axis and number of predicted incidences on the y-axis.

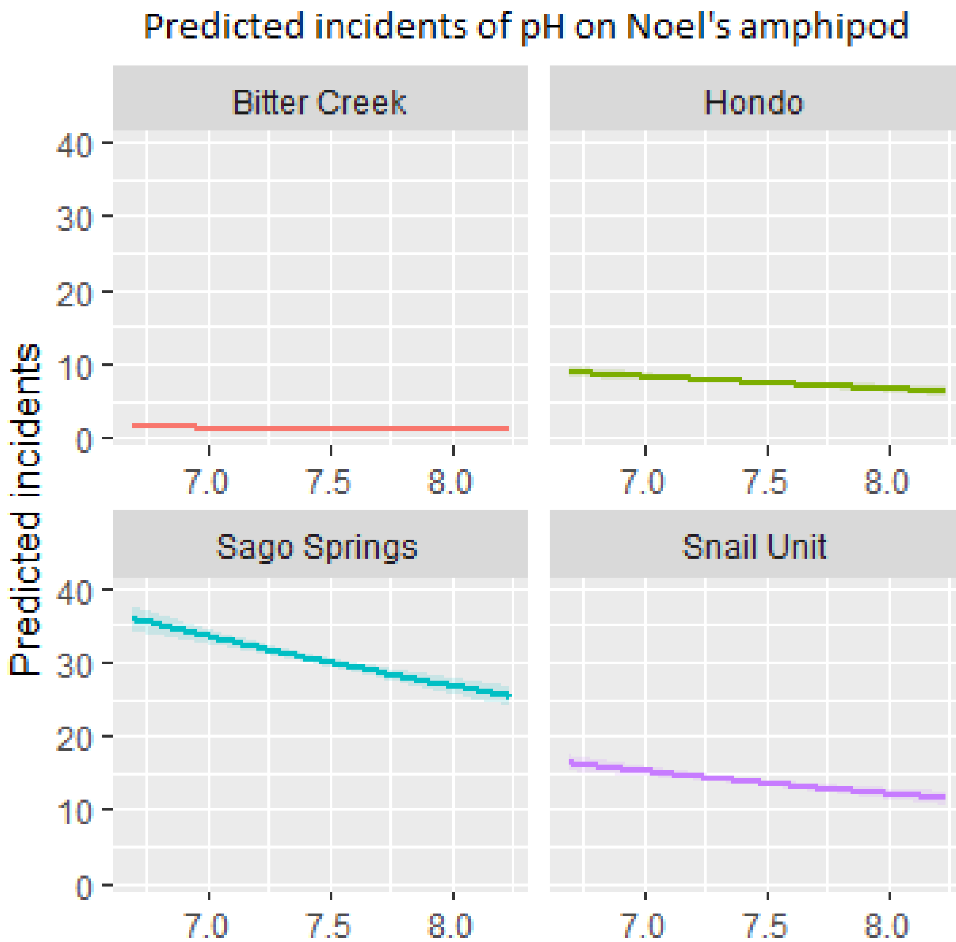

Figure 1.12: Predicted incidences of Noel's amphipod based on pH for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. pH is depicted on the x-axis and number of predicted incidences on the y-axis.

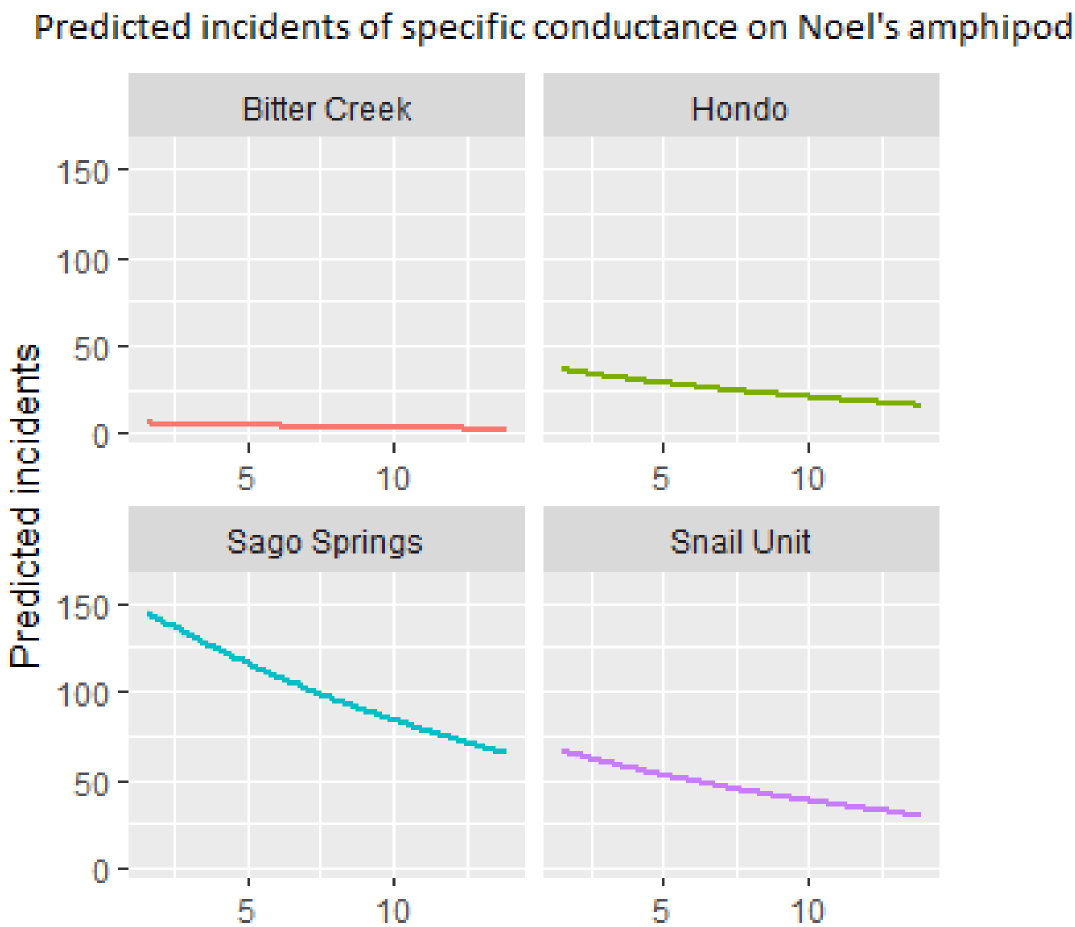

Figure 1.13: Predicted incidences of Noel's amphipod based on specific conductance (mS) for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Specific conductance (mS) is depicted on the x-axis and number of predicted incidences on the y-axis.

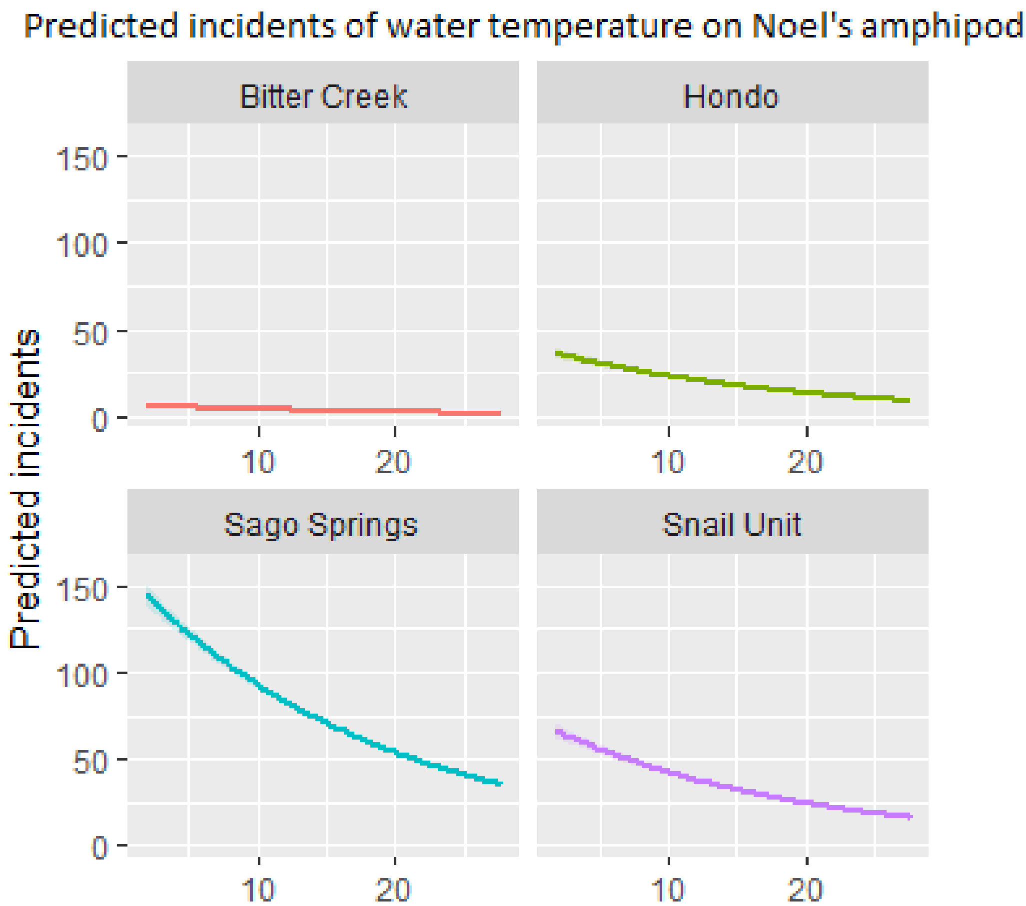

Figure 1.14: Predicted incidences of Noel's amphipod based on water temperature (ºC) for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Water temperature is depicted in cm on the x-axis and number of predicted incidences on the y-axis.

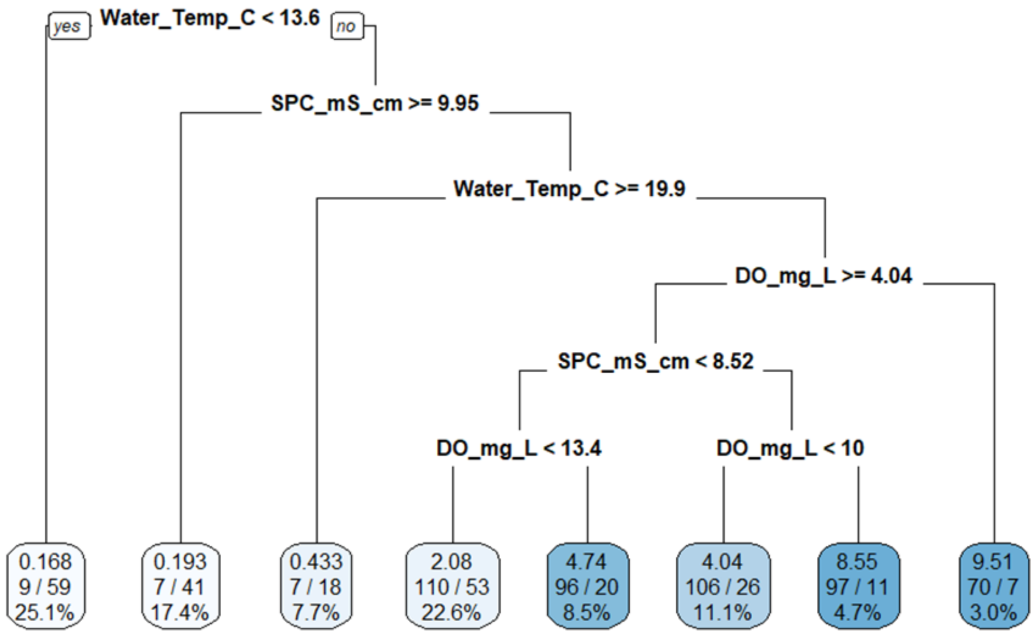

Figure 1.15: Classification and Regression Tree depicting how much influence water quality parameters are influencing abundances of Noel's amphipod on Bitter Lake National Wildlife Refuge between the years 2014-2017. Water_temp_C, SPC_mS_cm, and DO_mg_L stand for water temperature ºC, specific conductance (mS) and dissolved oxygen (mg/l). Each node at the bottom of the tree represents how much abundance can be attributed to the set of factors.

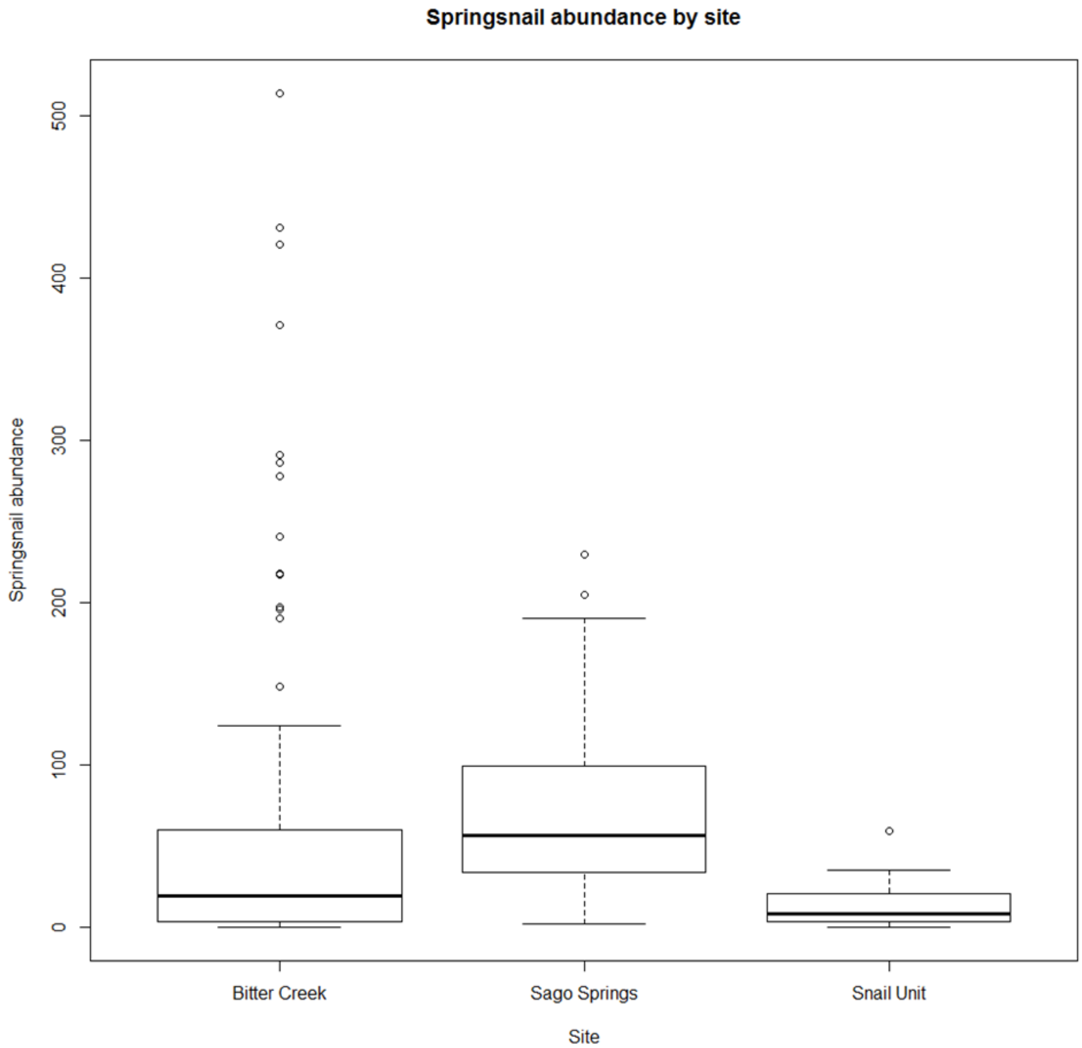

Figure 1.16: Box and whisker plot of springsnails by site (=system) concurring with monitoring efforts on Bitter Lake National Wildlife Refuge from 2014-2017. Outer bars depict minimum and maximum, boxes depict interquartile range 1 and 3, and dark lines depict medians. Circles indicate outliers.

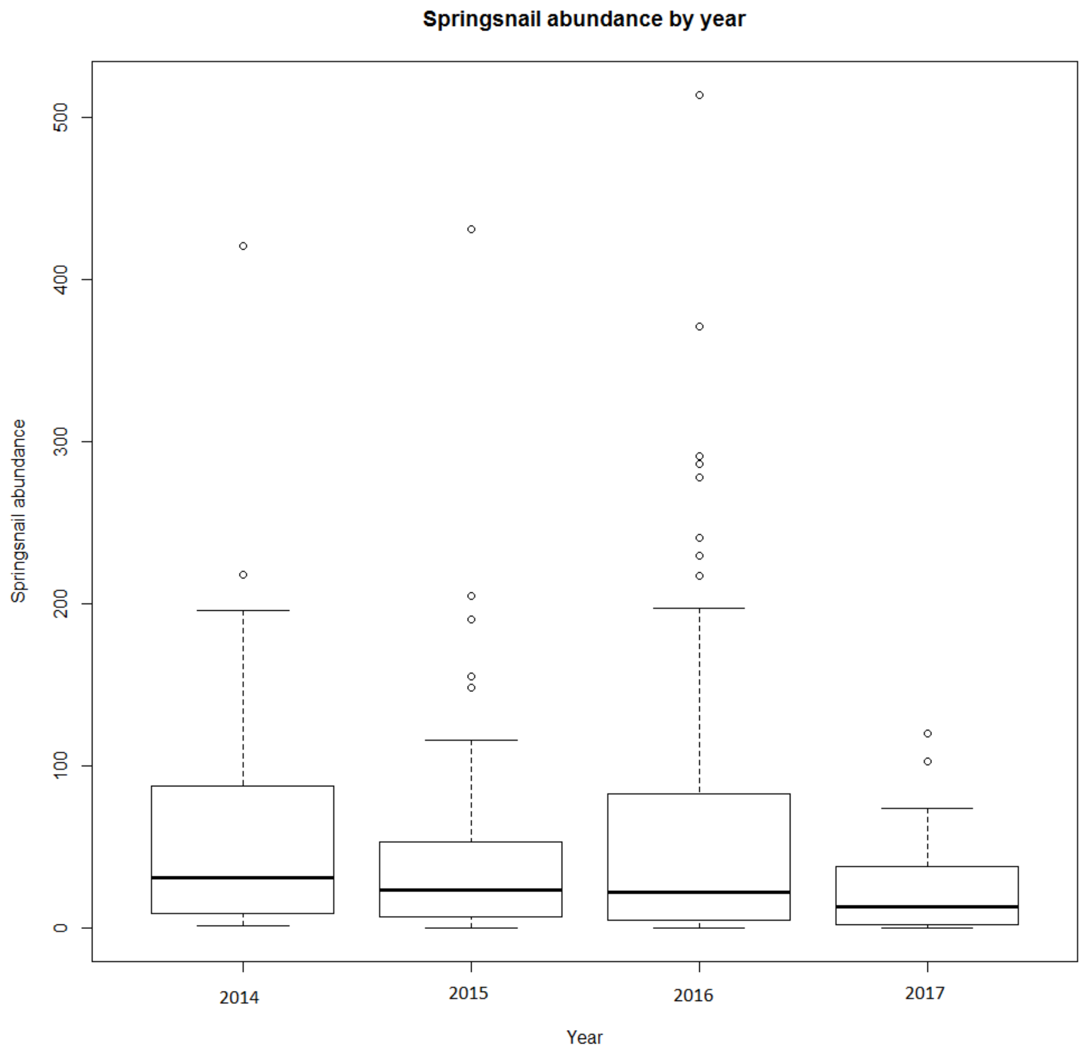

Figure 1.17: Box and whisker plot of springsnails by year concurring with monitoring efforts on Bitter Lake National Wildlife Refuge from 2014-2017. Outer bars depict minimum and maximum, boxes depict interquartile range 1 and 3, and dark lines depict medians. Circles indicate outliers.

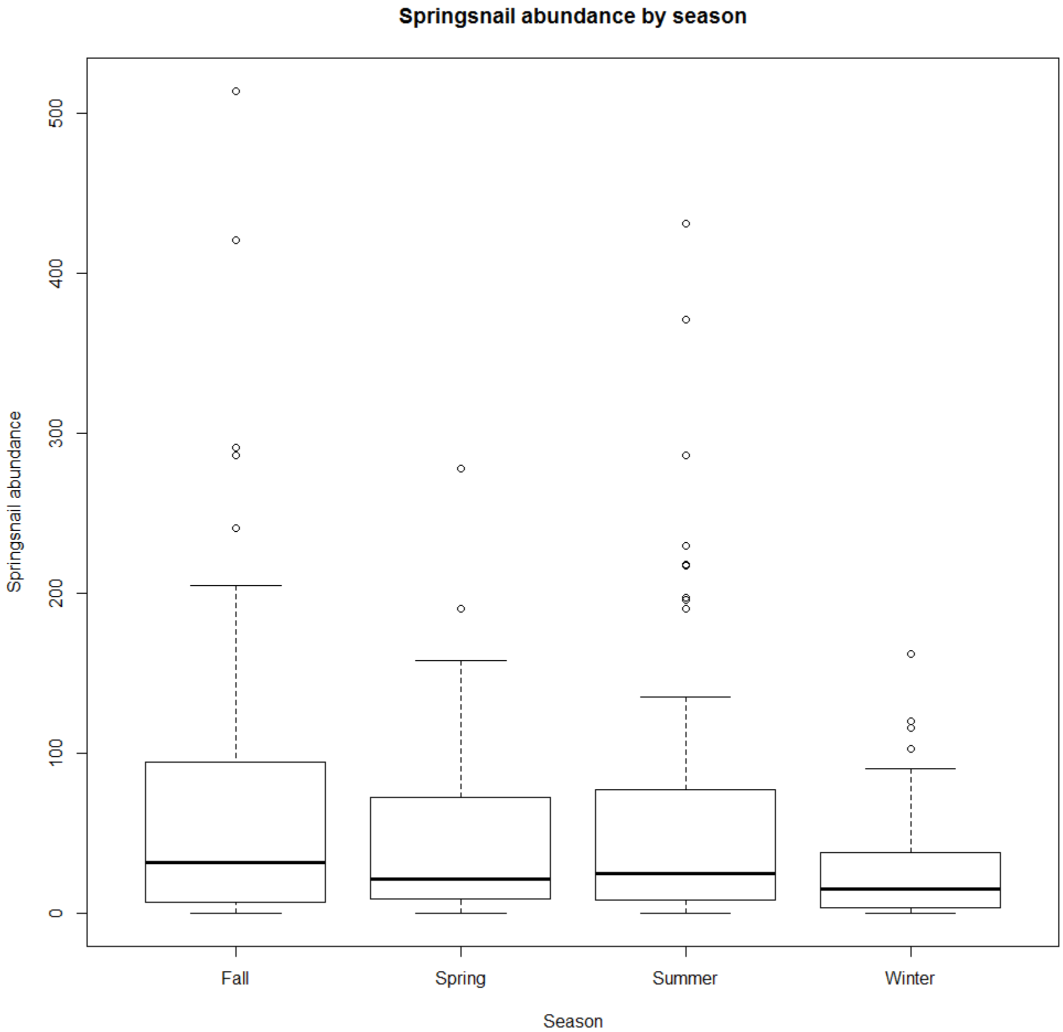

Figure 1.18: Box and whisker plot of springsnails by season concurring with monitoring efforts on Bitter Lake National Wildlife Refuge from 2014-2017. Outer bars depict minimum and maximum, boxes depict interquartile range 1 and 3, and dark lines depict medians. Circles indicate outliers.

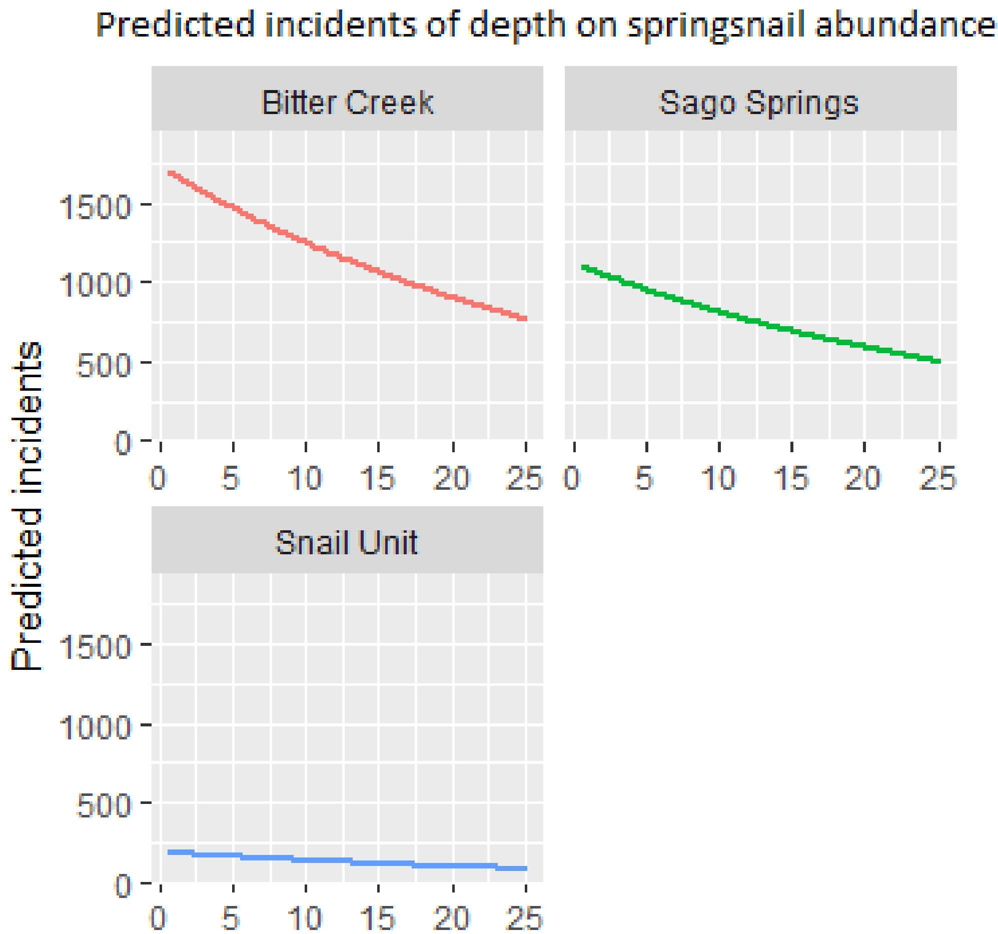

Figure 1.19: Predicted incidences of springsnails based on water depth (cm) for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Water depth is depicted in cm on the x-axis and number of predicted incidences on the y-axis.

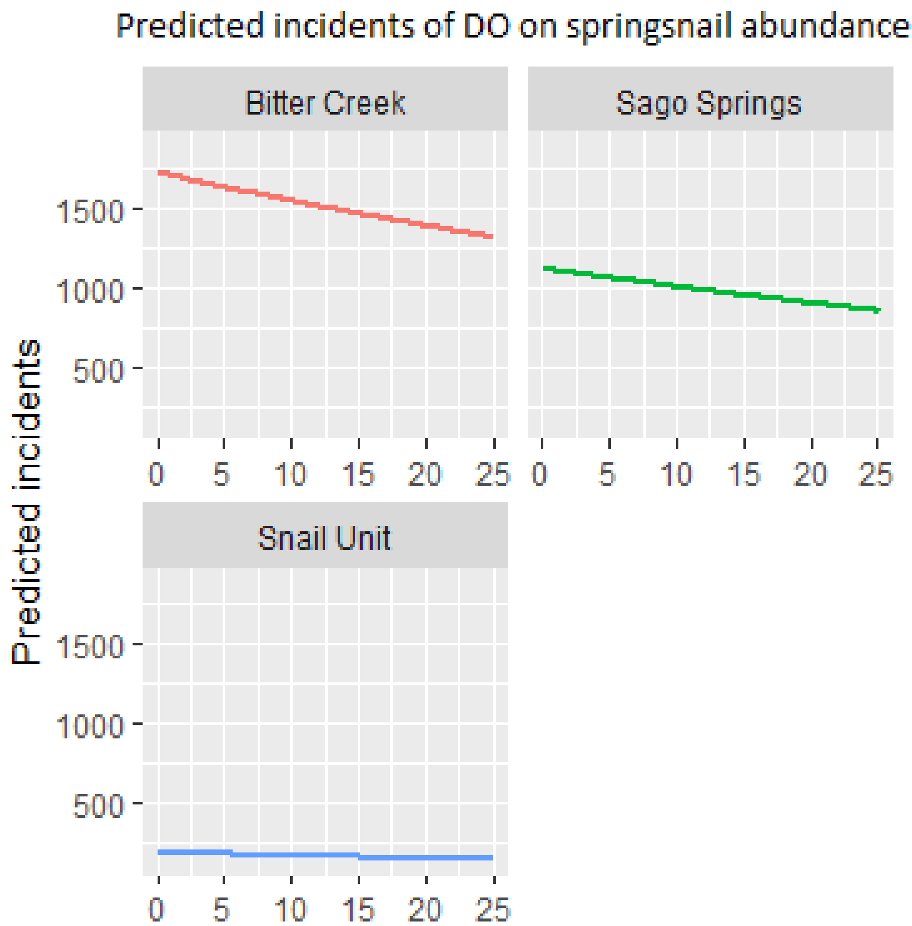

Figure 1.20: Predicted incidences of springsnails based on dissolved oxygen (mg/l) for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Dissolved oxygen is depicted in (mg/l) on the x-axis and number of predicted incidences on the y-axis.

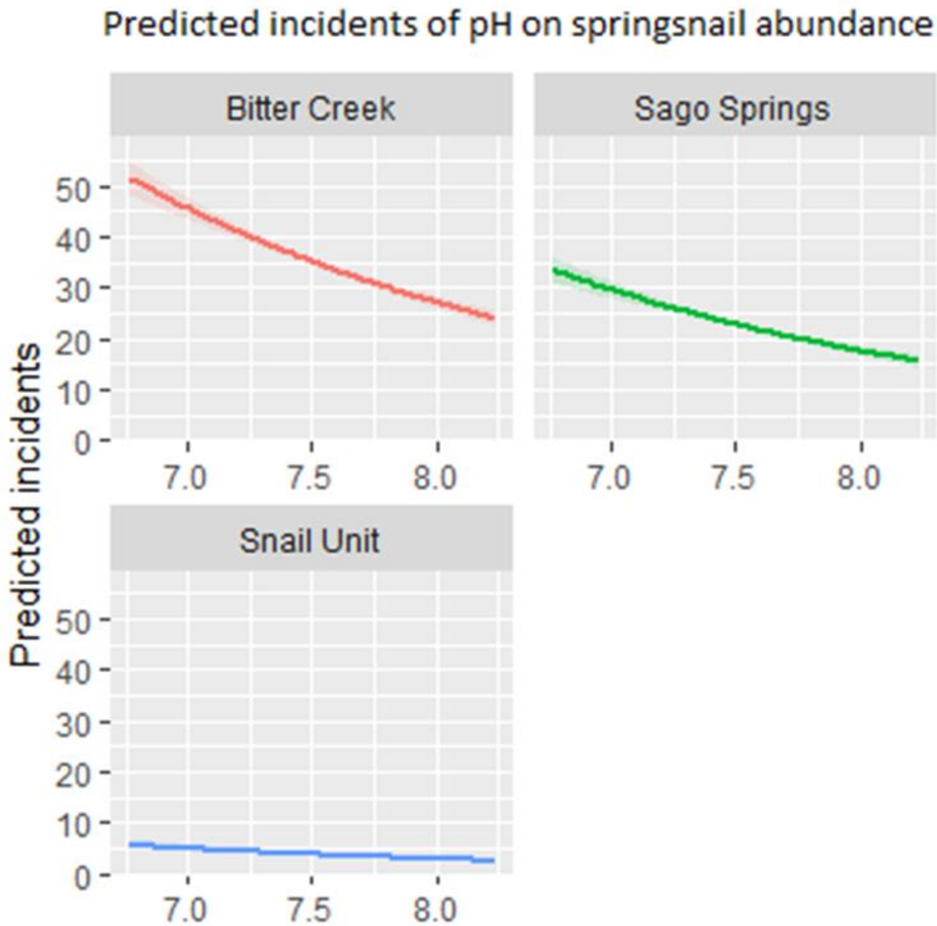

Figure 1.21: Predicted incidences of springsnails based on pH for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. pH is depicted on the x-axis and number of predicted incidences on the y-axis.

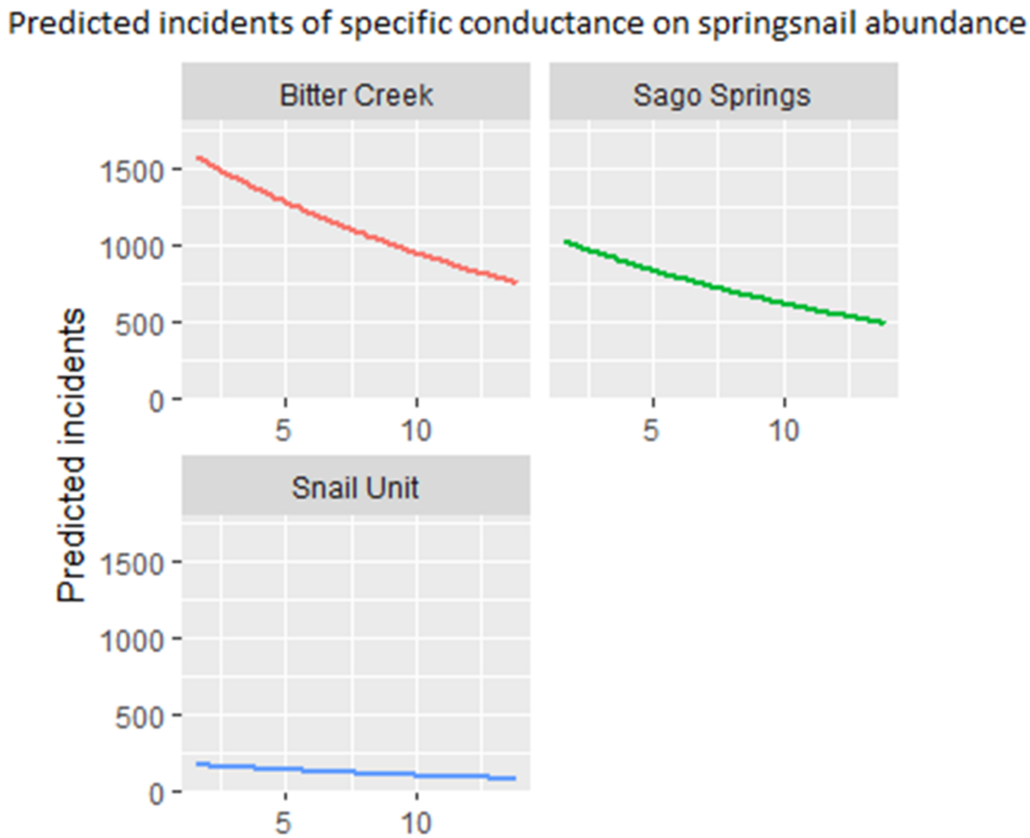

Figure 1.22: Predicted incidences of springsnails based on specific conductance (mS) for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Specific conductance is depicted in (mS) on the x-axis and number of predicted incidences on the y-axis.

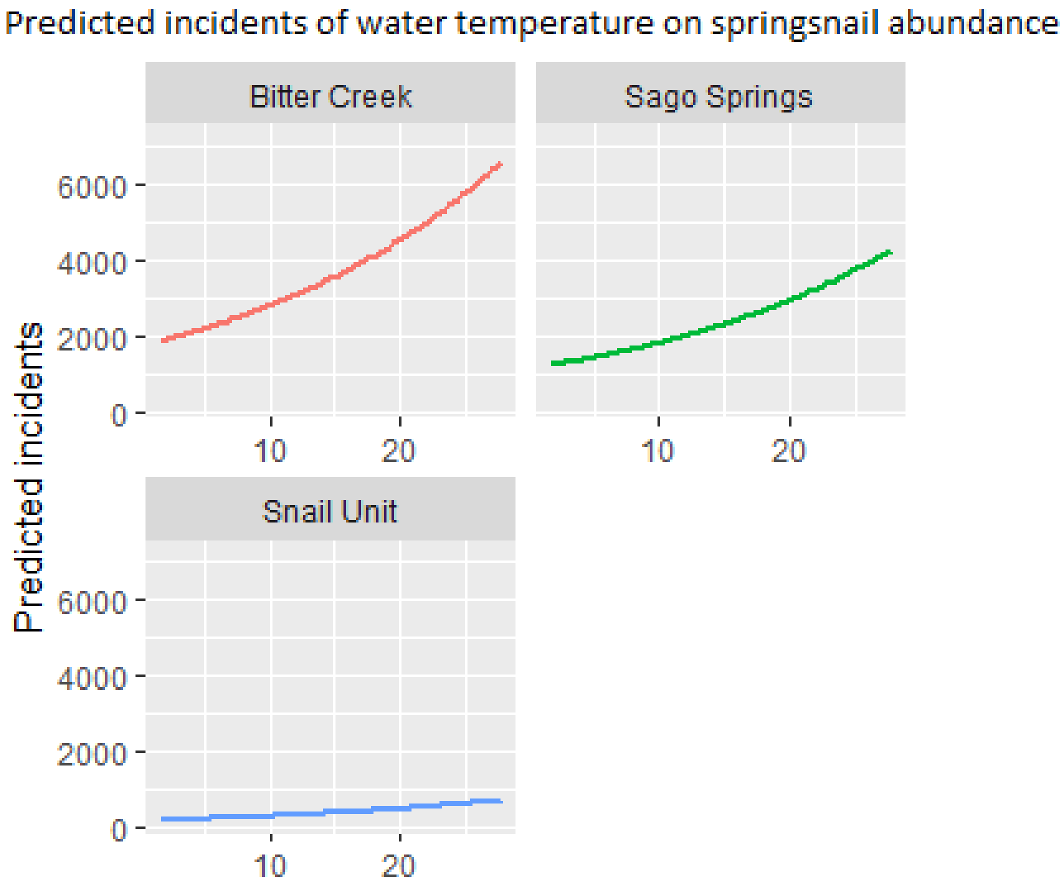

Figure 1.23: Predicted incidences of springsnails based on water temperature (ºC) for each system sampled concurring with monitoring efforts of aquatic invertebrates from 2014-2017. Water temperature (ºC) is depicted on the x-axis and number of predicted incidences on the y-axis.

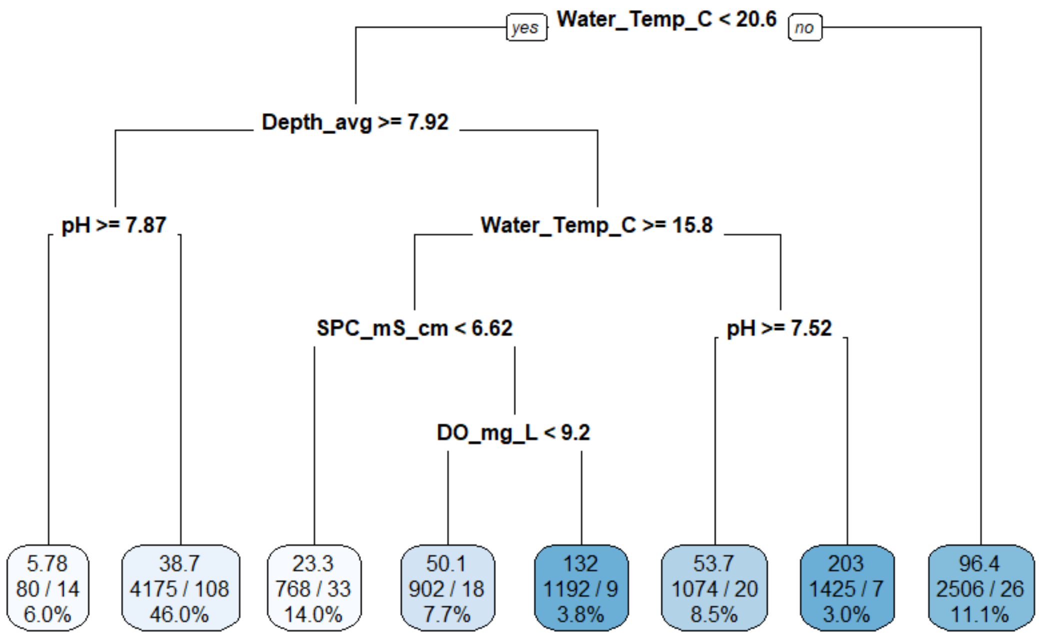

Figure 1.24: Classification and Regression Tree depicting how much influence water quality parameters are influencing abundances of Noel's amphipod on Bitter Lake National Wildlife Refuge between the years 2014-2017. Water_temp_C, SPC_mS_cm, pH and DO_mg_L stand for water temperature ºC, specific conductance (mS), pH and dissolved oxygen (mg/l). Each node at the bottom of the tree represents how much abundance can be attributed to these sets of water quality parameters.

CHAPTER II

EVALUATING OPERCULUM COLOR AS A DEFINING CHARACTERISTIC FOR ROSWELL SPRINGSNAIL AND KOSTER'S SPRINGSNAIL UTILIZING SANGER SEQUENCING

Bitter Lake National Wildlife Refuge (NWR) has 2 federally listed, endemic springsnails: Roswell springsnail (*Pyrgulopsis roswellensis*) and Koster's springsnail (*Juturnia kosteri*) (Taylor 1987, Morningstar et al 2014). Taylor (1987) noted that Roswell springsnail and Koster's springsnail were difficult to discern utilizing external morphological characters. However, he noted that Roswell springsnail has an amber operculum where Koster's springsnail has an opaque operculum (Taylor 1987). Current efforts to monitor population trends or establish new populations via translocation are complicated by the difficulty of separating the 2 species from each other. The snails are too small to differentiate in the field, and even under a dissecting microscope most morphological landmarks are not considered definitive. Thus, no attempt to separate the 2 species during monitoring efforts, meaning abundance estimates consider 2 species as 1. The inability to assign species identity to springsnails is an impediment to effective monitoring and management. For example, the 2 species could respond differently to management efforts, or if their population trends are moving in different trajectories, there is no efficient or practical way to measure this. Taylor (1987) suggested Roswell springsnail prefers lotic habitats where Koster's springsnail prefers lentic habitats.

Past attempts to link morphological landmarks to genetic determination of species have relied on snails that were first euthanized (Morningstar et al. 2014). Operculum color, however can only be determined in live snails. If operculum color alone could be used to differentiate species, this landmark could be incorporated into current monitoring efforts, allowing us to obtain accurate population estimates and responses to environmental variables. Before the refuge fully incorporates operculum color into monitoring efforts, the efficacy of the morphological landmark needs to be evaluated. Therefore, my objective was to determine the extent to which a simple landmark (operculum color) can be used to differentiate the two species of springsnails.

METHODS

Field methods

Snails were collected concurrently with quarterly monitoring efforts between 2015 and 2016. Ten-percent of snails were collected for each benthic grab with a maximum of 30 snails. Snails were immediately placed in ethanol to preserve the genomic DNA in 1.5 ml tubes. Snails were then brought back to the lab and sorted by one individual based off operculum color. A second individual would confirm the color of the operculum with no prior knowledge of the color sorted by the first individual. Once operculum color was agreed on, specimens were placed in a 1.5 ml tube based on operculum color and site.

Lab methods

For genetic analysis, whole-genome DNA was extracted from the entire specimen using CTAB/chloroform extraction followed by ethanol precipitation (Saghai-Maroof et al. 1984). Extracted genomic DNA was then calculated utilizing a nanospectrometer and subsequently diluted to 10 ng/µl. A 20 µl reaction of 10 µl GoTaq Master Mix (Promega Corporation) 1 µl of the forward primer (LCO1490), and 1 µl of the reverse primer (HCO2198), 6 µl of the molecular water and 2 µl of the template DNA (Folmer et al. 1994). Polymerase chain reaction (PCR) consisted of an initial 2-min denaturation step at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C for COI, 2 min at 72 °C, and a final extension step at 72 °C for 10 min.

Amplified DNA was visualized on a 2% agarose gel that was stained with ethidium bromide to check for length, quality and quantity. Bands were excised and then cleaned with a QIAquick Gel Extraction Kit following protocol (QIAGEN). Cycle sequencing reactions were 10-µl consisting of: 0.5-µl Big Dye, 0.875-µl of 10x sequencing buffer, 0.5-µl of the forward or reverse primer (listed above), 5.125 µl of molecular water and 3 µl of template DNA. The following cycling conditions were used: 96 °C for 2 min, then 30 cycles of 96 °C for 20 s, 45 °C for 20 s, and 60 °C for 4 min. Cycle sequencing products were purified following the EDTA/sodium-acetate/ethanol protocol from the BigDye kit and analyzed on an ABI Genetic Analyzer (Applied Biosystems, Forest City, California, USA). Sequences were assembled, edited, and aligned visually using the program Seqeuncher 5.4.6, and an open-reading frame for this gene and absence of the primer sequences were verified. Cleaned sequences were uploaded into GenBank as FASTA files and subsequently analyzed for similarity using GenBank nucleotide BLAST feature.

RESULTS

102 springsnails were successfully sequenced. Of these, 61 were field assigned to the "amber" operculum group and 41 were assigned to the "opaque" operculum color group. Based on genetic analyses 101 samples were 97-99% similar to *Juturnia kosteria*, and one sequence was similar at 68% to *Pyrgulopsis roswellensis*. Thus, use of operculum color suggested a 59:41 ratio of the 2 species while genetic sequencing suggested a 99:1 ratio of the 2 species.

DISCUSSION

My results suggested that operculum color, by itself, is not sufficient to diagnose springsnails at Bitter Lake NWR. Thus, it is not possible to determine ratios of these 2 species during field sampling using this character. Given that my DNA amplification produced high quality DNA, these results were unexpected and I recommend replication of my genetic analyses by an independent lab. Additionally, clarification of the morphological delineation of these 2 species of snails seems warranted. Because the original descriptions of the 2 species are extremely vague, identification of other morphological characters to be used either in conjunction with or instead of operculum color is necessary. Until additional morphological and genetic analyses are conducted, research that has relied on operculum color to differentiate these 2 species of springsnails should be considered suspect.

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