# COMMON CARP (*CYPRINUS CARPIO*) AFFECT WATER QUALITY AND MACROINVERTEBRATE COMMMUNITES IN NEBRASKA SANDHILL LAKES

By

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### Abstract

Invasive species like the common carp (*Cyprinus carpio*) threaten the health and integrity of aquatic ecosystems. The Nebraska Sandhills region consists of 57,000 km<sup>2</sup> of grassstabilized sand dunes and topographic lows between the dunes are generally occupied by shallow lakes, wetlands, or wet meadows. These wetlands and their associated shallow lakes are an ideal environment to evaluate the impacts of carp introduction and removal because of the large number of water bodies in the Sandhills that are vulnerable to carp invasions. To help understand the influence of carp on these ecosystems, I collaborated with the Nebraska Game and Parks Commission (NGP) to conduct an ecological assessment of 20 Sandhills lakes. I collected water quality samples and characterized benthic and littoral macroinvertebrate community data from 10 lakes infested with carp (3 medium density and 8 high density), and 10 carp free lakes in 2018 and 2019. I created a macroinvertebrate Index of Biological integrity (IBI) that shows carp had a significant negative effect on both benthic and littoral macroinvertebrate communities in these lakes. Non-carp lakes had lower turbidity, higher submergent vegetation coverage, and lower phytoplankton biomass. Heavy carp had higher turbidity, high phytoplankton biomass, and less submersed vegetation. Lakes with low to medium carp densities varied. Our study suggests that in order to improve water quality, and maximize invertebrate and plant resources, efforts should be made to eradicate carp from Nebraska Sandhill lakes.

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### CHAPTER I

# COMMON CARP (CYPRINUS CARPIO) AFFECT WATER QUALITY AND MACROINVERTEBRATE COMMUNITES IN NEBRASKA SANDHILL LAKES

### Introduction

Invasive species, those organisms that are not native to a specific location, have dramatic effects on natural resources, ecosystem function, human health, and the economy (Juliano and Lounibos 2005). Freshwater ecosystems are especially vulnerable to biological invasions and species extinctions because of their high degree of isolation and endemism (Richter et al. 1997). Aquatic invasive species have been intentionally and unintentionally introduced into the United States. When non-native species become established, they can negatively impact the functioning of an ecosystem. For example, Asian carp Hypophthalmichthys spp. was accidently introduced and has since expanded throughout much of the Mississippi River and its tributaries. These filter-feeding species target the base of the food web, altering energy flow throughout the system (Sampson et al. 2009). Other invasive species have been deliberately introduced for specific reasons such as biological control agents, food sources, or as pets. In the United States, approximately 50,000 foreign species are estimated to have been introduced, many with unintended consequences (Pimentel et al. 2004). The red swamp crayfish, Procambarus clarkii, native to northern Mexico and south-central United States, has been introduced in 25 countries for aquaculture reason (Gheradi et al. 1995). Even at low densities, this

invasive crayfish can reduce biodiversity and increase biotic homogenization in a short time (Gheradi 2007) In addition to ecological effects, invasive species cause billions of dollars in damage annually. For example, substantial damages have resulted from the introduction of zebra mussel *Dreissena polymorpha* to the Great Lakes. This one invasive species alone cost the Great lakes' power region \$3.1 billion in damages between 1993-1999 (Pimentel et al. 2004).

The common carp *Cyprinus carpio* is one of the most widely distributed fish species in the world. It is native to Eastern Europe and Asia, but has been introduced to every continent except Antarctica (Welcomme 1988). The U.S. Fish Commission imported common carp from Germany in 1877 and for the next two decades stocked and distributed it as a food source throughout much of the United States and its territories (National Park Service 2015). Common Carp are gape-limited consumers that primarily feed on macroinvertebrates but will also consume macrophytes such as Chara aspera (Miller and Provenza 2007), detritus, seeds, plants tissues, and even large tadpoles when macroinvertebrate resources are scarce (Kloskowski 2011, Garo and Zambrano 2004). Common carp are also habitat generalists, allowing them to survive in a wide range of conditions (i.e., temperature, dissolved oxygen, pH, turbidity) (Crivelli 1981). Carp also have many population characteristics that help make them successful invaders. Females reach sexual maturity in two years and can produce up to two million eggs per clutch (Swee and McCrimmon 1996). Common carp Juveniles mature early, between 2 and 3 years of age, and grow rapidly reaching 166 mm by age 1 and 366 mm by age 3 in North America (Panek 1987, Jackson et al. 2008). Common carp have evolved life histories that reduce egg predation by laying eggs in shallow waters that experience winter hypoxia

and have low densities of native egg-predators that otherwise dominate these locales (Bajer et al. 2012). The combination of rapid growth and maturation, high fecundity, high environmental tolerance, and the lack of native predators allow common carp populations to expand rapidly and attain high densities.

Common carp can influence invaded ecosystems in multiple ways. However, the influence of common carp on macroinvertebrates is unclear as different studies have found contradictory results. Parkos el al. (2009) showed that common carp at low densities reduced abundances of annelids, chironomids, and odonates. However, Miller and Crowl (2005) found higher densities of chironomids and oligochaetes in the presence of carp. They attributed this increase to the increase in detrital resources that were exposed by carp foraging. Therefore, the impacts of common carp on macroinvertebrates may be contingent upon lake characteristics such as detritus-rich benthic zones or sand bottoms. Common carp can also alter plant communities and water quality in shallow freshwater environments both directly and indirectly, but the extent of damage depends on the density of carp and the types of macrophytes present (Pipalova 2002)

Many shallow lakes exist in one of two alternative stable states, a clear water state with lush macrophyte growth, or a turbid state dominated by planktonic algae. (Jolley 2013). Macrophytes are important in helping stabilize these shallow aquatic ecosystems (Blindow et al. 2014). Common carp act as ecosystem engineers in shallow lakes (Jones et al. 1994) and their infestations and subsequent removal of macrophytes help shift lakes from the desired clear water state, to the turbid state. These shifts can have detrimental effects across multiple trophic levels. Many features, including turbidity and water quality, abundances of fish, invertebrates, macrophytes, and waterfowl usage change

considerably as a lake shifts between clear and turbid states (Hanson et al. 2010). Zooplankton use macrophytes as refugia from predation by zooplanktivorous fish and are important for maintaining the macrophyte dominated state in shallow lakes by maintaining water clarity at least in part through the grazing of pelagic phytoplankton (Perrow et al. 1999). Submerged macrophytes in the littoral zone of lakes also provide important habitat for fishes and macroinvertebrates as they provide structure and cover for protection from predators, as well as, invertebrate prey for fish and predatory macroinvertebrates (Randall 1996). Waterfowl and shore birds also rely on these macrophytes for habitat, nesting, and a food source (Knapton 1999) Macrophytes cannot re-establish in carp-invaded systems because the constant benthic foraging behaviors of the carp which uproots plants (Hootsman 1999).

Common carp influence turbidity in three ways. First, carp foraging behavior directly increases turbidity through bioturbation and stirring up of sediments while rooting through the benthos. Secondly, carp indirectly influence turbidity by reducing macrophytes, which allows for wave-induced sediment resuspension and turbidity in shallow aquatic systems (Lougheed et al. 1998). Third, carp can directly and indirectly increase water column nutrients as a result of benthic foraging activities, excretion, and destruction and subsequent decomposition of aquatic macrophytes (Carpenter and Lodge 1986, Cline et al. 1994, Lammarra 1975). These processes increase nutrient levels that can cause algal blooms that further increase turbidity. However, it is important to note that the impacts of carp on turbidity depend on water depth, sediment-type, carp biomass, and carp population density (Weber and Brown 2009). Because the presence of common carp can cause changes in water quality, macrophyte abundance and composition,

invertebrate richness and abundance, and waterfowl usage of a lake (Zambrano *et al.* 2003, Bajer et al.2009), state and federal agencies are working to renovate lakes and remove carp populations.

Management and control of common carp has been well documented through much of North America (Meronek et al. 1996) with millions of dollars invested on research and control (Pimentel et al. 2000). Removal projects include mechanical harvest by netting (Pinto et al. 2005), water level manipulation (Wanner 2009), exclusion from spawning habitat, and piscicide application (Meronek et al. 1996). Northern pike *Esox lucius* have also been used as a biological tool to control common carp recruitment in the Sandhill lakes in Nebraska (Paukert et al. 2003). Each of these methods of carp control have had varying levels of success (Meronek et al. 1996).

The purpose of my study was to determine how common carp influence water quality and macroinvertebrate communities in lakes of the Nebraska Sandhills. Relatively little is known about aquatic macroinvertebrate communities from Sandhills lakes in general, therefore, this research will also provide a baseline for Sandhill lake macroinvertebrate communities. I hypothesize that Sandhill lakes with no carp will be in the clear water state (i.e., lower turbidity, lower algae and chlorophyll *a* concentrations) than lakes with carp. Lakes with no carp will also have greater macrophyte cover than lakes without carp. Finally, I hypothesis that lakes with no carp will have higher macroinvertebrate richness and abundances than lakes with carp.

This project is part of a larger Aquatic Habitat Rehabilitation project taking place in Nebraska, by Nebraska Game and Parks (NGP) and the United States Fish and Wildlife Service (USFWS). The goals of the NGP rehabilitation efforts are to improve water quality and fish and waterfowl habitat by eliminating common carp from lakes and preventing their re-invasion. Water control structures, diking and dredging are other components of the project that have been completed to help limit movements of fish between vulnerable lakes. The second phase of the project will be lake renovation using rotenone to remove carp from in hopes of improving habitat. The NGP is conducting waterfowl and fish surveys on the lakes, which they will use in conjunction with my macroinvertebrate study with goals of better understanding the influence of carp and their removal on these Sandhill lakes.

### CHAPTER II

### METHODS

Study Area – The Nebraska Sandhills is the largest dune field in the Western Hemisphere, occupying 57,000 km<sup>2</sup>, in west central Nebraska (Figure 1). The Sandhills formed during an arid climate period between 8,000 and 5,000 years ago when wind sculpted the fine grains of sand into dunes that are now held in place by grass (loope and Swinehart 2000). These porous sand dunes overlay the Ogallala Aquifer, and topographic lows between the dunes are generally occupied by groundwater-fed shallow lakes, wetlands, or wet meadows (Ahlbrandt and Fryberger 1981). The Nebraska Natural Legacy Project (Schneider et al. 2005), which aims to implement a blueprint for conserving Nebraska's flora, fauna and natural habitats, has designated the lakes and wetlands in the Sandhills as biologically unique landscapes (BUL). The watersheds are primarily mixed- and tall-grass prairie, and livestock grazing is the principal land use (Bleed and Flowerday 1989). Water level fluctuations in these systems could influence primary productivity by releasing nutrients into each system as a result of ground water or precipitation events (McEwen and Butler 2010). Submergent vegetation coverage in Sandhill lakes is variable, commonly ranging from approximately 15% to nearly 100% (Paukert and willis 2003, Jolley 2009, Jolley and willis 2009). The fish communities in these lakes are relatively simple. Common species collected in the past include yellow

perch *Percaflavescens*, black bullhead *Ameiurus melas*, bluegill *Lepomis macrochirus*, largemouth bass *Micropterus salmoides*, northern pike *Esox Lucius*, and black crappie *Pomoxis nigromaculatus*. (Paukert and Willis 2003, Jolley 2009, Nebraska Game and Parks, unpublished data).

<u>Site selection</u> – Nebraska Game and Parks (NGP) designated a total of 20 natural lakes ranging from non-infested to highly-infested with carp to be surveyed for their carp renovation project (Table 1). Five of these carp lakes are scheduled to be renovated between 2019 and 2020 (Cody, Hackberry, Dewey, Clear, Goose). All lakes were located within the Valentine National Wildlife Refuge or on NGP Wildlife Management Areas. The lakes varied in surface areas from 15 to 477 ha and were shallow (mean maximum depth 1.5-3 m). I coordinated with NGP to obtain carp density estimates from each lake based on number of carp observed per hour of electrofishing during summer of 2018. The number of carp observed was used to categorize each lake in one of three density categories: no carp (n=10), low carp (n=3, <75 carp/hr), or high carp (n=7, >75 carp/hr).

<u>Field Survey and Sampling</u> – Macroinvertebrates were collected from the 20 study lakes on five sample dates: spring (June 2-5 2018, June 3-6 2019), summer (August 8-10 2018, July 23-25 2019), and fall (October 18-20 2018). Because most of the lakes had low accessibility, samples were primarily collected near boat ramps or at the most accessible portion of the lake relative to the road. At each lake, both benthic and littoral macroinvertebrates were collected. I used a standardized multi-habitat sampling approach to sample littoral macroinvertebrates. Different microhabitats were sampled in proportion

to their relative abundance in each lake because taxonomic groups show strong affinities to vegetation and sediment structure (GarciaCriado and Trigal 2005). During the first sampling event (June 2-5, 2018) I collected littoral macroinvertebrate samples using a Dnet (425 µm mesh) by taking 1-minute continuous sweeps from three points that were located at least 50 meters apart. These samples were then homogenized in a single jar and preserved in 95% ethanol. However, this method was difficult to use in different vegetation types that were located far apart and there were concerns that the net could possibly become clogged in some lakes especially if there were algal blooms. Therefore, I modified the littoral sampling procedure for subsequent sampling trips so that I collected six 1m sweeps per site within each lake (6 sweeps x 3 sites = 18 sweeps total per lake). The 18 sweeps at each lake were stratified according to estimated percent occurrence of major vegetation types (open water, emergent vegetation, and submergent vegetation). For example, if 30% of the littoral zone was occupied by emergent vegetation, then 6 of the 18 sweeps were taken from that habitat class. All 18 sweeps were combined into one jar and preserved in 95% ethanol.

Benthic macroinvertebrates were collected with an Eckman grab sampler from three sites in the pelagic zone of each lake. I washed the samples through a No.18 testing sieve (Cole-Palmer) to remove sediment, and combined the material collected in the sieve from all three sites into a single jar and preserved the samples in 95% ethanol. Upon returning to the laboratory, the samples were rinsed, sorted through, and invertebrates were stored in fresh 95% ethanol for later identification. Macroinvertebrates were identified to family level using Merritt & Cummins (1996), and other applicable

invertebrate taxonomic resources. Voucher specimens were stored at Oklahoma State University.

<u>Water quality</u> – Water quality measurements were collected from each lake during macroinvertebrate collection. I used a Hydrolab water quality probe to measure salinity (mS/cm), turbidity (NTU), dissolved oxygen (mg/L), and pH from 1m. A Secchi Disk was used to measure water clarity. Water was collected in a brown bottle from 1m in the pelagic zone at each lake for analysis of chlorophyll *a* (algae) and phosphorus (total and dissolved).

<u>Elevation Map</u> – I obtained elevation data from Nebraska Department of Natural Resources and used ArcMap10.7.1 to create a digital elevation model of Valentine National Refuge in order to delineate the watersheds in this region to examine connections between lakes that may account for variations in water quality (Figure 2).

<u>Vegetation Assessment</u> – Data on the aquatic plant communities in the study lakes was obtained from NGP. In 2018, NGP established transects across each lake and recorded water depth, plant taxa richness, and percent emergent and submergent plant cover. The transects were 500 meters apart on lakes less than 100 hectares, and 1km apart on lakes that are over 100 hectares. Points were spread out every 100m along those transects on the smaller lakes, and every 200m along transects for the bigger lakes. Percent emergent and submergent cover were calculated by NGP's digitally by photographing (Olympus 1030SW, 314 dpi) a 50x50 cm quadrat prior to collecting samples. The digital photo was then viewed on a 39.1 cm monitor at full screen under a 1 cm transparent dot grid and percent cover was determined by calculating the percent of points that covered vegetation

within the quadrat (de Szalav and Resh 2000). I used the percent submergent and emergent vegetation coverage of these lakes to see if macroinvertebrate richness and abundances relate to percent vegetation cover.

<u>Statistical analysis</u> – I used two way Multivariate Analysis of Variance (MANOVA) to compare water quality variables among seasons (spring, summer, fall) for lakes with and without carp to determine if the interaction of carp and season together had significant effects on water quality. Assumptions of MANOVA were tested before analyses. For the MANOVA analysis, water quality variables (chlorophyll *a*, TP, STP, Secchi disk depth) were log-transformed to normalize. I used two way Analysis of Variance (ANOVA) to determine if the presence of carp alone, or season alone influenced water quality variables.

I used one way Analyze of Variance (ANOVA) with Tukey's post hoc comparisons to separately compare benthic and littoral macroinvertebrate metrics (i.e., total taxa, number of families, Shannon diversity index, Chironomidae abundance, etc.) from lakes with no carp, medium carp, and heavy carp densities. I also combined the average number of individuals collected from the most common orders for benthic and littoral macroinvertebrates and analyzed with ANOVA. Order Odonata was split into the suborders Anisoptera and Zygoptera.

Principal Component Analysis (PCA) was used to identify major sources of variation in physical and chemical variables across the 20 studied lakes with no carp, medium carp, and heavy carp. We performed a partial forward selection Canonical Correspondence Analysis (CCA; 999 Monte Carlo permutations) using CANOCO 5.04

on the macroinvertebrate community compositions. Only environmental variables that significantly explained community variation (p = 0.05 with Bonferroni correction) were incorporated into the CCA. All the above analyses used the same environmental variables (lake area, chlorophyll *a*, TP, STP, turbidity, Secchi disk depth, emergent vegetation coverage, submersed vegetation cover, number of unique plant species, ph, and conductivity).

The plant vegetation data was only collected from 12 lakes within Valentine National Wildlife Refuge. Therefore, I categorized the vegetation data into two categories, non-carp (n=5) and carp (n=7) invaded lakes and preformed t-tests to determine if percent cover and plant richness differed between lakes with and without carp.

<u>Index of Biotic Integrity</u> – I created an Index of Biological Integrity (IBI), which relates anthropogenic impacts with macroinvertebrates in a water body, to determine impacts of carp. Macroinvertebrates are indicators of aquatic ecosystems health and commonly used to create IBI's because they are abundant, taxonomically diverse, and exhibit a wide range of tolerance to various stressors (Rosenberg and Resh 1993). IBIs were created for both the benthic and littoral macroinvertebrate communities in the 20 lakes. First, I compiled a list of macroinvertebrate metrics from the literature based on functional feeding groups, species richness and abundances, and tolerance measures and examined them for potential inclusion in the IBIs (Table 2). Functional feeding groups and tolerance values for the macroinvertebrates collected from the study lakes were taken from Merrit and Cummins (1996) and Maret (1988). These candidate metrics were then calculated for each macroinvertebrate sample and examined following the procedure of

Lunde (2012) to determine which metrics would be included in the IBIs. Specifically, a given metric needed to meet the following three criteria: (1) it was significantly correlated with carp abundance; (2) it had adequate range within metric scores; and (3) it lacked redundancy with other significant metrics. To test for relationships with carp density, ordinary least squares (OLS) regression was used. Selection criteria included wedge-type response or linear relationship, as well as an  $R^2 \ge 0.10$  (Lunde 2012). I retained metrics with range abundance values greater than or equal to 4 and percentage metrics with a range greater than 10%. Redundancy among metrics was tested using a Pearson correlation matrix. If two metrics were highly correlated with each other (r>0.7)they were deemed redundant and the one with a higher  $R^2$  value based on its (OLS) relationship with carp density was retained for the IBI. Metrics that passed all three criteria were then included in the IBI (Table 3). Scores were assigned to each metric by trisecting a box and whiskers plot after omitting the 5th and 95th percentiles to exclude the effects of outlier or extreme values (McDonough and Hickman, 1999). Scores that fell in the upper third were assigned a value of 5, indicating high quality, scores in the middle third received a value of 3, and scores in the lower third received a value of 1 indicating poor quality (McDonough and Hickman, 1999). I used one way Analyze of Variance (ANOVA) to compare benthic and littoral IBI scores for lakes with no carp, medium carp, and heavy carp densities.

### CHAPTER III

### RESULTS

Water Quality - Water quality variables differed among lakes (Table 4), but were structured by the presence of carp and season. Two way MANOVA using Wilk's Lambda test statistic revealed that the interaction of season and presence of carp together was not significant when structuring water quality (P=0.153) (Table 5). However, Two way MANOVA showed the presence of carp alone had a significant effect (P<0.001) and accounted for 63% of the variation in overall water quality parameters. Season alone had a significant effect (P<0.001) and accounted for 23% of the variation in water quality (Table 5). Two way ANOVA revealed carp lakes had significantly less dissolved phosphorous (P< 0.001), greater concentrations of chlorophyll *a* (P< 0.001), and lower Secchi Disk depth (P<0.001) (Table 6). Partial Eta Squared values suggest that the presence of carp explained 24% of the variation in dissolved phosphorus, 47% of variation in chlorophyll a, and 49% of the variation in Secchi depth. Season had a significant effect on total phosphorous (P=0.04), chlorophyll a (P=0.01), Secchi Disk depth (P=0.007). Partial Eta Squared values suggests 13% of variation in total phosphorous, 25% of the variation in chlorophyll a, and 18% of Secchi Disk variation were explained by season (Table 6).

Macroinvertebrates -- For benthic macroinvertebrates, one way ANOVA with Tukey's

post hoc comparisons showed that were many significant differences between non-carp, medium carp, and heavy carp lakes (Table 7). Non-carp lakes had significantly more benthic families, greater abundances of macroinvertebrates, higher Shannon Diversity and Ginni-Simpson Diversity than both medium and heavy carp lakes. Non-carp lakes had significantly more percent Ephemeroptera, Odonata, and Trichoptera (EOT) and abundances of Chironomidae than heavy carp lakes, but was not significant between noncarp to medium carp lakes. There were no differences between medium and heavy carp for these benthic metrics (Table 8).

For littoral macroinvertebrates, one way ANOVA with Tukey's post hoc comparisons showed that were many significant differences between non-carp, medium carp, and heavy carp lakes (Table 9). Non-carp lakes had significantly more macroinvertebrates collected than both medium and heavy carp lakes. Non-carp lakes had significantly more littoral families, higher percent Ephemeroptera, Odonata, and Trichoptera (EOT), higher Shannon diversity, higher Ginni-Simpson diversity, and greater abundance of Amphipoda than heavy carp lakes, but were not significant between non-carp to medium carp lakes. There were no differences between medium and heavy carp lakes for these littoral metrics (Table 10).

For average number of individuals collected from the most common orders, there were significantly greater abundances of Coleoptera (P=0.012), Diptera (P=0.01), Trichoptera (P=0.04), sub-order Zygoptera (P=0.037), and Hemiptera (P=0.002) found in non-carp lakes when compared to medium and heavy carp lakes. Abundances of Anisoptera and Ephemeroptera were not significantly different between non-carp, medium carp, and heavy carp lakes (P>0.05) (Table 11).

PCA/CCA multivariate statistics results - Principal Component Analysis (PCA) showed a clear separation of lakes based on the environmental variables (Figure 3). The first axis (PCA1) explained 35% of the variation and was mainly determined by chlorophyll a, turbidity, and total lake size. Variables secondarily associated with PCA1 were % submergent vegetation, ph, and Secchi Disk depth. The second axis (PCA2) explained 25% of the variance and was influenced by total phosphorous. Lakes that grouped on the positive side of PCA1 were larger lakes with high turbidity, lacked submergent vegetation coverage, and had high carp densities (Center, Clear, Hackberry, Twenty one, and VNWR Willow). Lakes with greater Secchi Disk depth and higher percent submergent vegetation were non-carp lakes and grouped on the negative end of PCA1 (Avocet, Defair, Frye, Little Hay, and Watts). Two non-carp lakes that had elevated phosphorous levels, high Secchi Disk depth, and low submergent vegetation grouped together on PCA2 (Rat, Beaver). Two non-carp lakes that had elevated phosphorous levels and high percent submergent vegetation grouped together (West long, Duck). The three medium carp density lakes (Willow, Dewey, Homestead) did not group together by physical or chemical properties (Figure 3).

The first and second axes of the overall CCA explained 18.79% (eigenvalue = 0.34) of the variation in macroinvertebrate communities (Figure 4). Macroinvertebrate families Crambidae, Sciomyzidae, Pleidae, Lampridae, Limnephilidae, Arrenuridae, Libuelidae, Glossiphoniidae, and Amphipods were positively correlated with the environmental variables %submergent vegetation coverage and Secchi Disk depth and grouped on the positive side of axis one. Macroinvertebrates families Halipidae, Caenidae, Mesovilidae, Dytiscidae, Hydrophilidae, and Notonectidae were correlated

with carp density on the negative side of axis one. Macroinvertebrates Lestidae, Baetidae, Gyrnidae, and Leptoceridae were grouped on the positive end of axis two and were negatively correlated with turbidty, while macroinvertebrates Belostomatidae, Aeshnidae, Curculionidae, and Gerridae were positively correlated with turbidity (Figure 4).

<u>Vegetation</u> - The percentage of submergent vegetation in carp lakes  $(9.7\% \pm 2.2 \text{ SE})$  was significantly lower (P=0.001) than in lakes with no carp  $(47.1\% \pm 7.8 \text{ SE})$ . The average number of aquatic plant species identified (Table 12) in lakes with no carp  $(6.4\pm 1.3 \text{ SE})$ was also significantly higher than in lakes with carp  $(3.5\pm 0.29 \text{ SE})$  (P=0.03). Emergent vegetation did not differ between lakes with and without carp. Lakes with carp averaged  $(12.7\% \pm 2.1 \text{ SE})$  emergent vegetation compared to lakes with no carp that averaged  $(8.3\% \pm 1.8 \text{ SE})$  emergent vegetation.

Indexes of Biotic Integrity - The IBIs showed that carp had a significant negative effect on both benthic and littoral macroinvertebrate communities in the Sandhills lakes. The calculated IBI scores for the benthic zone ranged from 10 (severely degraded) to 46 (least-impacted) out of a possible range of 10-50 (Figure 5). The calculated IBI scores for the littoral zone ranged from 15 (severely degraded) to 53 (least-impacted) out of a possible range of 15-55 (Figure 6). Both littoral and benthic macroinvertebrate communities were structured by carp densities. Non-carp lakes had overall higher scores than lakes with carp, except for two carp lakes (Hackberry & Goose) that had higher littoral zone scores than the other carp lakes. The mean IBI littoral score for non-carp lakes was  $(44\pm2.6 \text{ SE})$  and significantly higher than medium carp lakes (average IBI =  $26\pm5.1 \text{ SE}$ , P=0.01) and heavy carp lakes (average IBI =  $23\pm4.2 \text{ SE}$ , P<0.001). The mean IBI benthic score for non-carp lakes was  $(39\pm1.9 \text{ SE})$  and was significantly higher than

medium carp lakes (average IBI =  $16\pm2.8$  SE, P<0.001) and heavy carp lakes (average IBI =  $16\pm3.8$  SE, P<0.001) (Figure 7).

### CHAPTER IV

### DISCUSSION

Invasive common carp have strong negative effects on the water quality, aquatic vegetation, and macroinvertebrates in Sandhill Lakes. The PCA ordination revealed alternative ecosystem states exist in these Sandhill lakes across a gradient of carp density from clear water to turbid water, with some lakes intermediate and in a possible state of hysteresis (Figure 3) (Scheffer 1998).

Previous studies have shown that carp increase water column phosphorus due to benthic foraging activities, excretion, and/or destruction and subsequent decomposition of aquatic macrophytes (Lammarra 1975, Carpenter and Lodge 1986, Cline et al. 1994). However, lakes with carp in this study had significantly lower concentrations of phosphorus compared to some non-carp lakes, which is consistent with other lakes in the region (Jolley 2009, Jolley 2013). Lakes in the Sandhills region generally have higher nutrient concentrations relative to nearby ecoregions (Hayford 2011), regardless of the presence of carp. For example, all the study lakes had total phosphorous concentrations that were indicative of eutrophic (35-100  $\mu$ g/L) or hypereutrophic (>100  $\mu$ g/L) systems (Carlson and Simpson 1996). The range of total phosphorous concentrations in the study lakes varied greatly, even between non-carp lakes that were in close proximity to each

other. For example, the average total phosphorous concertation in West Long Lake was 346  $\mu$ g/l, while the average concentrations in Watts Lake was 67.3  $\mu$ g/l; these two lakes are located only three miles apart within the Valentine National Wildlife Refuge.

A factor likely contributing to lower phosphorous levels in heavy carp lakes was the presence of algal blooms as indicated by the elevated chlorophyll *a* concentrations and visual observations in heavy carp lakes compared to non-carp lakes during sampling (Figure 8). Dissolved phosphorus is a limiting nutrient that is taken up by algal cells during blooms and is essential in photosynthetic processes (Adey et al. 1993). Algal blooms were notable in heavy carp lakes and likely used any dissolved phosphorous as it became available in the water column.

Season and geomorphology of these lakes also plays an important role in nutrient cycling (Jolley 2009, Wanner 2009). Phosphorous and chlorophyll *a* concentration were lowest, and Secchi Disk depth was highest in both carp and non-carp lakes in the spring. By summer, non-carp lakes had high amounts of submergent macrophytes (Figure 9) that help stabilize the sediment and provide habitat for phytoplankton grazers like zooplankton and Amphipods that are important for helping to maintain the macrophyte dominated clear water state (Perrow et al. 1999). As water temperatures begin to decrease in fall, the submergent vegetation begins to die and decompose releasing phosphorous into the water column. This naturally occurring nutrient cycle leads to elevated algal concentrations and decreased water clarity during the fall in non-carp lakes. The geomorphology of these lakes may also play a major role in the variable phosphorus levels. The digital elevation model revealed a steep elevation gradient decreasing from southwest to northeast across the refuge (Figure 2). Lakes in the south west corner of the

refuge (West long, Homestead, Rat, Beaver, and Duck) are surrounded by higher sand dunes that make the basins of these lakes completely closed with no out flow. Elevated phosphorous levels in these refuge lakes as indicated by the environmental grouping of the lakes based on the PCA may result from absence of outflow. Lakes in the northern part of the refuge (Watts, Dewey, Hackberry, Clear, and VNWR Willow) form basins, but are not closed off completely and instead have dams and water control structures that manipulate the flow of water and likely influence nutrients in these lakes (Wanner 2007, Wanner 2009).

The overall abundance and diversity of macroinvertebrates was significantly greater in lakes without carp. These results are consistent with previous studies showing carp reduce invertebrate abundances, diversity, evenness, and richness (Lellak 1978, Wilcox and Hornbach 1991, Parkos et al. 2003, Stewart and Downing 2008). However, carp did not negatively affect all macroinvertebrate families. Our CCA analyses suggest Dytiscidae, Corixidae, Caenidae, Nototnectidae, Hydrophilidae, and Mesovelidae had a positive relationship with carp density. Notonectidae, Hydrophilidae, and Dytiscidae are taxa that require open water habitats (Wells et al. 1981, Hosseinie 1995, Davy-Bowker 2002) at different stages in their lifecycle. The presence of carp decreases submergent vegetation which may provide these taxa access to the open water column. Corixidae are mainly herbivores and feed on algae, diatoms, and bottom detritus (Sweeney 1977) and are tolerant of a wide range of environmental variables (Scudder 1976). Caenidae are collector-gatherers (Merritt and Cummins 1996) are often found in disturbed aquatic ecosystems and may be tolerant of carp effects (Hilsenhoff 1987, Puckett and Cook 2004). Lakes with carp in this study had elevated algal concentrations which may provide a food source for Corixidae and Caenidae and explain their positive relationship with the presence of carp.

Submergent vegetation is important for many macroinvertebrates but can depend on macrophyte biomass and vegetation type (Van den Berg 1997). In this study, percentsubmerged vegetation and Secchi Disk depth were the main factors in carp free lakes that influenced macroinvertebrate communities. Macroinvertebrates that generally grouped with lakes in the clear water state were Crambidae, Leptoceridae, Sciomyzidae, Polycentropodidae, Hyalellidae, Lestidae, Libulelidae, Pleidae and Baetidae. Caddisflies are important for waterfowl diets, and were an abundant taxa in non-carp lakes in this study. Leptoceridae which is an important food source for Ring-necked ducks Aythya collaris (Hohman 1985) was mainly found in Duck, Little hay, Watts, and West long lakes which also had the submerged macrophyte *Potamogeton crispus*. Ninety-three percent of Leptoceridae were collected from these four lakes which suggests that Leptoceridae may be using this plant. Limnephilidae, another caddisfly that is important for waterfowl diets (Scheauhammer et al. 1997) showed relationships with sediment type and lake depth. Seventy-four percent of collected Limnephilidae came from Rat/Beaver Lake. Rat and Beaver lakes are two interconnected lakes without carp that are shallow enough (average depth <0.5m) that wind and wave induced sediment resuspension doesn't allow for macrophytes to establish. Limnephilidae build their cases out of sand and gravel (MacKay 1977, Boyer and Barnard 2004). Rat and Beaver lakes have sandy bottoms that allowed Limnephilidae to build their cases.

Chironomidae are an important food source for most species of waterfowl. The Rudy duck *Oxyura jamaicensis* diet is comprised almost entirely of Chironomidae Larvae

(Woodin and Swanson 1989). In this study, Chironomidae abundances were significantly higher in non-carp. Hyalellidae (Amphipoda) are important components of the invertebrate fauna of semi-permanent wetlands and permanent lakes in the prairie pothole region and are also a major food item in the diet of some species of waterfowl (Swanson 1984). Hyalellidae have been shown to be positively correlated with percent submerged aquatic vegetation (Anteau 2011). Hyalellidae were the most abundant taxa in our study with a significantly greater number collected in non-carp lakes. Baetidae are an intolerant family of mayfly and are often used as indicators of water quality in aquatic systems (Bowles 2013). Baetidae have been shown to be positively correlated with pH, dissolved oxygen, and water clarity, but negatively correlated with conductivity, temperature, and turbidity (Buluta et al. 2010). Baetidae were significantly more abundant in non-carp lakes suggesting that they are intolerant to common carp disturbance.

It is important to consider that the study lakes had diverse fish populations communities, and variations in fish species other than carp can also influence macroinvertebrate communities. For example, Rice, Watts, and West long lake had an abundance of piscivorous largemouth bass *Micropterus salmoides* and northern pike *Esox lucius* (Jolley 2009). These lakes with piscivorous dominated fish communities had relatively clear water, dense vegetation, high invertebrate abundance, and low phytoplankton levels. These characteristics are likely influenced by the entire fish community, not just carp (Ward et al. 2008). Bluegill *Lepomis macrochirus* populations were abundant in two non-carp Frye and Duck lakes, but did not exist in two non-carp Rat and Beaver Lakes (Nebraska Game and Parks, unpublished data 2018). Crowder and Cooper (1982) suggested that total benthic macroinvertebrate biomass was reduced by

bluegill predation, but their impact was related to bluegills selectively feeding on larger invertebrates (e.g., Amphipods and Odonates). Therefore, the impacts of common carp on macroinvertebrates in these lakes may be more complicated than just presence and absence of carp, and future studies should consider how other species of fish that are present impact macroinvertebrates.

It is also important to note that there was historic flooding in Nebraska in 2019 (Cooper and Shulski 2009). Between the Months of May and August 2019, Valentine National Wildlife Refuge received 20.12 inches of rain, which was 8.04 inches more than normal for this span (usclimatedata.com). Water level fluctuations in these systems could influence primary productivity by releasing nutrients into each system as a result of ground water or precipitation events (McEwen and Butler 2010). These nutrients could influence macroinvertebrate communities. Flooding of wetland habitats has been shown to increase macroinvertebrate abundances and may be related to the death of the belowground components of the emergent vegetation, the availability of coarse organic litter early in flooding, and the development of fine particulate organic matter during flooding. (Whiles and Goldowitz 2005, Murkin and Kadlec 1986).

These floods may have also allowed for the movement of common carp into lakes that carp had not previously been established or had previously been removed. Valentine National Wildlife Refuge has a history of lakes becoming full and flowing over. These high water events have resulted in many of the lakes becoming interconnected, and fish movements between lakes have been observed (Wanner 2007).

This study advances our understanding of how invasive carp impact shallow lake ecosystems. Carp reduced invertebrate abundances and diversity and alter community structure in infested lakes. Carp also influenced water quality parameters, but these impacts may have been more variable due to season and geomorphological characteristics associated with different Sandhill lakes. It is also important to note the severe flooding that occurred during this study that might have influenced our results. The Comprehensive Conservation Plan for the Valentine National Wildlife Refuge states goals of maximizing invertebrate and plant food resources to provide an appropriate food base for indigenous wildlife including migratory birds (USFWS 1999). Our study suggests in order to maximize invertebrate and plant resources, efforts should be made to eradicate carp from Sandhill lakes.

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# Tables

Table 1. Characteristics of study lakes in the Nebraska Sandhills region. Categories of carp density were provided by the Nebraska Game and Parks.

Lake Name	County	No Carp	Low Carp Density	High Carp Density	Scheduled for Renovation	Total Size (ha)	Palustine (ha)
Cody Lake	Cherry			Х	2019/20	310.3	155.0
Cottonwood/Steverson WMA	Cherry			Х		279.9	17.5
Frye Lake WMA	Grant	Х				105.1	6.1
Avocet WMA	Grant	Х				62.5	6.0
De Fair Lake WMA	Grant	Х				43.9	13.6
VNWR - Watts Lake	Cherry	Х				191.3	123.3
VNWR - Hackberry Lake	Cherry			Х	2019	322.6	114.5
VNWR - Dewey Lake	Cherry		Х		2020	477.4	281.9
VNWR - Rice Lake	Cherry	Х				19.8	10.8
VNWR - Duck Lake	Cherry	Х				32.3	6.2
VNWR - West Long Lake	Cherry	Х				42.4	10.1
VNWR - Clear Lake	Cherry			Х	2021	225.2	12.3
VNWR - Willow Lake	Cherry			Х		196.2	45.2
VNWR - Little Hay Lake	Cherry	х				15.1	6.0
VNWR - Center Lake	Cherry			Х		83.5	57.1
VNWR - Twentyone Lake	Cherry			Х		115.7	85.8
VNWR - Homestead Lake	Cherry		Х			13.6	1.9
Beaver & Rat Lake	Cherry	Х				193.2	3.6
Willow Lake B.C. WMA	Brown		Х			154.7	18.8
Goose Lake WMA	Holt			Х	2019	99.5	15.3

Table 2. Candidate macroinvertebrate metrics that were tested for inclusion in benthic and littoral Indexes of Biotic Integrity for the Nebraska Sandhill study lakes. See text for description of how metrics were selected for IBIs.

	Functional Feeding Group
Abundance Metrics	Metrics
Total Individuals	Percent Filterers
Family Richness	Filterer Richness
Shannon-Weaver	Percent Gatherer
Simpson's Heterogeneity	Gatherer Richness
	Percent Predators
Sensitivity Metrics	Predator Richness
Hilsonhoff Biotic Index	Percent Scraper
Percent 2 Dominant	Scraper Richness
	Percent Shredders
Taxonomic Composition	
Metrics	Shredder Richness
Percent Baetidae	Percent Herbivores
Percent Corixidae	Herbivore Richness
Corixdae Richness	
Odonata Richness	
Percent EOT	
EOT Richness	
Percent Chironomidae	
Percent Trichoptera	
Percent Amphipoda	
Chironomidae abundance	
Trichoptera abundance	

Littoral				Benthic			
	Scoring	l .			Scoring		
Metric	Criteria	9		Metric	Criteria		
Abundance	5	3	1	Abundance	5	3	1
1. # of families	>10.5	7.5-10.5	0-7.5	1. # of families	>4.5	2.5-4.5	<2.5
2.Simpson's index	>.65	.5365	<.53	2. Simpson's index	>.4	.24	<.2
3. Total taxa N	>193	103-193	13.4-103	3. Total Taxa N	>75	39-75	<39
4. Shannon				4. Shannon diversity			
diversity index	>1.5	1.25-1.5	<1.25	index	>.83	.4583	<.45
Taxon							
composition				Taxon composition			
5. % Corixidae	<10%	10%-20%	>20%	5. % EOT	>34%	17%-34%	<17%
				6. Trichoptera			
6. % EOT	>50%	31%-50%	<31%	abundance*	>17.8	7.8-17.8	<1.3
Sensitivity				Sensitivity			
7. %2 Dominant							
Таха	<63%	63%-79%	>79%	7. %2 dominant taxa	<.80%	80%-90%	>90%
8. Hilsenhoff				8. Hilsenhoff biotic index			
Biotic index (HBI)	<5.7	5.7-6.6	>6.6	(HBI)	<6.34	6.34-7.17	>7.17
Functional				Functional feeding			
feeding group				group			
				9. Chironomidae			
9. % Gatherer	>38%	20%-38%	<20%	abundance*	>50	26-50	<26
10. % Predator	>34%	24%-34%	<24%	10. % Shreddar	>19%	2%-19%	<%2
11. % shreddar	>20%	10%-20%	<10%				

Table 3. Scoring criteria for selected metrics for inclusion in the Index of Biological Integrity for the benthic and littoral macroinvertebrates in the Sandhills lakes.

Table 4. Water quality parameters from no carp, medium carp, and heavy carp Sandhill lakes. Data are average from the entire study period  $\pm$  standard error with mean, and max and min values in parentheses.

Water Quality	No Carp	Medium Carp	Heavy Carp
Temperature (C)	19.17±6.11(6.92-30)	18.98±6.14(8.14-27.7)	18.61±6.18(7.89-27.73)
Turbidity (NTU)	10.2±11.98(0.3-41)	38.36±30.81(3.2-105)	51.68±69.03(4.4-348.4)
Secchi depth (m)	1.03±.44(0.25-1.8)	.64±.33(0.3-1.3)	.45±.20(0.2-1)
рН	9.35±.87(7.6-10.73)	8.83±.71(7.42-10.14)	8.81±0.77(7.53-10.37)
Dissolved oxygen (mg/L) Conductivity	9.8±1.53(6.13-12.85)	8.91±1.35(6.82-10.28)	8.96±1.59(6.63-11.63)
(mS/cm)	0.33±0.16(0.14-0.593)	.27±.09(0.12-0.436)	.33±.08(0.204-0.455)
Total phosphorus (µg/l) Soluble reactive	271.23±208.03(24.3-585.9)	231.92±127.77(48.4-450.8)	141.22±41.92(22-490.1)
phosphorus (µg/l)	208.57±190.35(10.1-431.5)	161.63±103.09(23.6-373.4)	26.74±18.71(2.8-83.5)
Chlorophyll a (µg/l)	15.91±19.18(0.89-87.14)	24.14±23.34(3.41-78.84)	45.6±79.11(3.17-376.43)

Table 5. Two-way MANOVA results, Use Wilk's lambda, season\*presence not significant on water quality, season by itself is significant, presence of carp is significant on overall water quality.

Effect	Test Statistic	Value	F	Sig.	Partial Eta Squared
Intercept	Wilks' Lambda	0.022	507.205 <sup>b</sup>	0.001	0.978
season	Wilks' Lambda	0.586	3.523 <sup>b</sup>	0.001	0.234
presence	Wilks' Lambda	0.371	19.486 <sup>b</sup>	0.001	0.629
season*presence	Wilks' Lambda	0.777	1.545 <sup>b</sup>	0.153	0.118

Table 6. Two-way ANOVA results. Showing season and carp presence have significant effect on which logged transformed water quality parameters

Source	Dependent Variable	F	Sig.	Partial Eta Squared
season	Total Phosphorus	3.444	0.04	0.123
	Soluble Phosphorus	2.593	0.085	0.096
	Chlorophyll a	7.994	0.001	0.246
	Secchi Disk depth	5.498	0.007	0.183
presence	Total Phosphorus	0.381	0.54	0.008
	Soluble Phosphorus	15.838	0.001	0.244
	Chlorophyll a	42.596	0.001	0.465
	Secchi Disk depth	47.436	0.001	0.492

Benthic ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Total Taxa	10363.04	2	5181.518	8.532	0.003
Families	43.863	2	21.931	14.957	0.001
Shannon Diversity	1.482	2	0.741	8.535	0.003
Simpson Diversity	0.489	2	0.244	11.033	0.001
Percent EOT	0.283	2	0.142	6.002	0.011
Chironomidae	2971.139	2	1485.569	3.61	0.049

Table 7. Results from one-way ANOVA for benthic metrics tested.

Table 8. Results of Tukey's post hoc comparisons for benthic metrics tested.

Dependent Variable	(I) CarpLevel	(J) CarpLevel	Mean Difference (I-J)	Std. Error	Sig.
Families	No carp	Medium carp	2.8333*	0.7164	0.003
		Heavy carp	3.0417*	0.6253	0.001
	Medium	No carp	-2.8333*	0.7164	0.003
	carp	Heavy carp	0.2083	0.7816	0.962
	Heavy	No carp	-3.0417*	0.6253	0.001
	carp	Medium carp	-0.2083	0.7816	0.962
TotalTaxa	No carp	Medium carp	40.627	16.30464	0.058
		Heavy carp	42.775*	14.23186	0.021
	Medium	No carp	-40.6292	16.30464	0.058
	carp	Heavy carp	2.14583	17.78982	0.992
	Heavy carp	No carp	-42.775*	14.23186	0.021
		Medium carp	-2.14583	17.78982	0.992
ShannonDiversity	No carp	Medium carp	.55731*	0.17434	0.014
		Heavy carp	.53563*	0.15218	0.007

	Medium	No carp	55731*	0.17434	0.014
	carp	Heavy carp	-0.02168	0.19022	0.993
	Heavy	No carp	53563*	0.15218	0.007
	carp	Medium carp	0.02168	0.19022	0.993
GiniSimpson	No carp	Medium carp	.31988*	0.08807	0.006
		Heavy carp	.30776*	0.07687	0.003
	Medium	No carp	31988*	0.08807	0.006
	carp	Heavy carp	-0.01212	0.09609	0.991
	Heavy	No carp	30776*	0.07687	0.003
	carp	Medium carp	0.01212	0.09609	0.991
PercentEOT	No carp	Medium carp	0.1536	0.09088	0.238
		Heavy carp	.27060*	0.07933	0.009
	Medium	No carp	-0.1536	0.09088	0.238
	carp	Heavy carp	0.117	0.09916	0.481
	Heavy	No carp	27060*	0.07933	0.009
	carp	Medium carp	-0.117	0.09916	0.481
Chironmidae	No carp	Medium carp	17.2875	12.0019	0.343
		Heavy carp	27.39167*	10.47612	0.045
	Medium	No carp	-17.2875	12.0019	0.343
	carp	Heavy carp	10.10417	13.09514	0.725
	Heavy carp	No carp	- 27.39167*	10.47612	0.045
		Medium carp	-10.1042	13.09514	0.725

Littoral ANOVA	Sum of	df	Mean	F	Sig.
	Squares		Square		
Families	62.7	2	31.35	4.891	0.021
TotalTaxa	106257.2	2	53128.59	9.401	0.002
Shannon diversity	0.58	2	0.29	8.657	0.003
Amphipoda	29587.56	2	14793.78	4.289	0.031
Simpson diversity	0.091	2	0.045	9.473	0.002
Percent EOT	0.209	2	0.104	4.031	0.037

Table 9. One-way ANOVA with Tukey's post hoc comparisons for littoral metrics tested.

Table 10. One-way ANOVA with Tukey's post hoc comparisons for littoral metrics tested.

Dependent	(I)	(J)	Mean	Std. Error	Sig.
Variable	CarpLevel	CarpLevel	Difference		
			(I-J)		
Families	None	Medium	3.2583	1.517	0.11
		Heavy	3.5833*	1.3242	0.038
	Medium	None	-3.2583	1.517	0.11
		Heavy	0.325	1.6552	0.979
	Heavy	None	-3.5833*	1.3242	0.038
		Medium	-0.325	1.6552	0.979
TotalTaxa	None	Medium	151.37917*	44.54998	0.009
		Heavy	141.40000*	38.88641	0.005
	Medium	None	-	44.54998	0.009
			151.37917*		
		Heavy	-9.97917	48.60801	0.977
	Heavy	None	-	38.88641	0.005
			141.40000*		
		Medium	9.97917	48.60801	0.977
ShannonDiversity	None	Medium	0.25041	0.10308	0.045

					<u> </u>
		Heavy	.40049*	0.08998	0.001
	Medium	None	-0.25041	0.10308	0.045
		Heavy	0.15008	0.11247	0.396
	Heavy	None	40049*	0.08998	0.001
		Medium	-0.15008	0.11247	0.396
Amphipoda	None	Medium	81	33.67432	0.068
		Heavy	79.63333 <sup>*</sup>	29.39335	0.038
	Medium	None	-81	33.67432	0.068
		Heavy	-1.36667	36.74169	0.999
	Heavy	None	-79.63333*	29.39335	0.038
		Medium	1.36667	36.74169	0.999
GiniSimpson	None	Medium	0.09862	0.04115	0.069
		Heavy	.14951*	0.03592	0.002
	Medium	None	-0.09862	0.04115	0.069
		Heavy	0.0509	0.0449	0.507
	Heavy	None	14951*	0.03592	0.002
		Medium	-0.0509	0.0449	0.507
PercentEOT	None	Medium	0.17723	0.09542	0.181
		Heavy	.21733*	0.08329	0.046
	Medium	None	-0.17723	0.09542	0.181
		Heavy	0.0401	0.10411	0.922
	Heavy	None	21733*	0.08329	0.046
		Medium	-0.0401	0.10411	0.922

	Sum of	df	Mean	F	Sig.
	Squares		Square		
Zygoptera	368.403	2	184.201	4.066	0.037
Anisoptera	4.144	2	2.072	1.636	0.226
Ephemeroptera	61.785	2	30.893	0.473	0.632
Hemiptera	1433.659	2	716.83	8.978	0.002
Diptera	3490.32	2	1745.16	6.189	0.01
Trichoptera	16926.65	2	8463.327	3.937	0.041
Coleoptera	49.019	2	24.509	5.909	0.012

Table 11. One-way ANOVA results for Carp level on average abundances of macroinvertebrates collected in each order for littoral and benthic macroinvertebrates combined.

	Little Hay	Duck	Homestead	watts	Clear	West Long	Rice	VNWR Willow	Hackberr	Dewey	Twenty one	center	fixe	Defair	Avocet
Myriophyllum sibiricum	х	х		х	х	X	х	X	Х	х	X	х	X	х	
lemna trisulca	Х			Х	Х	Х		Х	Х	Х		Х			
phragmites.*								Х	Х		Х	Х	Х	Х	Х
Elodea <u>nuttallii</u>															Х
Juncus articus				Х	Х										
Potamogeton crispus *	х	х		х		х									
Sagittaria Iatifolia		х													
Sagittaria cuneata	х			х											
Potamogeton Illinoensis	х														
Myriophyllum verticallatum							х								
Potamogeton zosteriformis						х									
Juncus vasevi						Х									
Sagittaria calycina						х									
Schoenplectus heterochaetus						х									
Lemna aequinoctialis						х									
Sagittaria brevirostra				х											
Cattails	х			х	Х	х		х	х		х	х		х	
Willows			х										х		
Stuckenia Pectinata				х											
Polygonum amphibium							х								
Potamogeton filiformis											х				
Nymphaeaceae												Х			

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Table 12. Plant Taxa Identified by Nebraska Game and Parks in Valentine National Wildlife Refuge lakes.

## Figures.

Figure 1. Location of study lakes across the Nebraska Sandhills. Valentine National Wildlife Refuge was centrally located.

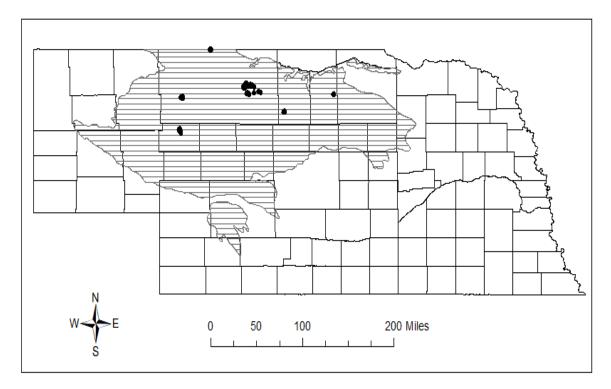


Figure 2. Digital Elevation map of Valentine National Wildlife Refuge revealing an elevation decrease from west to east across the refuge.

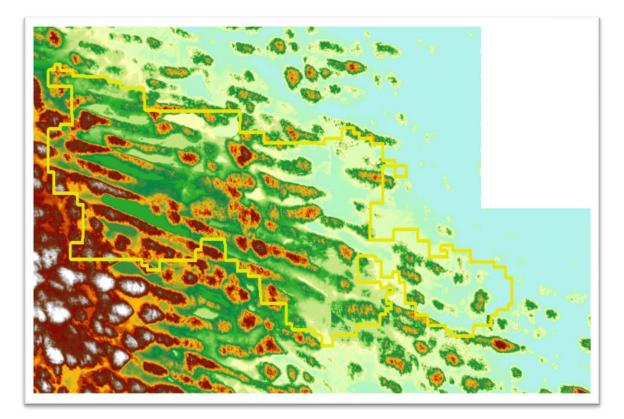


Figure 3. Principal components analyses on environmental variables for study lakes. Non-carp lakes are represented by the white dots, mid-level carp lakes are grey, and heavy carp lakes are the black dots.

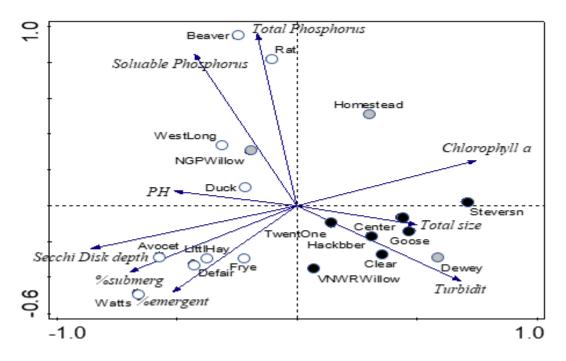


Figure 4. Partial forward selection CCA for study lakes. Eigenvalue = 0.34; explained variation = 18.79%; contribution to explained variation: Carp Density = 15.2%, Secchi Disk Depth = 14.9%, %submergent veg = 13.5%, Total Size = 10%, Turbidity = 8.5%.

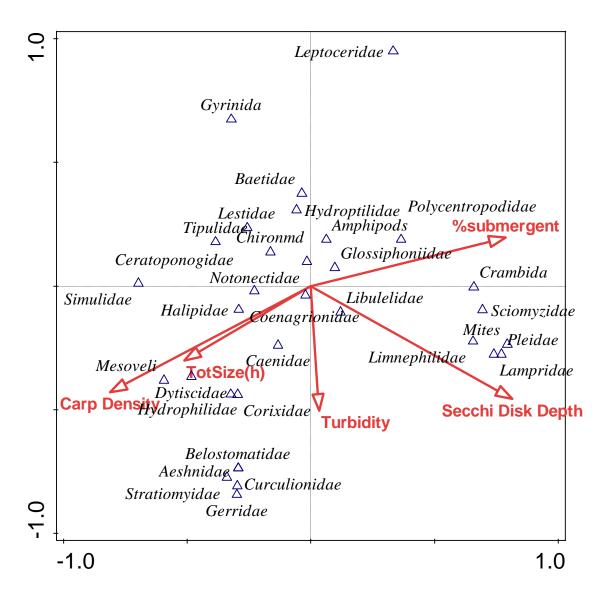


Figure 5. Benthic Index of Biological Integrity scores for studied carp lakes. Blue lakes ( $\Omega$ ) are non-carp lakes, green lakes ( $\lambda$ ) are medium carp density lakes, red lakes ( $\Delta$ ) are heavy carp density lakes.

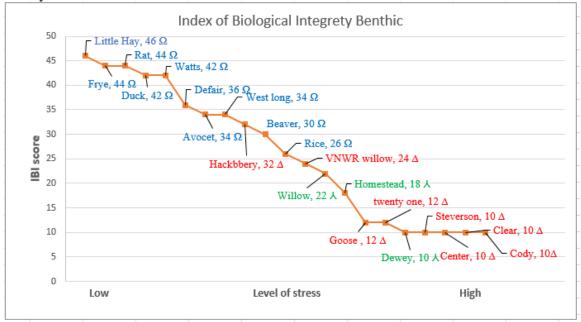
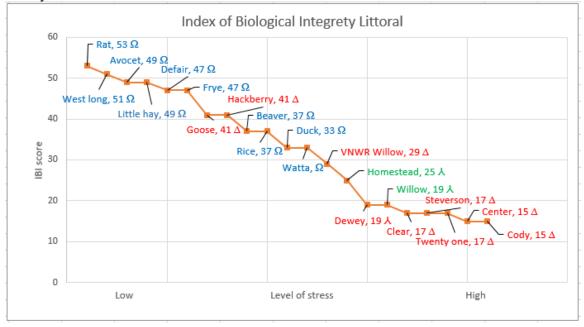


Figure 6. Littoral Index of Biological Integrity scores for studied carp lakes. Blue lakes ( $\Omega$ ) are non-carp lakes, green lakes ( $\lambda$ ) are medium carp density lakes, red lakes ( $\Delta$ ) are heavy carp density lakes.



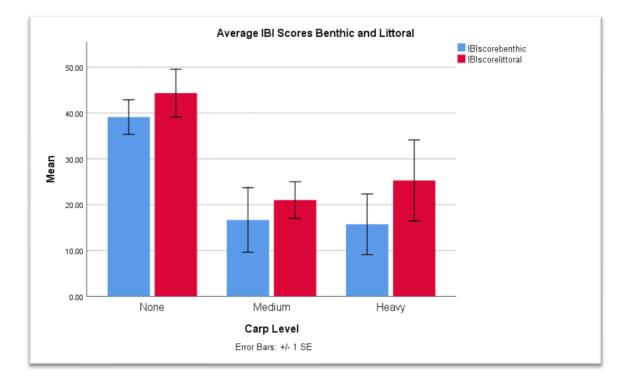


Figure 7. Average IBI benthic and littoral scores for non-carp, medium carp, and heavy carp density lakes +/- 1 SE.

Figure 8. Image of heavy carp density lake, Clear Lake, in turbid water state with noticeable algal bloom.



Figure 9. Image of non-carp lake, Duck Lake, in clear water state with lush submergent vegetation



# APPENDICES

Littoral means	Families	Total taxa	Shannon Index	Amphipod abundance	Simpson Index	% EOT
Avocet	11.8	223.75	1.52	111	0.72	0.36
Beaver	10	392.5	1.5	295	0.66	0.37
Defair	13.5	141.5	1.88	58	0.77	0.26
Duck	8.5	244.75	1.31	34	0.72	0.64
Frye	11.3	165.25	1.75	88	0.77	0.4
Little Hay	11.3	89.75	1.64	44	0.73	0.68
West long	11.5	241.25	1.73	50	0.71	0.6
Rice	8	72	1.52	30	0.68	0.7
Watts	7.5	94.75	1.42	44	0.61	0.55
Rat	14.8	277.25	1.7	130	0.72	0.48
Dewey	6.5	42.75	1.16	10	0.56	0.29
Willow	6	40.25	1.25	13	0.63	0.37
Homestea	7	29.25	1.32	3	0.66	0.4
Steverso	5.5	29	1.25	4.2	0.59	0.15
Twenty	6.8	87.75	1.21	18	0.6	0.38
one						
VNWR wil	8.3	71.5	1.3	7	0.62	0.57
Center	5.4	35.6	1.02	5	0.42	0.22
Clear	4.4	13.4	1.02	2	0.43	0.13
Goose	8	59.33	1.65	15	0.58	0.3
Hackbberry	9.2	80	1.37	38	0.68	0.35

Table A1. Mean scores for common Littoral metrics across 5 sample dates.

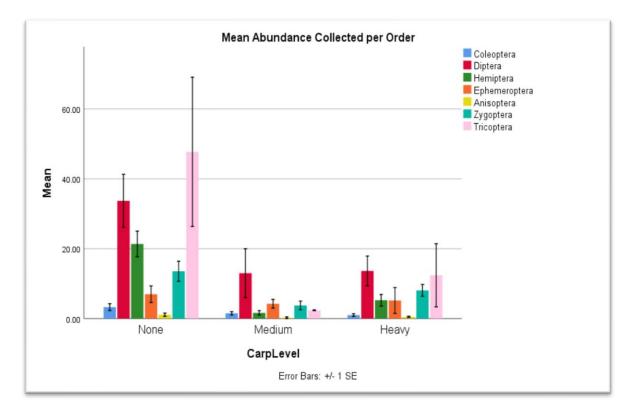
Benthic	Families	Total	Shannon	Simpson	% EOT	Chironomidae
means		Taxa	Index	Index		abundance
Avocet	6.3	111.25	0.74	0.35	0.15	92
Beaver	4.3	81.25	0.71	0.4	0.38	35
Center	1.5	12	0.08	0.04	0	3
Clear	0.8	3.5	0.09	0	0.07	6
Defair	5.8	45.25	0.83	0.4	0.23	27.25
Dewey	2.3	34.25	0.2	0.11	0.06	32
Duck	5.3	45	1.48	0.71	0.21	22
Frye	6.5	44	1.12	0.59	0.49	23.5
Goose	2	9.5	0.25	0	0.3	7
Hackberry	5	73	0.8	0.39	0.22	19
Homestead	2.5	6.75	0.25	0.17	0.2	5
Little Hay	6	42.5	1.19	0.54	0.49	18
Rat	6	33.5	1.14	0.58	0.62	12
Rice	3	35	0.54	0.28	0.51	16
Steverson	1	4.75	0.2	0.07	0.04	2.5
Twenty	2.5	13.5	0.37	0.21	0.03	8.25
one						
VNWR	3	13.5	0.55	0.3	0.4	6
willow Watta	C	7175	0.55	0.6	0.25	20.75
Watts	6	74.75	0.55	0.6	0.35	29.75
West long	4.3	115.67	0.55	0.32	0.51	73
Willow	3.3	38.25	0.61	0.34	0.4	26.25

Table A2. Mean scores for common Benthic metrics across 5 sample dates.

Order	Family	Benthic	Littoral	Carp Benthic	No Carp Benthic	Carp Littoral	No Carp Littoral
Amphipoda	Hyalellidae	492	4219	130	362	762	3457
Coleoptera	Curculionidae	1	22	1	0	4	18
Coleoptera	Dytiscidae	0	79	0	0	32	47
Coleoptera	Elmidae	6	3	2	4	0	3
Coleoptera	Gyrinidae	0	6	0	0	3	3
Coleoptera	Halipidae	2	42	2	0	8	34
Coleoptera	Hydrophilidae	3	20	0	3	9	11
Coleoptera	Lampridae	1	19	0	1	5	14
Diptera	Athericidae	0	33	0	0	0	33
Diptera	Ceratoponogidae	12	71	3	9	34	37
Diptera	Chaoboridae	25	0	16	9	0	0
Diptera	Chironmidae	1767	1066	643	1124	497	569
Diptera	Sciomyzidae	0	6	0	0	0	6
Diptera	Stratiomyidae	0	97	0	0	23	74
Diptera	Tabanidae	1	3	0	1	3	0
Diptera	Tipulidae	0	22	0	0	7	15
Diptera	Simulidae	4	5	0	4	5	0
Ephemeroptera	Baetidae	9	117	1	8	12	105
Ephemeroptera	Caenidae	67	527	29	38	351	176
Hemiptera	Belostomatidae	0	115	0	0	10	105
Hemiptera	Corixidae	1	346	0	1	155	191
Hemiptera	Gerridae	0	14	0	0	4	10
Hemiptera	Mesoveliidae	0	108	0	0	19	89
Hemiptera	Nepidae	0	31	0	0	10	21
Hemiptera	Notonectidae	1	66	0	1	24	42
Hemiptera	Pleidae	3	374	0	3	24	350
Arhynchobdellida	Hirudinidae	32	20	0	8	4	6
Hydrachinidia	Arrenuridae	0	61	0	0	32	29
Lepidoptera	Crambidae	9	34	0	6	11	18
Megaloptera	Corydalidae	0	5	0	0	3	2
Odonata	Aeshnidae	2	52	1	1	10	42
Odonata	Coenagrionidae	79	727	32	47	261	466
Odonata	Lestidae	1	55	0	1	3	52
Odonata	Libulelidae	4	71	2	2	2	69
Rhynchobdellida	Glossiphoniidae	35	24	6	1	5	3
Tricoptera	Hydroptilidae	22	44	4	18	26	18
Tricoptera	Leptoceridae	371	1532	40	331	18	1514
Tricoptera	Limnephilidae	123	100	12	111	8	92
Tricoptera	Polycentropodida	134	62	9	125	14	48

Table A3. Total Macroinvertebrates collected in the Littoral and Benthic zones of carp lakes

Figure A1. Mean abundance of taxa collected from the most common Orders for benthic and littoral macroinvertebrates combined.



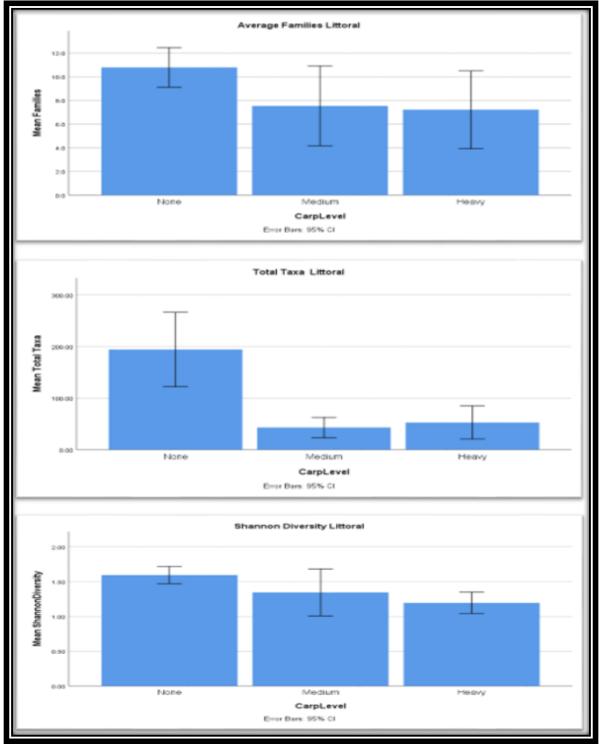


Figure A2. Average Littoral Families, Total Taxa collected, and Shannon Diversity index value for non, medium and heavy carp lakes over 5 sample trips.

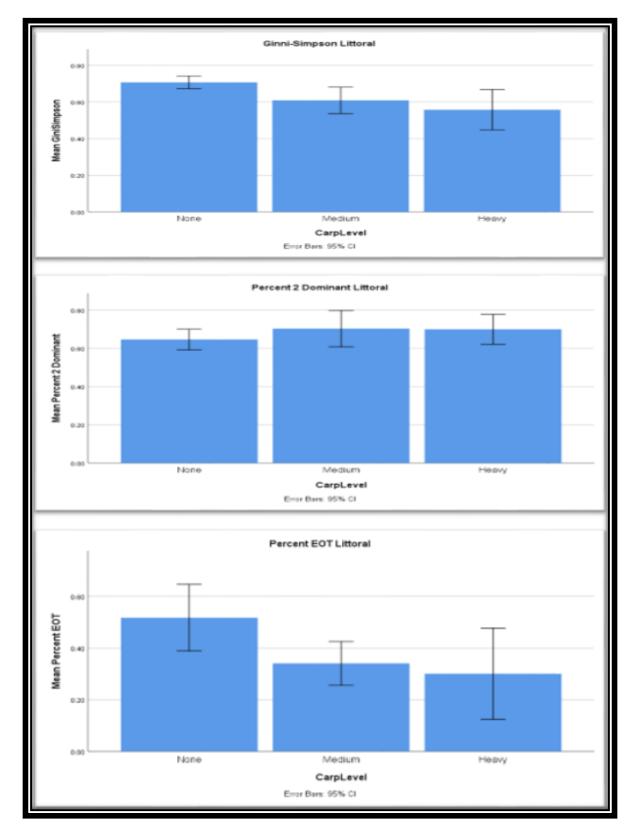
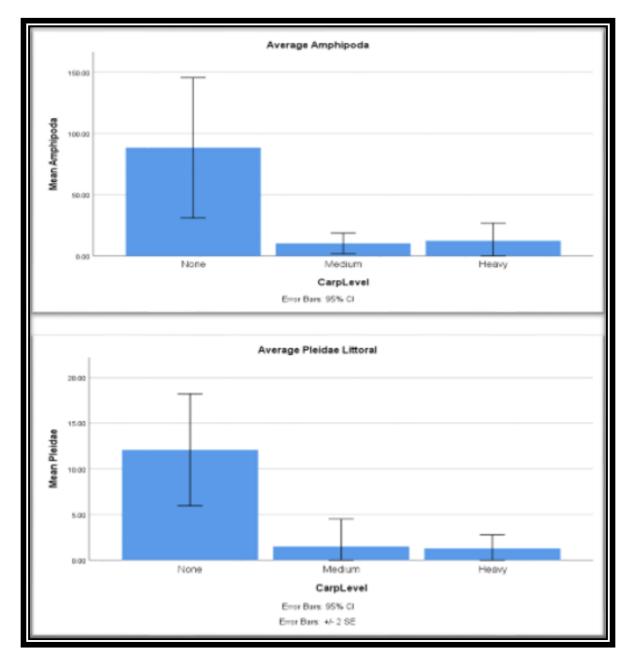


Figure A3. Average Littoral Simpson Index, %2 Dominant taxa collected, and % EOT value for non, medium and heavy carp lakes over 5 sample trips.

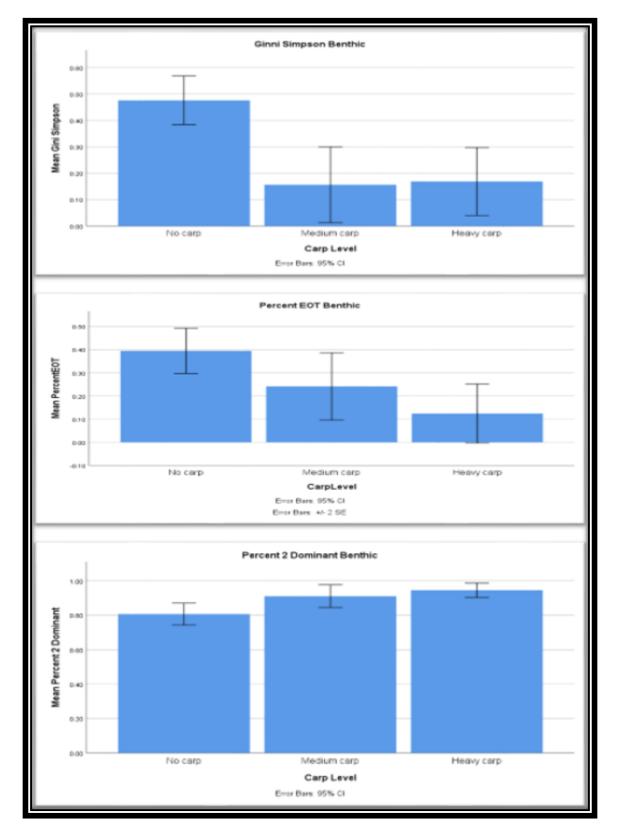
Figure A4. Average Littoral Amphipod Abundance and Pleidae abundance value for non, medium and heavy carp lakes over 5 sample trips



Total Taxa Benthic 100.00 80.00 Mean TotalTaxa 60-00 40.00 20.03 0.00 No carp Medium carp Heavy carp Carp Level Error Bars: 95% CI Average Families Benthic Mean Families Collected No carp Medium carp Heavy carp CarpLevel Error Bars: 95% CI Error Bars: 4/- 2 SE Shannon Divirsity Benthic 1.20 1.00 Mean Shannon Diversity 0.93 0.93 0.43 0.20 0.00 No carp Medium carp Heavy carp Carp Level Error Bars: 95% CI

Figure A5. Average Benthic Total Taxa collected, Family abundance, and Shannon Diversity index value for non, medium and heavy carp lakes over 5 sample trips.

Figure A6. Average Benthic Simpson Index, %EOT, and %2 dominant taxa value for non, medium and heavy carp lakes over 5 sample trips.



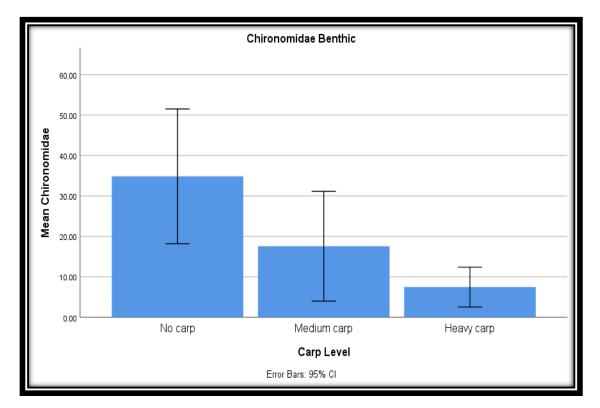


Figure A7. Average Benthic Chironomidae abundance for non, medium and heavy carp lakes over 5 sample trips.

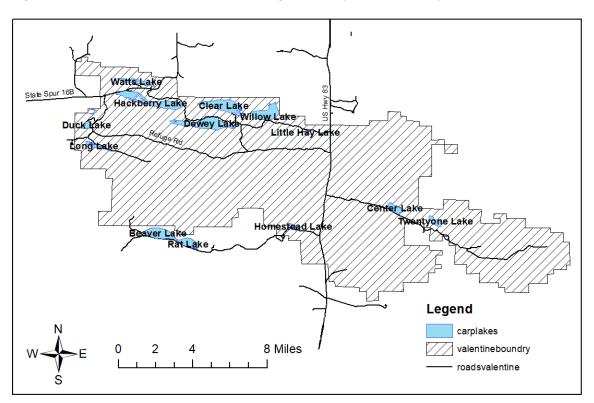


Figure A8. Valentine National Wildlife Refuge boundary, size, and study lake location. .

### VITA

### JASON DAVID GLOVER

Candidate for the Degree of

#### Master of Science

### Thesis: COMMON CARP (*CYPRINUS CARPIO*) AFFECT WATER QUALITY AND MACROINVERTEBRATE COMMMUNITES IN NEBRASKA SANDHILL LAKES

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Biographical:

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