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Can zooplankton on the North American Great Plains ‘keep up’ with climate-driven salinity change?

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Can zooplankton on the North American Great Plains ‘keep up’ with climate-driven salinity change?

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Declaration of co-authorship

The following authors contributed to the process and completion of chapter 2 of this thesis.

Chapter 2:

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Primary Author:

Mariam Elmarsafy: Planned, prepared, and maintained and conducted all experiments (unless otherwise stated) and wrote the first draft of the manuscript.

Co-authors:

Derek Gray: Assisted in execution of statistical analysis and interpretation of experiments and editing the manuscript.

Kayla Tasky: Assisted with ec50 toxicity experiments and the making of chemical treatments, and the maintenance of zooplankton cultures.

Abstract

The Great Plains of North America are projected to become more arid as the climate changes over the next century. Salinity levels for lakes in this region are linked to climate, suggesting that lakes will become more saline as the climate becomes drier. One group of organisms that might be affected by increased salinity levels are the zooplankton, aquatic invertebrates that transfer energy from primary producers to macroinvertebrates and fish. Little is known about the ability of zooplankton to adapt to long-term increases in lake salinity levels. For my thesis, I used resurrection ecology to examine how a common zooplankter, *Ceriodaphnia dubia*, responded to long-term salinity changes in Moon Lake, North Dakota; a lake that has experienced fluctuating salinity levels and for which a history of salinity levels has been reconstructed from diatoms. My thesis had three objectives: 1) To determine how salinity influences the rate of hatching of *C. dubia* resting eggs in sediment core slices of different depths; 2) To determine if the salinity tolerance of *C. dubia* has changed through time; and 3) To determine if *C. dubia*, has existed in Moon Lake throughout the last 170 years, or if the species was absent from the lake during periods of high salinity. I collected seven sediment cores from Moon Lake and counted the number of *Ceriodaphnia* eggs present in 1 cm intervals of each core over the last 170 years. I then performed experiments to determine the salinity levels that were most likely to induce hatching in *C. dubia* eggs through time, and I performed toxicity tests to determine if salinity tolerance of the species has changed through time in concert with the salinity history of the lake. My egg counts showed that *C. dubia* was present in Moon Lake throughout all core layers during the past 170 years, even through periods of intense drought and high lake salinity levels. My experiments also showed that *C. dubia* eggs preferred to hatch in saltier water following periods of drought and in fresher water following wet periods. Similarly, my toxicity experiments showed that EC50 values for *C. dubia* were higher following episodes of drought. The presence of *C. dubia* eggs throughout the sediment core during the last 170 years combined with their ability to adapt to changing salinity levels, suggests that they will likely be able to persist through coming droughts. This is a positive result, as it suggests that ecosystem functions provided by this species, including the maintenance of water clarity through filtration and the provisioning of food for other invertebrates and juvenile fish, can be expected to continue during the coming period of drought. However, since my study focused on a single species, it is not possible to determine how other zooplankton species common to Prairie lakes will respond to coming drought conditions. Future studies that examine the limits of salinity tolerance for a variety of a species using distributional data and laboratory evolution experiments would be helpful for understanding which species are likely to thrive through the droughts expected during the middle of this century.

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Chapter 1: General Introduction

Can zooplankton on the North American Great Plains ‘keep up’ with climate-driven salinity change?

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1.1 Lakes on the Great Plains of North America

The North American Great Plains is a vast stretch of land extending west from the Missouri River to the Rocky Mountains and from the Texas Panhandle to the Canadian Prairies (Figure 1.1) (Osterkamp 1987). It has a flat grassy landscape that acts as an expressway for warm fronts from the Gulf of Mexico and cold fronts from the North to meet without any obstruction, causing extreme and variable weather (Clark et al. 2002). Not only this, the Rocky Mountains west of the Great Plains block moist air from the Pacific Ocean from moving across the region, leaving the area dry and vulnerable to drought. Due to the arid climate, the Great Plains have experienced recurrent droughts within the last century during the 1930s, 1980s, and 2000s that have negatively affected rural communities, water quality and availability, and threatened freshwater resources with salinization (Sauchyn et al. 2002; Prabhakar et al. 2014). Paleolimnological studies indicate that these recurrent droughts have naturally occurred over the course of many millennia (Woodhouse and Brown 2001).

Due to climate change, the Great Plains are predicted to experience an increase in air temperatures and a decrease in precipitation within the next 50 years (Norwine 2014). Though the entire region of the Great Plains is forecasted to experience the effects of climate change, North Dakota is of particular concern because the average air temperature has increased faster than any state in America (Norwine 2014). As aridity increases and evaporation exceeds precipitation, freshwater resources can be threatened with salinization (Dai 2011; Randsalu-Wendrup et al. 2016). Currently, one third of the lakes in the Canadian Prairies are considered freshwater (0-3‰), one third are saline (3-30‰) and the remaining third are hypersaline (>30‰) (Last and Ginn 2005). On the Great Plains, lake salinity levels are tightly linked with climate. A paleolimnological reconstruction of salinity levels for several lakes in the region showed that over thousands of years salinity levels fluctuated in concert with precipitation levels (Fritz et al. 2000).

1.2 Zooplankton ecology

Zooplankton are a ubiquitous and diverse community of heterotrophic invertebrates that live in aquatic habitats and serve to transfer energy from primary producers to larger macroinvertebrates and fish (Alcaraz and Calbet 2009). They vary in size, taxonomic composition, and have diverse morphological features. Zooplankton can be found in oceans, lakes, ponds and some rivers and streams, and fill a variety of trophic niches (Alcaraz and Calbet 2009). Zooplankton are considered indicator organisms because they have a broad sensitivity to environmental changes and can therefore be used to understand and assess impacts of multiple environmental stressors (Attayde and Bozelli 1998). Some members of zooplankton are identified as ‘holoplankton’, indicating they spend their whole life in the water column with other plankton, while others are identified as ‘meroplankton’, indicating they spend only part of their life in the water column, and will eventually experience metamorphosis and in some cases, can spend part of their life cycle on land (Brierley 2017). In bodies of freshwater, the three common groups of zooplankton are cladocerans, copepods and rotifers (Ismail and Mohd Adnan 2016). Zooplankton float, drift or weakly swim in the water column, but many species undergo significant daily vertical migration to avoid visual predators and ultraviolet light (Alcaraz and Calbet 2009). Zooplankton have diverse food preferences which can include a combination of feeding on phytoplankton or algae, bacteria, detritivory (dead material), and carnivory. Many cladocerans, such as *Daphnia*, are herbivorous, while cyclopoid copepods are often opportunistic feeders, and many rotifers feed on bacteria (Work and Havens 2003). In many lakes, grazing by abundant herbivorous zooplankton can increase water clarity by preventing the overgrowth of algae (Schoenberg and

Carlson 1984). In terms of life history, cladocerans have short generation times of 2-10 days and their average lifespan ranges from 4-150 days, depending on the species (Suhett et al. 2015). Cladocerans can produce neonates every 2-3 days and can produce multiple offspring at a time; however reproductive rates are highly dependent on environmental conditions (Sipaúba-Tavares et al. 2014). Cladocerans can reproduce both asexually and sexually, and often produce resting eggs at the end of each season and/or when environmental conditions become unfavorable. If conditions are unfavourable, resting eggs will remain dormant until favoured conditions return. Calanoid copepods typically have longer lifespans than cladocerans as they can live up to 7-12 months, however some species only live for one week (Herzig et al. 1980). Calanoid copepods can only reproduce sexually, and they produce eggs which hatch into nauplius larvae (LeBlanc et al. 1997). As with cladocerans, come species of calanoid copepod can produce resting eggs. In both cladocerans and copepods, resting eggs are able to withstand harsh environmental conditions (e.g. freezing) and are resistant to desiccation (Radzikowski 2013).

1.3 *Ceriodaphnia dubia* ecology

Ceriodaphnia dubia are small (<1 mm length), herbivorous cladocerans that are found in freshwater lakes and ponds around the world. Like most zooplankton, they transfer energy from primary producers to larger macroinvertebrates and fish (Alcaraz and Calbet 2009). Depending on environmental conditions, their lifespan can range from 30 to 125 days and they are able to reproduce every 2-3 days (Cowgill et al.1985; Stewart and Konetsky 1998). Their lifespan and reproductive rate can be affected by temperature, as Filenko et al. (2011) found that a decrease in temperature from 25 to 18°C significantly increased their lifespan and reproductive rates. Due to their sensitivity to changes in the environment, *C. dubia* is recognized as a bioindicator and is often used to monitor toxins in aquatic environments (Pakrashi et al. 2013). Laboratory studies show that *C. dubia* has a low salinity tolerance. Both Cowgill and Milazzo (1991) and Armstead et al. (2016) found that fairly low salinities (< 3‰) affected the survival and reproduction of this species. Armstead et al. (2016) also found that *C. dubia* were more sensitive to NaCl than other zooplankton species such as *Daphnia magna*. However, *C. dubia* may be more tolerant of other salts. For example, a study by Lasier and Hardin (2010) found that sulfate is significantly less toxic than chlorine and bicarbonate in low and moderately-hard waters, and chloride toxicity decreases with an increase in sodium levels. Many lakes on the Great Plains are dominated by bicarbonate, sulphate, and magnesium rather than sodium and chloride (Rawson and Moore 1944), suggesting that these ions are likely of more importance for zooplankton in this region. In addition, the toxicity of contaminants for *C. dubia* is highly dependent on food availability (i.e. algal density) (Stewart and Konetsky 1998; Rodgher and Espíndola 2008). Overall, past studies suggest that understanding the salinity tolerance of *C. dubia* is not straightforward, and that the ability of this species to effectively osmoregulate likely depends on both the amount of salts in the water and the compositions of those salts.

1.4 Zooplankton in Great Plains' lakes

The chemical and physical variables that determine the structure of zooplankton communities on the Great Plains include depth, water clarity, temperature, and salinity, with salinity being the major driver (Bowman and Sachs 2008; Beaver et al. 2014). High salinity lakes tend to have a lower richness and diversity of zooplankton than low salinity systems (Hammer 1993). On Great Plains lakes, species diversity declines as salinity increases between 3-50‰ until only one or two species are present at the highest salinities. Harpacticoid copepods and *Artemia* tend to dominate high salinity lakes, while low salinity lakes can support species of *Daphnia*, *Ceriodaphnia*, and calanoid copepods (Wissel et al. 2011). These distributional patterns suggest that there is an upper salinity limit above which many zooplankton species cannot survive; however, little research has been conducted to determine survival thresholds for individual species. In addition, no research has been done to determine if there is variation in salinity tolerance within a species, or whether salinity tolerance for a species might change through time.

Since zooplankton are important members of aquatic food webs and are sensitive to changes in the environment, recent studies have been conducted to understand how they respond to acute and chronic stressors such as salinity, microplastics and chemical dispersants (Gonçalves et al. 2007; Dugan et al. 2017; Toyota et al. 2017; Ziajahromi et al. 2017). Laboratory studies of *Daphnia* have demonstrated tolerance to salinity levels ranging from 2.9‰ to 5.9‰, with larger species such as *Daphnia magna*, having shown a higher salinity tolerance. Though *Daphnia* can tolerate salinity increases to some degree, higher salinities have an adverse impact on their ability to reproduce and survive (Gonçalves et al. 2007). This is because salinity induces a physiologically demanding response that interrupts ion regulation and osmoregulation, which in turn decreases zooplankton fitness (Lee et al. 2003). Unfortunately, most studies of zooplankton salinity tolerance have been performed on species from a single genus (*Daphnia*) and with animals sourced from long-term laboratory cultures or freshwater sources. Therefore, the salinity tolerance levels found in these studies may not be applicable to regions where zooplankton experience salinity levels that vary significantly over space and time. For zooplankton to persist in lakes experiencing salinization, they must exhibit some degree of phenotypic plasticity or undergo evolution in response to salinity changes. As discussed above, there is evidence that zooplankton can tolerate a narrow range of salinity levels, suggesting some degree of phenotypic plasticity. The degree to which evolution can drive changes in salinity tolerance is less clear. In theory, zooplankton have the potential for rapid evolution because they exist in large populations, are capable of sexual reproduction and have short generation times. In changing environments, the probability of beneficial mutations occurring and being passed on to offspring are increased accordingly (Dudycha and Tessier 1999; Hairston et al. 1999; Peijnenburg and Goetze 2013). Past studies show that zooplankton can evolve tolerance to changes in pH, temperature, and eutrophication (Vehmaa et al. 2012; Frisch et al. 2014; Hann and Salki 2017), but few studies have examined salinization. One recent study provided evidence of rapid evolution to salinity tolerance in a laboratory setting, showing that *Daphnia pulex* could evolve to salinities of up to 1‰ within 5-10 generations (Coldsnow et al., 2017). Other studies have demonstrated that zooplankton may be able to adapt to salinity levels over longer time scales. For example, Weider and Hebert (1987) showed that *Daphnia pulex* from different Arctic ponds had different threshold limits to varying salinities. In addition, Ortells et al. (2005) showed variation in salinity tolerance for *Daphnia magna* hatched from lake sediments, suggesting that variation in lake salinity levels may drive the evolution of salinity tolerance in zooplankton.

1.5 Are all salts the same?

How does the composition of salts in water affect biota? Much of the recent literature has focused on salt pollution dominated by sodium due to the use of NaCl in road salt (Van Meter and Swan 2014; Hintz et al. 2019). However, the composition of salts in lakes depends on watershed geology, hydrology and groundwater sources that vary greatly among regions (Khatri and Tyagi 2015). Unlike many lakes across the world, Great Plains' lakes are sulfate and carbonate rich (Last and Ginn 2005). Since most salt-based toxicity tests are conducted using NaCl, it becomes problematic to generalize whether these tests can apply to animals that do not often interact with NaCl. To examine if different types of salts may have different effects on invertebrates, Zalizniak et al. (2006) conducted LC50 toxicity tests on freshwater invertebrates using four different types of salts and compared them with commercial sea salt. Their results showed that there were no differences in salinity tolerance related to different salt compositions, suggesting that the total amount of salt is a better indicator of toxicity rather than the composition of those salts (Zalizniak et al. 2006). In addition, Davies and Hall (2007) found interactions between sodium and Ca:Mg ratios (i.e. harder water) did not have a significant influence on the mortality rates of *Hyalella azteca* and *Daphnia magna*. Conversely, Lasier and Hardin (2010) found that *Ceriodaphnia dubia* were more tolerant to sulfate compared to bicarbonate in waters that have low-medium hardness. Taking these past studies together, it appears that more investigation is needed to determine if the composition of salts is an important consideration when discussing the salinity tolerance of zooplankton.

1.6 Physiological response of zooplankton to increasing salinity

Ionic stress can make it difficult for zooplankton to maintain internal homeostasis, which leads to significant water loss across the cell membrane (Aladin and Potts 1995). Upon disruption of their physical equilibrium, zooplankton can experience increased mortality rates, reduced lifespan, and lower rates of reproduction (Brown et al. 2004; Sarma et al. 2005). Freshwater zooplankton do have the ability to control water and salt concentrations through osmoregulation that can allow them to persist through episodes of salt pollution (Aladin and Potts 1995). Branchiopods, which include the cladocerans, have sensory organs such as the nuchal gland and epipodites which allow them to mediate salt exchange internally and maintain osmotic homeostasis (Aladin & Potts, 1995). However, physiological responses that might help zooplankton persist can have side effects, such as developmental and growth delays (Latta et al. 2012). Growth delays are a typical response common in invertebrates, whereby energy that would have been used for growth is instead used to maintain homeostasis in stressful conditions (Buchwalter et al. 2019). Slower growth may increase time to maturity, resulting in lower reproductive rates. For example, research shows that freshwater zooplankton populations that evolved to tolerate sodium chloride are 65-67% less abundant than populations that were not affected by elevated salinities (Hintz et al. 2019). The authors suggested that less grazing and overall abundance triggered cascade effects that lead to algal blooms in the affected lake (Hintz et al. 2019).

1.7 Power of the past

Climate change is expected to cause large-scale negative impacts on aquatic ecosystems in the future, but most studies lack long term-data, making it more difficult to determine the severity and timing of these expected ecosystem changes (Smol 2010). To solve this problem, paleolimnologists have used sediment profiles to better understand historical lake and landscape development, the timing and scale of anthropogenic disruptions, and temporal changes in biological communities (Michelutti *et al.*, 2009; Anderson, 2014; Koff *et al.*, 2016). This type of paleolimnological evidence has been valuable in gaining community and government support for lake mitigation proposals and monitoring programs (Moos and Ginn, 2016). In addition, lake sediments that contain seed or egg banks have been used to better understand ecological and evolutionary responses of organisms to change (Burge et al. 2018). Eggs that are layered within the sediment from different climate periods have been used to assess the long-term evolutionary traits and tolerance limits invertebrates have to ecological changes (i.e. resurrection ecology). This can be performed on zooplankton because they deposit resting eggs in the sediment that can remain dormant for centuries (Burge et al. 2018). Although this field of paleolimnology has gained attention, much work remains. On the Great Plains, climate-driven salinity change is common and a threat for freshwater zooplankton species. However, resurrection ecology has not yet been utilized to determine survival thresholds for individual species that live in this region.

Understanding how freshwater zooplankton will respond to salinization will involve using paleolimnology to measure how salinity has changed in the past coupled with climate models and drought indexes to reconstruct dry and wet periods in a specific area through time.
One strategy that could be used to examine the long-term evolution of salinity tolerance in zooplankton is to resurrect individuals that existed during periods of high or low salinities and run experiments to look for differences in salinity tolerance (i.e. resurrection ecology) (Figure 1.2) (Kerfoot et al. 1994; Korosi et al. 2017). Many species of zooplankton deposit dormant diapausing eggs that remain viable in lake sediment for decades. Organisms can be hatched from these diapausing eggs to assess how evolution has occurred in response to environmental change (Hairston et al. 1999; Frisch et al. 2014; Turko et al. 2016). The eggs can be isolated by collecting sediment cores and later can be divided into layers by depth that reveal the history of events inside and outside of the lake over millennia. With this approach, it is possible to determine how an organism might respond to a stressor in the future based on how they responded to that same stressor in the past.

For my MSc thesis, I used resurrection ecology in combination with laboratory experiments to determine if phenotypic plasticity or evolution might allow a common zooplankton species to persist in lakes as salinity increases. These experiments have filled in the two research gaps I identified above: 1) the lack of studies on salinity tolerance for zooplankton living in environments where salinity varies in space and time; and 2) the lack of studies on the long-term evolution of salinity tolerance in zooplankton. My study revealed that zooplankton can respond to climate-driven increases in salinity through evolutionary change.

1.8 Research objectives

To examine if zooplankton can tolerate long-term changes in salinity and if they show evidence of evolution in response to elevated salinity levels, I conducted the three objectives below. I have listed my hypotheses below for each objective.

1. Determine how salinity influences the rate of hatching of *Ceriodaphnia dubia* resting eggs in sediment core slices of different depths (ages) to evaluate if salinity preferences for hatching eggs have changed through time.

    Null hypothesis 1: Hatching rates in the different salinity treatments will be consistent through time, suggesting no evolutionary change.

    Alternative hypothesis 1: Hatching rates in the different salinity treatments will change through time, suggesting evolutionary change.

2. Determine if the salinity tolerance of *C. dubia* has changed through time to evaluate if this species has evolved in response to fluctuating salinity levels in Moon Lake.

    Null hypothesis 2: Salinity tolerance will be the same regardless of the layer the individual hatched from, suggesting no evolutionary change.

    Alternative hypothesis 2: Salinity tolerance will differ among individuals hatched from sediment layers of different ages, suggesting evolutionary change.

3. Examine the egg bank of Moon Lake to determine if *C. dubia* has been present in Moon Lake throughout the last 170 years, or if the species was absent from the lake during periods of high salinity.

    Null hypothesis 3: *Ceriodaphnia dubia* eggs will be present throughout all layers of the core, suggesting that adults could survive through high salinity events.

    Alternative hypothesis 3: *Ceriodaphnia dubia* eggs will be absent from sediment layers representative of high salinity periods in the history of my study lake, suggesting that adults did not survive at high salinity levels.

1.9 Structure of thesis

In the first chapter, I have provided background on my study system. I reviewed the importance of climate patterns for lake salinity levels, the ecology of zooplankton and their response to changing salinities, and the usefulness of paleolimnology and resurrection ecology for understanding how organisms have responded to past environmental changes. In the second chapter, I present a manuscript describing the experiments I conducted to examine if a common zooplankton species can tolerate long-term changes in salinity, and if the species shows evidence of evolution in response to those changes. In the third chapter, I present a summary describing my results, and I discuss the significance and future direction of my project. In my third chapter I also review how my project is integrative in nature.

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Figures

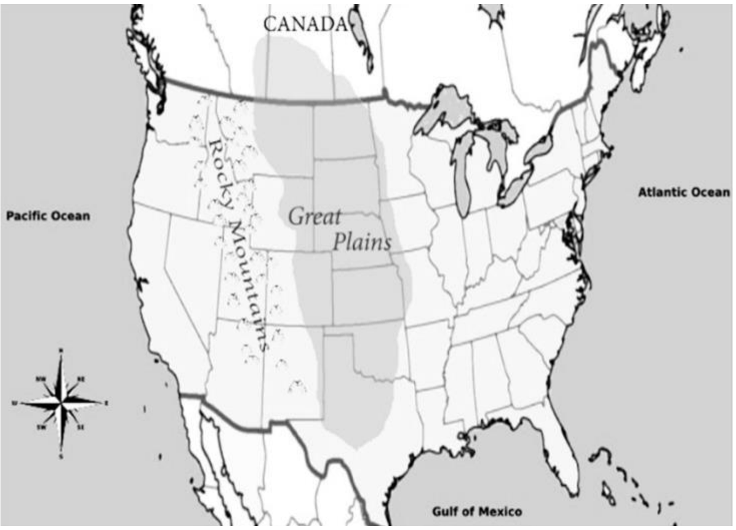

Figure 1.1. Map of North America which illustrates the location of Great Plains (shaded) and the Rocky Mountains.

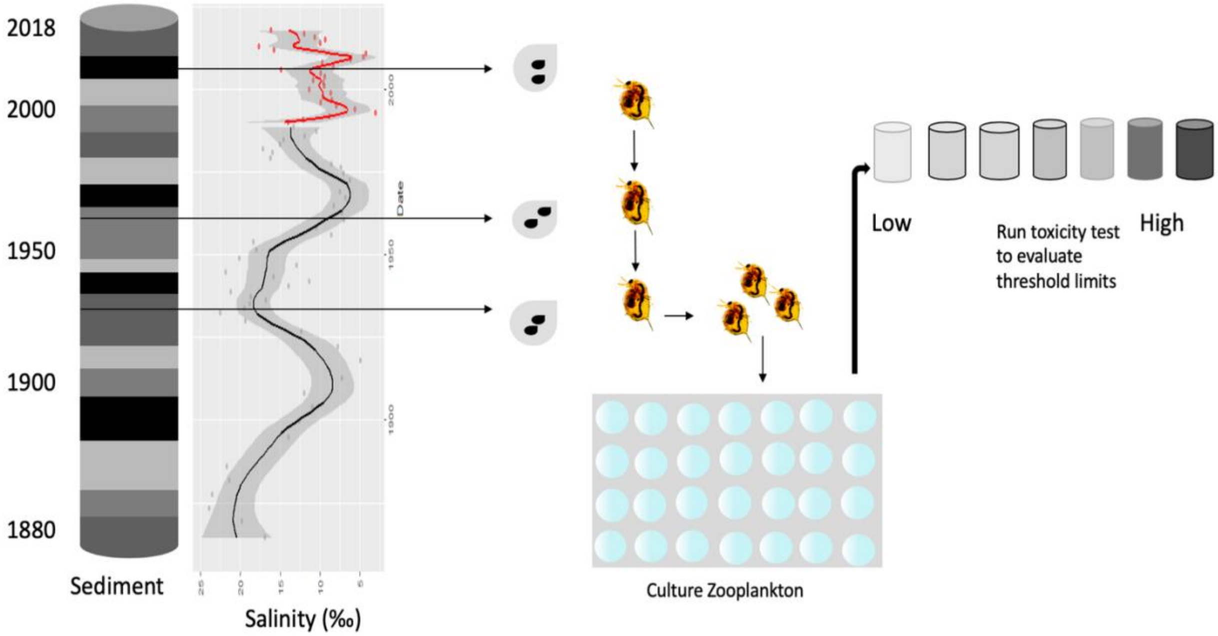

Figure 1.2. Schematic diagram of how to conduct resurrection ecology. The cylinder of on left side of the diagram represents a sediment core with various ages of layered sediment. The graph represents how salinity has fluctuated through time (black) and how to will increase in the future (red). Arrows ejecting from the sediment are pointing to isolated eggs from the sediment that can be hatched and cultured in lab. Series of cylinders on the right side represent toxicity tests from low to high salinities that can be conducted using cultured zooplankton. (Diagram adapted from image by John Megahan)

Chapter 2: Evolution of salinity tolerance in zooplankton on the Great Plains of North America

Can zooplankton on the North American Great Plains ‘keep up’ with climate-driven salinity change?

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2.1 Abstract

The Great Plains of North America are projected to become more arid over the next century. Paleolimnological studies show that lake salinity levels in this region are tightly linked with climate, and that lakes will become more saline as the climate becomes drier. One group of organisms that might be affected by increased salinity levels are the zooplankton. Although recent studies suggest that zooplankton can evolve to tolerate small increases in salinity over short time periods, few studies have examined how they respond when experiencing large increases over longer time frames. For this study, we used resurrection ecology to examine how a common zooplankter, *Ceriodaphnia dubia*, has responded to long-term salinity change in Moon Lake, North Dakota over the last 150 years. We ran experiments to determine the salinity levels that induced *C. dubia* eggs to hatch and we ran toxicity experiments to determine the salinity tolerance of adults. These experiments showed that *C. dubia* eggs hatched in saltier water during periods of drought and in fresher water during wet periods. Similarly, our toxicity experiments showed that EC50 values for *C. dubia* were higher during episodes of drought. The presence of *C. dubia* eggs throughout the sediment core during the last 150 years combined with their ability to adapt to changing salinity levels, suggests that they will likely be able to persist through coming droughts. Further studies will be needed to determine if other common zooplankton species in Great Plains' lakes are similarly adaptable.

2.2 Introduction

Increases in the concentration of dissolved salts in freshwaters – termed “salinization syndrome” – has become a concern in many regions of the world (Latta et al. 2012; Dugan et al. 2017; Kaushal et al. 2018). There are many causes of freshwater salinization, including runoff from agricultural operations, saltwater intrusion, excess use of road de-icing salt, and natural changes in precipitation and evaporation regimes (Cañedo-Argüelles et al. 2019). In some regions of the world, natural variability in lake salinity levels associated with climate cycles is expected to increase due to warming temperatures and decreases in precipitation (Schallenberg et al. 2003; Kaushal et al. 2018). For example, in regions vulnerable to drought, such as the North American Great Plains, lakes may exhibit significant oscillations in salinity levels as the climate shifts between wet and dry periods (Fritz et al. 2000). Evidence for long-term shifts in lake salinity levels has been provided by paleolimnological studies using diatoms to reconstruct salinity. These studies show that salinity levels in an individual lake can fluctuate between freshwater (0-3‰) and saline conditions (3-30‰) in response to prolonged droughts and flooding (Laird et al., 1998). If climate change leads to increases in salinity levels for Great Plains' lakes (Sereda et al. 2011), what will this mean for the organisms that live in them?

One group of organisms known to be sensitive to lake salinization are zooplankton. Zooplankton transfer energy from primary producers to larger invertebrates and fish, making them critical to lake food webs (McManus and Woodson 2012). On the Great Plains, species diversity declines as salinity levels increase between 3-50‰ until only one or two species are present at the highest salinities (Hammer 1993; Wissel et al. 2011). Harpacticoid copepods and *Artemia* tend to dominate high salinity lakes, while low salinity lakes can support a diversity of taxa, including species of *Daphnia*, *Ceriodaphnia*, *Bosmina*, calanoid copepods, and many others (Wissel et al. 2011). These distributional patterns suggest that there is an upper salinity threshold above which many zooplankton species cannot survive. Laboratory studies have shown a range of salinity tolerance levels for freshwater zooplankton species, with most tolerating less than 5‰ (Nielsen et al. 2003; Sarma et al. 2006). However, most studies of zooplankton salinity tolerance have been performed on species from a single genus (*Daphnia*) and with animals sourced from long-term laboratory cultures or freshwater habitats that exhibit little temporal variability in salinity levels (Schuytema et al. 1997; Liu and Steiner 2017; Toyota et al. 2017). Therefore, the salinity tolerance levels found in these studies may not be relevant to regions such as the Great Plains where zooplankton experience salinities that vary significantly over space and time.

For zooplankton to persist in lakes experiencing salinization, they must exhibit some degree of phenotypic plasticity and undergo evolution in response to salinity changes. As discussed above, there is evidence that zooplankton can tolerate a narrow range of salinity levels, suggesting some degree of phenotypic plasticity. However, the degree to which evolution can drive changes in salinity tolerance is less clear. In theory, zooplankton have the potential for rapid evolution because they exist in large populations and have short generation times, increasing the likelihood that mutations will arise to allow for evolutionary change (Dam 2013; Peijnenburg and Goetze 2013; Lanfear et al. 2014). Previous studies show that zooplankton can evolve in response to changes in pH, temperature, and eutrophication over many decades (Vehmaa et al. 2012; Frisch et al. 2014; Hann and Salki 2017). In addition, Coldsnow et al. (2017) showed that freshwater *Daphnia pulex* could evolve to tolerate slightly elevated salt concentrations (0.1-1‰) after 5-10 generations. In theory, the evolutionary response of zooplankton could be accelerated by the dispersal of salinity-tolerant genotypes from surrounding lakes (Cottenie and De Meester 2003), but little research has examined if intraspecific differences in salinity tolerance exist in natural populations (Huynh and Gray 2020).

Understanding how zooplankton may respond to long-term changes in salinity levels is limited by our ability to run long-term experiments in the laboratory that could simulate salinity change over decades, as happens naturally on the Great Plains (Last and Ginn 2005; Fritz 2013).

An alternative approach is to examine how zooplankton responded to past changes in salinity levels. Many species of cladoceran zooplankton deposit dormant diapausing eggs that remain viable in lake sediment for decades, and these eggs can be hatched in the laboratory to assess how evolution has occurred in response to environmental change (Hairston et al. 1999; Frisch et al. 2014). For this study, resurrection ecology was used to examine how a common zooplankton species responded to fluctuating salinity levels in the past. Sediment cores containing diapausing eggs of *Ceriodaphnia* were collected from Moon Lake, North Dakota; a lake that has experienced fluctuating salinity levels and for which a history of salinity levels has been reconstructed from diatoms (Laird et al. 1998). Zooplankton eggs deposited during the last 170 years were used to examine if the salinity tolerance of adults, or the salinity of media required for the hatching of eggs, changed through time. Changes in salinity tolerance and the salinity of media required for hatching of eggs was then correlated with diatom-inferred salinity levels and a drought index. Our results suggest that zooplankton might evolve in response to changing salinity levels and that a common cladoceran species in Moon Lake appears to be resilient to long-term changes in salinity.

2.3 Materials

2.3.1 Study site

Moon Lake, North Dakota (46.855, -98.156) was selected for our study site, as past salinity levels have been reconstructed for this lake using diatoms (Laird et al. 1998), and climate change models predict that the northern Great Plains will experience rising temperatures and reductions in precipitation in the latter half of the 21st century (Wehner et al. 2011). The present salinity of Moon Lake is 2‰, however paleolimnological salinity estimates show that levels have fluctuated substantially through time, exceeding 20‰ during the droughts of the 1930s (Laird et al. 1998). In order to gather data on the salinity history of Moon Lake, we took two approaches. First, we used data from Laird et al. (1998), which allowed for estimates of salinity levels between ~1864-1988. This data set was extended using data provided by North Dakota Fish and Game, which allowed us to complete the dataset to 2019. The data from Laird et al. (1998) allowed for estimates of salinity at a frequency of about one observation every 2.5 years, while the Fish and Game data allowed for an average salinity measurement every 0.6 years. This combined dataset was then converted to a yearly salinity estimate by first fitting a generalized additive model (GAM) to describe the relationship between the salinity and year, and then using the fitted model to provide yearly salinity estimates. The GAM was fit using the gam function in the mcgv library in R, with cubic spline as the option for smooth class, a k value of 30, and with generalized cross validation for parameter estimation (Wood 2011). We used the predict_gam function in the tidymv package to provide estimates of salinity a yearly scale (Coretta 2020).

The second approach we used to estimate salinity levels in Moon Lake through time was to use the Standardized Precipitation-Evapotranspiration Index (SPEI) as a proxy for lake salinity. The SPEI is a drought index used to quantify the intensity and duration of drought (Tirivarombo et al. 2018). Monthly average temperature and precipitation data used to calculate SPEI were sourced from nearby weather stations dependent on availability of temperature and precipitation data. Between 1896-1927 data were taken from Jamestown State Hospital (station USC00324418, 39 km from Moon Lake), between 1928-2010 data were obtained from Valley City (station USC00328937, 14 km from Moon Lake), and from 2011-2019 data were obtained from Jamestown Municipal Airport (station USW00014919, 39 km from Moon Lake). These stations measured temperature daily using a standard electronic Maximum Minimum

Temperature System and precipitation with a standard rain gauge or Automated Heating Tipping Bucket system. Full details on the equipment and frequency of measurements can be found for each station at the National Center for Environmental Information website (https://www.ncdc.noaa.gov/cdo-web/). To fill in gaps in monthly precipitation or temperature data, we used the na_interpolation function in the imputeTS library (Moritz and Bartz-Beielstein 2017) to linearly interpolate values. Only 2.2% of precipitation values and 2.5% of temperature values were missing and required interpolation. We used the spei function in the SPEI package (Vicente-Serrano et al. 2010) to calculate SPEI. For calculating the SPEI, the scale was set at 120 months, such that the SPEI would use data from a window of values over ten years. A scale of 120 months was chosen to correspond with the age range represented by each 1 cm slice of our sediment cores, which represented approximately 8-10 years (see dating methods below). High SPEI values were then indicative of periods of wet conditions while low values indicated droughts.

2.3.2 Sample collection

We collected seven sediment cores from Moon Lake, North Dakota in July 2018 and 2019 using a universal gravity corer. Cores were collected from the deepest area of the lake at ~12 m depth. Each core was sliced into 1 cm layers on shore, transferred to Whirlpak bags, and stored in the dark at 4 ºC. We used 24 cm of each core for the experiments described below, as hatching of zooplankton did not occur from deeper layers. One core was dated by measuring ^{210}Pb levels using an EGG Ortec 476A alpha counter and the Constant Rate of Supply (CRS) model (Appleby and Oldfield 1978). The CRS model was used because the excess ^{210}Pb did not decay monotonically (Figure S1) and this model has been used in past studies of Moon Lake (Laird et al. 1996). The calculations for ^{210}Pb dating were conducted using the serac package in R (Bruel and Sabatier 2020). The CRS model suggested that each 1 cm layer represented ~7-8 years of history in the lake, similar to the analysis by Laird et al. (1998), which suggested each 0.5 cm provided a resolution of approximately four years at the top of their core. Ideally, we would have conducted ^{210}Pb dating on all seven cores we collected, but due to cost and a desire to save as much sediment as possible for our experiments, we dated only one. Although the cores were collected from the same point on the lake, it is likely that spatial variation in sediment focussing could have produced differences in core chronology (e.g. Tylmann et al. 2013). Therefore, we must acknowledge that dating of layers from our cores are approximate and that differences in chronology among our cores was likely an important source of variation in our study. While at the lake in 2019 we checked for chemical stratification by collecting water at 1 m intervals down to 10 m depth using a Wildco Beta Sampler and measured conductivity of those samples with a Milwaukee MW302 conductivity meter (Milwaukee Instruments Inc.).

2.3.4 Culturing zooplankton

We maintained all zooplankton in an environmental chamber at 20°C on a 16:8 light: dark cycle. We fed the animals live *Chlamydomonas reinhardtii* algae cultured in a 0.01:1 mixture of Bold's Basal Medium and COMBO (Winner and Farrell 1976). To ensure consistency in the amount of food provided to animals for experiments that were conducted at different points in time, we measured absorbance of algae cultures on a spectrophotometer at a wavelength of 665 nm and adjusted the density of the culture until the absorbance was 1.5 before feeding. Cultures were given 10 mL of algae weekly, while smaller amounts were given to individuals during toxicity experiments (described below). Hatched zooplankton were cultured through at least three generations prior to running experiments to minimize the influence of maternal effects (Baird et al. 1989).

To create a culture medium with a composition of salts that was representative of that in Moon Lake, we collected a surface water sample from the lake and analyzed it using an inductively coupled plasma optical emissions spectrometer (ICP-OES) along with a YSI Chloride meter. To create media where the percentage of each element approximated what was found in Moon Lake, we developed an artificial salt mixture that was made up by weight of 41% KCl, 27% $NaHCO_3$, 19% $MgCO_3$, 5% Na_2SO_4, 3% KCO_3, 3% NaCl, and 2% $CaSO_4$. We created a high salinity stock solution (>50‰) by mixing these ingredients with Milli-Q water so that it could be added to COMBO medium (Kilham et al. 1998) to create lower salinity treatments for our experiments described below. The COMBO medium itself contains low concentrations of the elements found in our artificial salt mixture, but the salinity of COMBO itself is quite low (0.2‰) compared to the concentrations used for our treatments (1-10‰).

2.3.5 Experiments to determine optimum salinity for hatching of eggs

In order to separate dormant zooplankton eggs from the sediment, we washed each 1 cm layer in a 45 µm sieve to clear away fine sediment. We then extracted the eggs from the sediment using a sucrose flotation method that relies on differences in the density of eggs and inorganics (Lukić et al. 2016). After centrifugation, we poured the supernatant into a 30 µm sieve and washed it with deionized water to remove sucrose from the extracted eggs. Eggs found in the cores included *Daphnia* spp. and *Ceriodaphnia* spp. *Ceriodaphnia* eggs were more abundant, so we chose to focus on members of this genus. We transferred *Ceriodaphnia* eggs into square petri dishes containing a mixture of COMBO and the artificial salt mixture prepared as described below. We enumerated the *Ceriodaphnia* eggs in dishes and used a dissecting microscope to record the number present in each layer.

Treatments of varying salinities: 0.2, 1, 2, 3, 4, 5, 7, and 10‰ were created in the square petri dishes by adding the high salinity stock solution created from our artificial salt mixture to COMBO until the required salinity was reached. Once the petri dishes were filled with media adjusted the appropriate salinities, we divided the recovered eggs from each layer equally into these eight salinity treatments. We repeated this procedure for each layer in the top 24 cm of each core for seven separate cores for a total of 1344 individual petri dishes. We selected the salinity levels for this experiment based on the most common salinity ranges found throughout Moon Lake's history (Laird et al. 1998), as well as by examining salinity tolerances of zooplankton from Loureiro et al. (2012) and Salem et al. (2015). We checked the eggs in each treatment for hatching every second day for 14 days. To establish cultures for the toxicity experiments described below, we randomly selected 1-2 *Ceriodaphnia dubia* that hatched within the 14 day period from each salinity treatment and pipetted them into 1000 mL of COMBO with the salinity level that matched the medium in which they hatched (i.e. if a zooplankton egg hatched in 1‰, it was cultured in a medium of 1‰). We were able to successfully establish cultures of *C. dubia* from layers 0-1, 1-2, 2-3, 3-4, 4-5, 6-7, 8-9, 10-11, 13-14, 16-17, and 22-23 cm. *Ceriodaphnia dubia* was the most common *Ceriodaphnia* species (>95% of individuals hatched), so we did not attempt to establish cultures of other species. When each 14-day hatching experiment was completed, we transferred eggs that did not hatch to the fridge at 4°C for two weeks to provide a refractory period and then put them back into the environmental chambers with salinity levels matching those predicted from diatom reconstructions and/or measurements by North Dakota Fish and Game (Figure S2). This allowed eggs that were deposited during periods of drought the chance to hatch at salinities exceeding 10 ‰ (Figure S2). The eggs were checked every second day for hatching. We did not record any additional hatching in this second round.

To determine if the salinity level required to induce the hatching of eggs had changed through time, we calculated a salinity-weighted hatching index (W) core as follows:

$$W = \sum_{i=1}^{n} S_i \times \frac{H_i}{T}$$

Where S_i is the salinity of treatment *i*, H_i is the number of eggs that hatched in salinity treatment *i*, *T* is the total number of eggs hatched across all salinity treatments in that layer, and *n* is the total number of salinity treatments. A high value for this index indicated that eggs hatched in higher salinities, whereas a low value indicated that they tended to hatch in lower salt concentrations. Hatching index values were calculated for each replicate of each sediment layer. For example, for the seven sediment cores collected, we performed seven experiments on layers 0-1 cm. Therefore, salinity-weighted hatching index values were calculated for each of those seven experiments. If no eggs hatched during an experiment, a salinity-weighted hatching index value was not calculated for that particular experiment. As a result, some sediment layers in our final data set had fewer than seven hatching index values due to a lack of hatching in some experiments.

To check the alignment between the time series of salinity-weighted hatching index (*W*) values and the Standardized Precipitation-Evapotranspiration Index (SPEI) values or salinity estimates based on paleolimnological and measured data, we used cross-correlations. The cross-correlations allowed us to examine if the time series were in alignment, or if there were offsets or lags between them. The cross-correlations were done using the ggCcf function in the forecast package for R (Hyndman and Khandakar 2008). Since cross-correlations require an evenly spaced time series, we combined the hatching data from all seven cores and used the gam function in the mcgv library in R to fit a generalized additive model (GAM) to describe the relationship between the hatching index and year (Wood 2011). The model was fit using a cubic spline as the smooth class, a k value of 20, and with generalized cross validation for parameter estimation. In effect, GAMs allow for fitting a smooth function to the data, which can then be used to estimate values of the response variable (hatching index) at specific intervals. We used the predict_gam function in the tidymv package to provide estimates of the hatching index at a yearly scale (Coretta 2020). All cross correlations were conducted on time series between 1896-present due to the lack of climate data before this period that were needed to calculate SPEI.

To examine if there were significant relationships between our hatching index and SPEI or salinity values, we fit GAM models. We used the gam function in the mcgv library to fit separate models for the hatching index versus SPEI and the hatching index versus salinity. The models were fit using a cubic spline as the smooth class, with generalized cross validation for parameter estimation. A k value of 15 was used for SPEI and 10 for salinity. Salinity or SPEI values were time-shifted by seven and three years, respectively, based on the results of our cross correlations, which showed a maximum correlation at these time lags. This time shifting of the SPEI and salinity values allowed for proper alignment with the hatching index values. The gam.check function in the mcgv package was used to ensure values of k were appropriate for the data and to produce diagnostic plots to check that each GAM model provided a reasonable fit to the data.

We compared mean hatching index values between wet (SPEI>0) and dry (SPEI<0) periods using a Mann-Whitney U test (wilcox.test function in R). The Mann-Whitney test was chosen instead of a t-test because the mean hatching index values by layer were not normally distributed (Shapiro-Wilks test $p<0.05$) and could not be normalized through transformation.

2.3.6 Salt toxicity experiments

We ran acute toxicity experiments over 96 hours with *Ceriodaphnia dubia*. For these experiments, we maintained each animal individually in test tubes with 15 mL of media with a salinity of either 0.2, 1, 2, 3, 4, or 5‰. For each salinity treatment, we used 10 individuals, so that each salinity exposure was replicated 10 times. We fed each individual three drops (~0.20 mL) of *Chlamydomonas* algal culture per day for a duration of 96 h. The animals were checked at the end of the experiment to determine if they were mobile (alive) or immobile (assumed dead).

For each core slice, at least three separate acute toxicity experiments were run using different cultures. For example, if *Ceriodaphnia* only hatched in 0‰ water for one core slice, then we used animals from three of the cultures developed from that slice to run three separate acute toxicity experiments. However, we also had cases where *Ceriodaphnia* cultures were developed from eggs hatched in 1‰ or 2‰ water. In those cases, we included three replicate acute toxicity experiments for the additional higher salinity cultures. At most, we were able to establish cultures for two different salinity levels for a single layer, so that meant that three to six independent experiments (replicates) were conducted to measure EC50 for each layer. Layers with six replicates had *C. dubia* hatch in more than one salinity, necessitating three replicate toxicity experiments for cultures of each salinity. Our cultures were each started with a single individual that hatched from a resting egg, meaning that each culture was monoclonal. This meant that at a minimum, EC50 values were based on estimates from three clones hatched from each layer. We calculated EC50 values for each sediment layer using the drm and ED functions in the drc library (Ritz et al. 2015).

To check the alignment between the time series of EC50 values and the Standardized Precipitation-Evapotranspiration Index (SPEI) values or salinity estimates based on paleolimnological and measured data, we used cross-correlations. The cross-correlations allowed us to examine if the time series were in alignment, or if there were offsets or lags between them. The cross-correlations were done using the ggCcf function in the forecast package (Hyndman and Khandakar 2008). Since cross-correlations require an evenly spaced time series, we combined the EC50 data from the 3-6 replicates per layer and used the gam function in the mcgv library in R to fit a generalized additive model (GAM) to describe the relationship between EC50 and year (Wood 2011). The model was fit using a cubic spline as the smooth class, a k value of 13, and with generalized cross validation for parameter estimation. In effect, GAMs allow for fitting a smooth function to the data, which can then be used to estimate values of the response variable (EC50) at specific intervals. We used the predict_gam function in the tidymv package to provide estimates of the EC50 at a yearly scale (Coretta 2020). All cross correlations were conducted on time series between 1896-present due to the lack of climate data before this period that were needed to calculate SPEI.

To examine if there were significant relationships between our EC50 values and SPEI or salinity values, we fit GAM models. We used the gam function in the mcgv library to fit separate models for EC50 versus SPEI and EC50 versus salinity. The models were fit using a cubic spline as the smooth class, with generalized cross validation for parameter estimation. A k value of 10 was used for SPEI and 5 for salinity. SPEI values were time-shifted by nine years and salinity by seven years based on the results of our cross correlations, which showed a maximum correlation at +9 and +7 years, for SPEI and salinity, respectively. This time shifting of the SPEI and salinity values allowed for proper alignment with the EC50 values. The gam.check function in the mcgv package was used to ensure values of k were appropriate for the data and to produce diagnostic plots to check that each GAM model provided a reasonable fit to the data.

We compared mean EC50 values between wet (SPEI>0) and dry (SPEI<0) periods using a Mann-Whitney U test (wilcox.test function in R). The Mann-Whitney test was chosen instead of a t-test because the mean EC50 values by layer were not normally distributed (Shapiro-Wilks test $p<0.05$) and could not be normalized through transformation.

2.3.7 Forecasting SPEI levels for Moon Lake

In order to examine if zooplankton in Moon Lake might experience drought conditions in the future that differ from those over the last century, we obtained simulated temperature and precipitation data based on the Canadian Regional Climate Model using Coupled General Circulation Model 3 (Mearns et al. 2007). We calculated monthly mean temperature (surface temperature) and precipitation (precipitation flux) at Valley City, North Dakota based on simulated values provided at four-hour intervals over the period 2038-2061. The monthly temperature and precipitation data were then used with the spei function in the SPEI package (Vicente-Serrano et al. 2010) to calculate SPEI values for 2038-2061. To match with the SPEI values we had previously calculated for comparison with our core samples, we set the scale for calculating future SPEI values at 120 months, such that each SPEI value would be calculated based on conditions over a period of ten years.

2.4 Results

The salinity of Moon Lake was 2.1 and 2.4‰ during our visits in 2018 and 2019. Our vertical profiles of conductivity did not show evidence of a chemocline (Table S1). *Ceriodaphnia dubia* hatched from every layer of the top 22 cm of the cores except for 18-19 cm and 19-20 cm. Based on our ^{210}Pb dating, the top 22 cm covered a period of ~150 years, with each 1 cm layer representing approximately 7-8 years (Figure S1). We were able to successfully establish cultures of animals from 0-1, 1-2, 2-3, 3-4, 4-5, 6-7, 8-9, 10-11, 13-14, 16-17, and 22-23 cm. *Ceriodaphnia* eggs were present throughout all layers of the core (Figure 2.1).

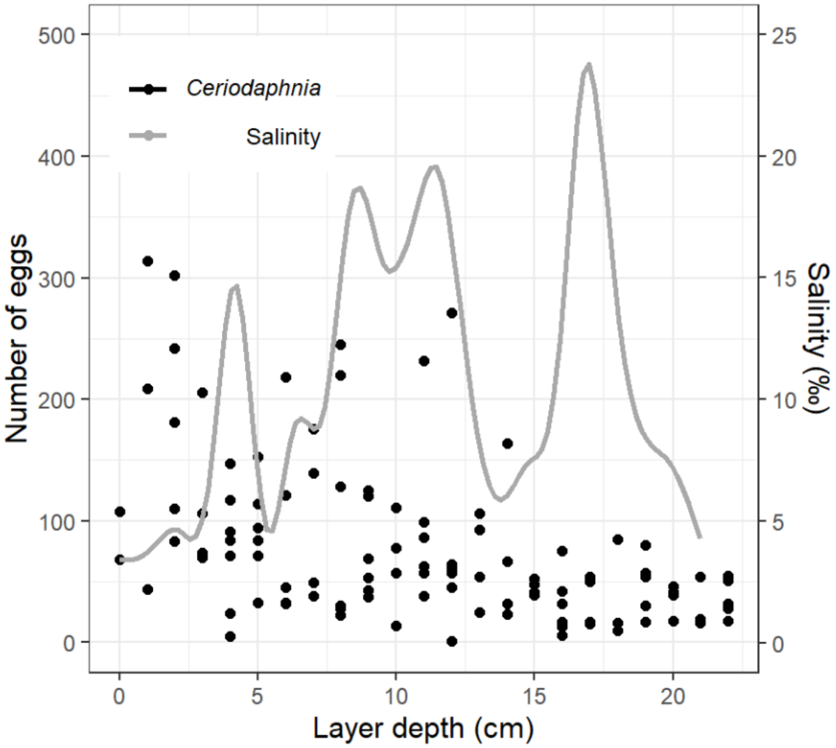

Figure 2.1 Number of *Ceriodaphnia* eggs found by layer depth in cores collected from Moon Lake, North Dakota. Also included is the diatom-based salinity estimate from Laird et al. (1998) along with recent salinity measurements provided by North Dakota Fish and Game.

2.4.1 Patterns of hatching versus salinity

The SPEI drought index exhibited a strong negative cross-correlation with the hatching index for *Ceriodaphnia* eggs with a lag of three years, over the last 120 years (Figure 2.2A, B, r= -0.62). In addition, results from our GAM showed that hatching index values were significantly negatively related to SPEI values (Figure 2.2C, Table 1). During periods of drought, the hatching index tended to be higher, while during wet periods, the index was lower. For example, during the 1930s dust bowl the hatching index value was high, indicating eggs collected from that time period hatched in saltier water than in wetter periods (Figure 2.2A). Similarly, eggs deposited in more recent periods when there was severe flooding in the region (2010s), tended to hatch in water with lower salinity levels (Figure 2.2A). Separating out our hatching index results based on wet (SPEI>0) and dry (SPEI<0) periods showed that the mean hatching index was lower during wet periods, but the difference between periods was only marginally significant (Figure 2.3A; Mann-Whitney test, p=0.06). Our hatching index also showed a positive cross-correlation with salinity levels reconstructed by Laird et al. (1998) with a time lag of seven years (Figure 2.4A, B; r= 0.55). The results from our GAM showed that hatching index values were significantly positively related to salinity values (Figure 2.4C, Table 1). Overall, 96% of the eggs that hatched did so in salinities <3‰, and no eggs in our experiments hatched in salinity treatments >7‰.

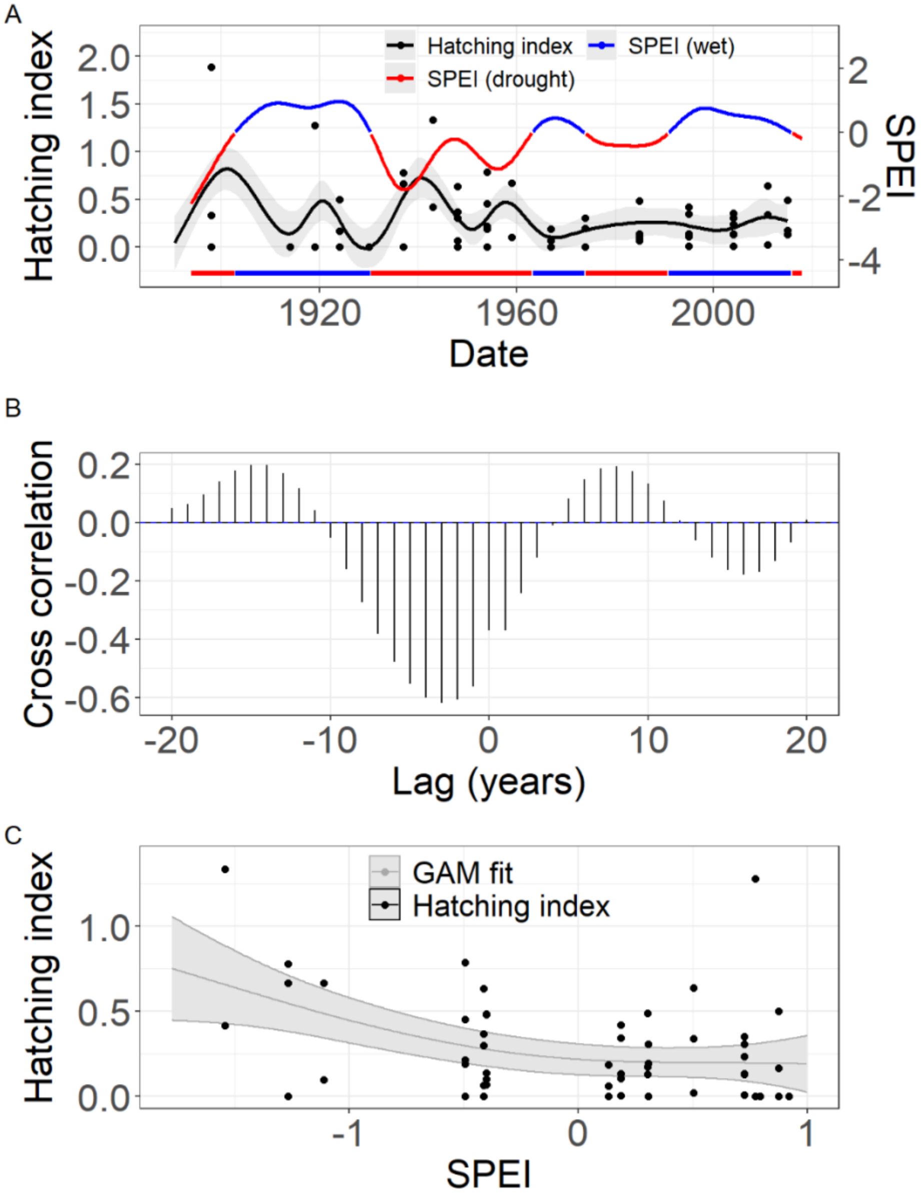

Figure 2.2 Relationship between SPEI and hatching index. Panel A shows changes in the hatching index and SPEI values through time. Lines were fitted using general additive models and the colour of the SPEI line and the bar along the x-axis identifies wet (blue) and dry (red) periods. Panel B shows the cross-correlation between the two time series. Panel C shows the relationship between hatching index and salinity using a fitted GAM model.

Table 2.1 General additive model results for hatching index and EC50 values for *C. dubia*. The predictors were smoothers for the SPEI drought index and salinity. e.d.f= effective degrees of freedom.

Model	Coefficients (Std. Error)		t (F)-value	P-value	Adj. R-square	e.d.f.
HatchingIndex~ f(SPEI)	Intercept	0.266 (0.033)	7.940	<0.0001	0.173	
	f(SPEI)	-	0.968	0.0010		1.957
HatchingIndex~ f(salinity)	Intercept	0.271 (0.033)	8.185	<0.0001	0.101	
	f(salinity)	-	0.792	0.0175		1.761
EC50~ f(SPEI)	Intercept	1.567 (0.068)	23.000	<0.0001	0.514	
	f(SPEI)	-	5.644	<0.0001		4.621
EC50~ f(salinity)	Intercept	1.631 (0.066)	24.360	<0.0001	0.592	
	f(salinity)	-	11.150	<0.0001		1.748

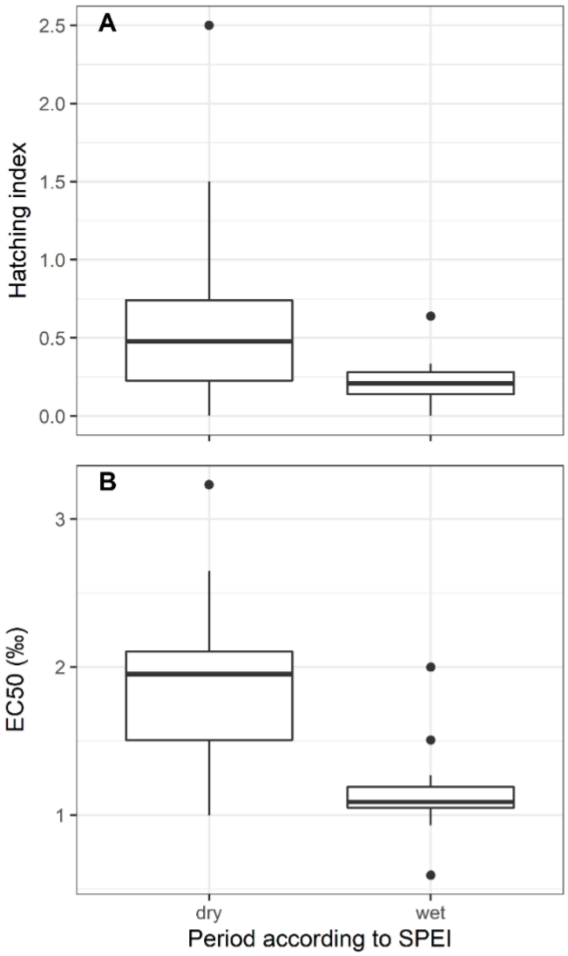

Figure 2.3 Comparison of hatching index values (panel A) and EC50 values (panel B) in wet versus dry periods in the history of Moon Lake as defined by SPEI values (SPEI >0 = wet; SPEI <0 = dry).

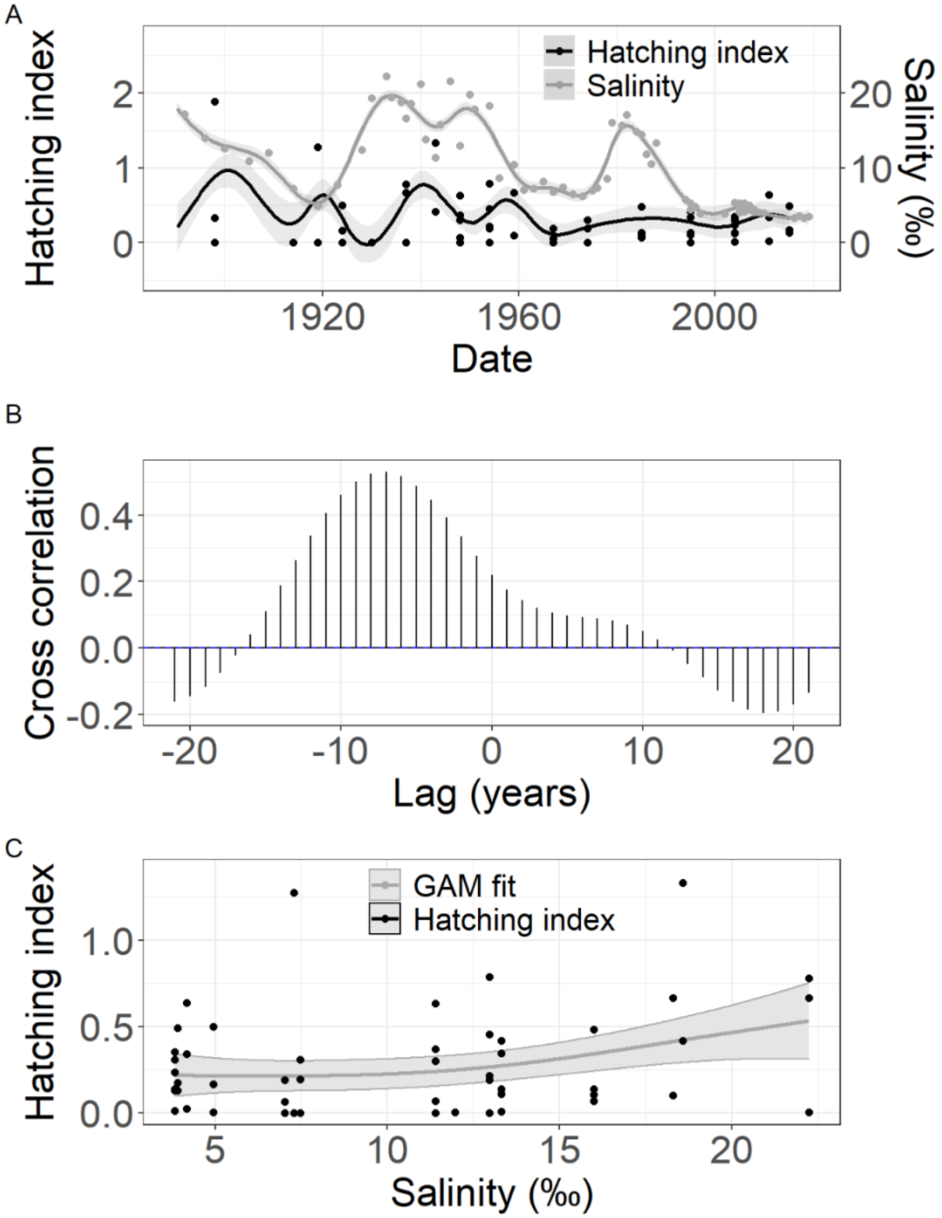

Figure 2.4 Relationship between salinity and hatching index. Panel A shows changes in the hatching index and salinity through time. Lines were fit using general additive models. Panel B shows cross-correlation between the two time series. Panel C shows the relationship between the hatching index and salinity using a fitted GAM model.

2.4.2 Changing salinity tolerances

The SPEI drought index exhibited a strong negative cross-correlation with *Ceriodaphnia* EC50 values with a maximum cross correlation occurring at +9 years over the last 120 years (Figure 5A, B, r= -0.92). In addition, results from our GAM showed that EC50 values were significantly negatively related to SPEI values (Figure 2.5C, Table 1). During periods of drought, zooplankton tended to have higher EC50 values, while the EC50 values were lower during wet periods (Figure 2.5A). For example, during extended droughts in the 1930s, EC50 values increased (Figure 2.5A). Similarly, in the 2010s when severe flooding hit eastern North Dakota, EC50 values went down (Figure 2.5A). Separating out our EC50 results based on wet (SPEI>0) and dry (SPEI<0) periods shows the magnitude of the difference between wet and dry periods (Figure 2.4B; Mann-Whitney test, p=0.0003). EC50 values also showed a strong positive cross-correlation with salinity levels reconstructed by Laird et al. (1998) with a lag of +7 years (Figure 2.6 A, B, r=0.91). The results from our GAM showed that EC50 values were significantly positively related to salinity values (Figure 2.6C, Table 1.1). Overall, no *Ceriodaphnia dubia* survived 96 h in salinity treatments >5‰.

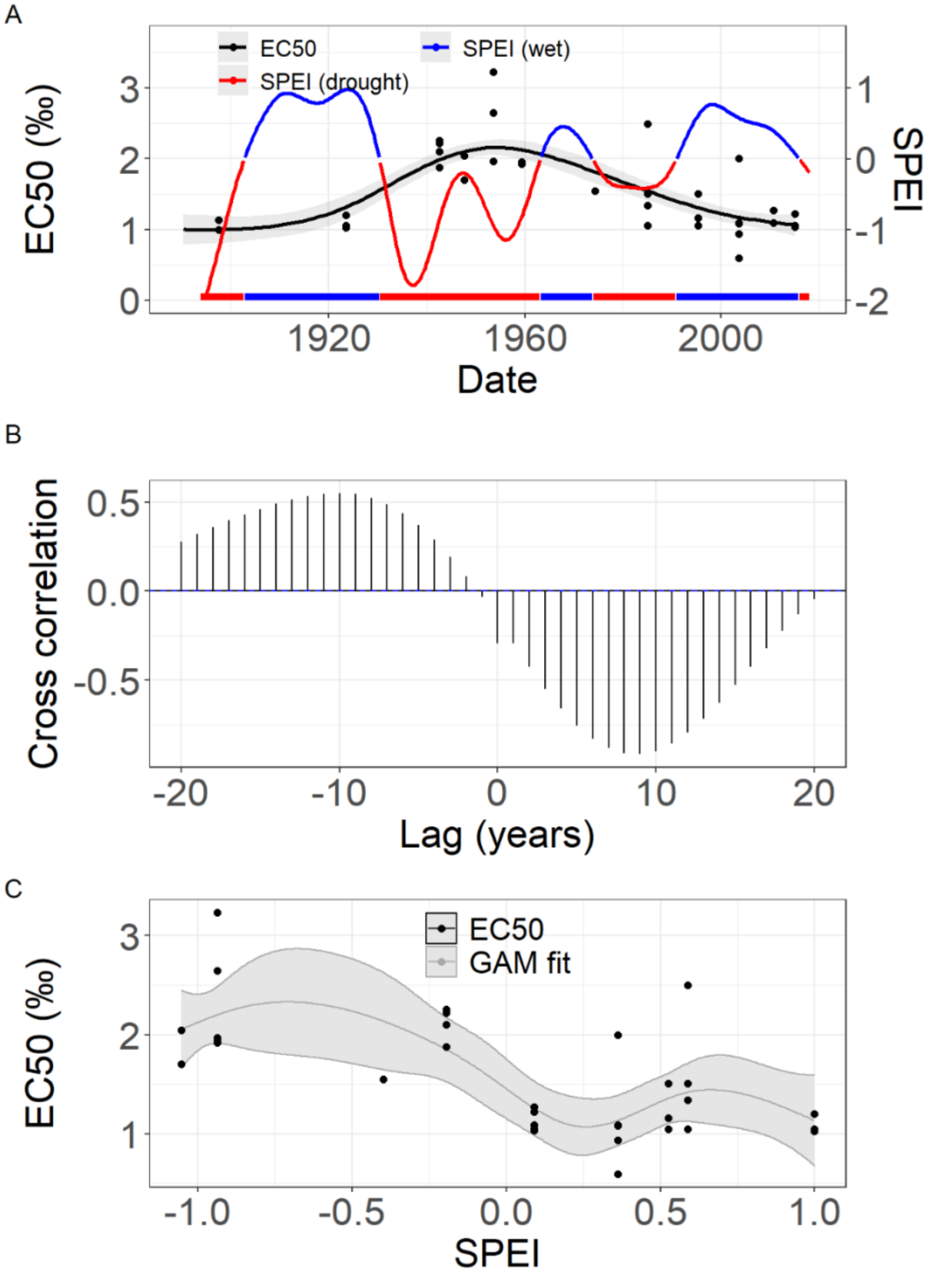

Figure 2.5 Relationship between SPEI and EC50 values. Panel A shows changes in EC50 and SPEI values through time. The lines were fit using general additive models, and the colour of the SPEI line and the bar along the x-axis identifies wet (blue) and dry (red) periods. Panel B shows the cross-correlation between the two-time series. Panel C shows the relationship between EC50 and SPEI using a fitted GAM model.

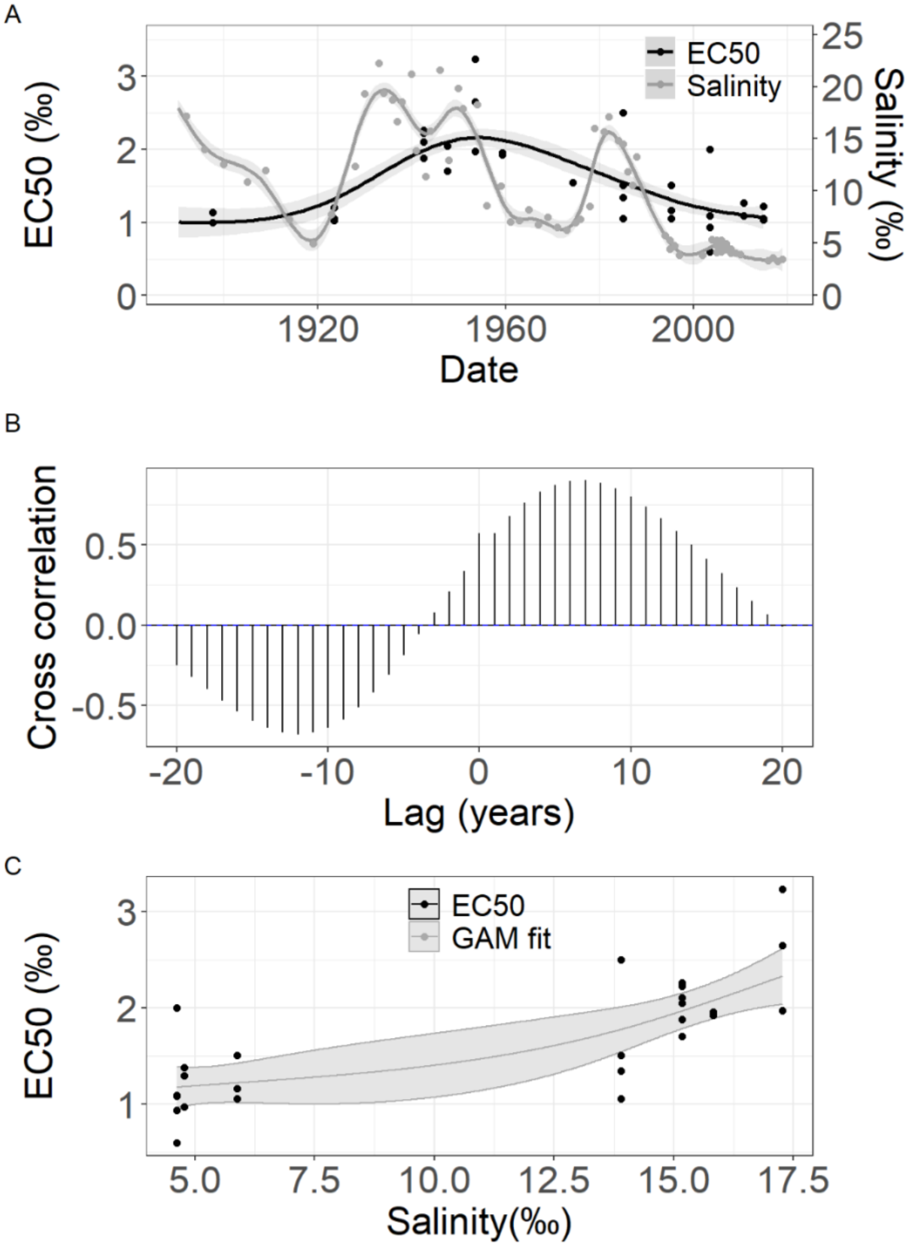

Figure 2.6 Relationship between salinity and EC50 values. Panel A shows changes in EC50 values and salinity levels through time. The lines were fit using general additive models. Panel B shows the cross-correlation between the two-time series, and Panel C shows the relationship between EC50 and salinity using a fitted GAM model.

2.4.3 Future droughts/SPEI results

The Standardized Precipitation-Evapotranspiration Index (SPEI) for the area surrounding Moon Lake showed that several cycles of wet and dry periods have occurred between 1896-2018 (Figure 2.7). The SPEI values we calculated using projections from the Canadian Regional Climate Model (2038-2061) suggested that this region will experience prolonged droughts that are more extreme than any experienced during the last 120 years (Figure 2.7).

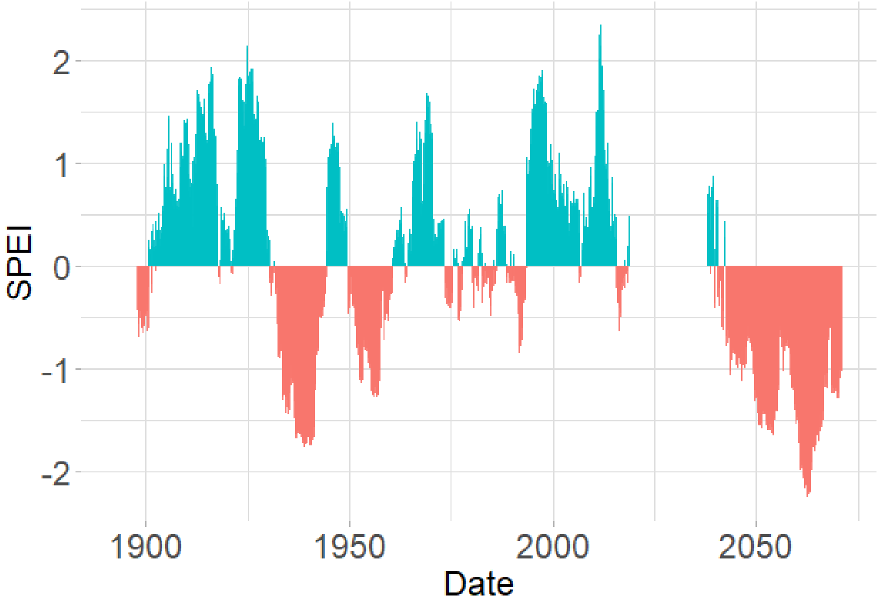

Figure 2.7 Standardized Precipitation-Evapotranspiration Index (SPEI) for the area surrounding Moon Lake. Time periods marked by blue bars have above average moisture, while those in red indicate drought conditions. Values for 1900-2018 were based on meteorological data, while the future projections (2038-2061) were calculated using data from simulations based on the Canadian Regional Climate Model (CRCM).

2.5 Discussion

Our results suggest that the salinity tolerance of *Ceriodaphnia dubia* in Moon Lake has changed through time, likely in response to fluctuating salinity levels over the last 120 years. Our hatching experiments revealed higher rates of hatching in saltier water for eggs deposited during periods of drought and higher lake salinities, while lower salinity media produced more hatching for eggs deposited during wet periods with lower lake salinities. In addition, our toxicity experiments demonstrated that EC50 values showed a similar association with fluctuating salinity levels, such that higher EC50 values were found for eggs deposited following periods of drought, and lower EC50 values followed wet periods with low lake salinity levels. Based on these results, we hypothesize that *C. dubia* has evolved in response to salinity changes through time. If our hypothesis is correct, our results suggest that the short-term evolutionary changes described by Coldsnow et al. (2017) in their laboratory experiments might take place over longer time scales in the field. Our results are also consistent with past studies that have found different tolerances to salinity depending on local habitat and history of salinity exposure (Weider and Hebert 1987; Arnér and Koivisto 1993; Sinclair and Arnott 2018), and they provide support to the idea proposed by Ortells et al. (2005) that differences in salinity tolerance of individuals resurrected from an egg bank are a result of the history of salinity changes in a system. Due to the mixed nature of their sediments, Ortells et al. (2005) were unable to link these changes to a history of changes in salinity, but here we confirm that there are relationships with lake salinity and drought, suggesting that environmental changes are likely the selection pressure driving evolution of salinity tolerance in *C. dubia*.

Although our experiments provided evidence that *Ceriodaphnia dubia* might have evolved in response to changes in lake salinity, the ability of adults to tolerate elevated salinity levels, and for eggs to hatch in elevated salinities, was surprisingly limited. Eggs did not hatch in salinity treatments >7‰, and our toxicity tests showed that *C. dubia* were unable to tolerate salinities >5‰ for 96 h. Past studies also show that *C. dubia* has a low salinity tolerance. Both Cowgill and Milazzo (1991) and Armstead et al. (2016) found that fairly low salinities (< 3‰) affected the survival and reproduction of this species. However, despite this evidence of low salinity tolerance, our egg count data, and the identity of hatched organisms, showed that *C. dubia* were present in Moon Lake throughout the past 170 years, even through periods where salinity levels were >20‰. We expected eggs to be absent during high-salinity periods because adults would be unlikely to survive, and therefore egg production would cease. So how might *C. dubia* have survived through high salinity periods, or might there be alternative explanations for our results?

One explanation for the survival of *C. dubia* through high salinity periods is that the lake may have been chemically stratified during periods of drought, resulting in lower salinity in the upper layer of the water column (Last and Slezak 2007). This could theoretically have provided a low salinity refuge for the zooplankton. However, lakes are more likely to chemically stratify (meromixis) when deeper, rather than in periods of drought when lake levels go down (e.g. Valero-Garcés and Kelts 1995). In addition, the prairies tend to experience high wind speeds (Gillette and Hanson 1989), which promote mixing, and make it difficult to maintain chemical or thermal stratification (Goodrich et al. 1987). We also did not detect a significant difference in conductivity with depth when we collected a vertical profile of conductivity measurements at Moon Lake in 2019 (Table S1), and surveys of saline lakes on the prairies show that salinity changes little with depth for most lakes (Rawson and Moore 1944). Nevertheless, it is difficult to completely discount vertical stratification of salinity as a potential explanation for the presence of *C. dubia* during periods of high salinity.

We considered several other possibilities to explain why *C. dubia* eggs were present during high salinity periods but exhibited a relatively low salinity tolerance in our experiments. First, we thought that bioturbation – the disturbance of layered sediments by living organisms – might have redistributed *C. dubia* resting eggs within the egg bank (Gyllström et al. 2008), leading to a false signal of presence during droughts. However, bioturbation by benthic invertebrates is often limited to the top 2 cm of the sediment (Kearns 1996), and periods of high and low salinity in Moon Lake were separated by several cm. In addition, the correlations we found between the SPEI drought index and measures of salinity tolerance suggested that the eggs were not mixed enough to remove that signal. Second, it is possible that individuals with the genotypes most fit for surviving in higher salinities did not hatch during our experiments. Given that we produced an artificial salt medium to match the composition of salts in Moon Lake, and that we provided opportunities for these organisms to hatch in a range of salinities, we think this is unlikely. However, we only tested 3-6 clones from each layer of sediment, which likely provided an underestimate of the range of salinity tolerance in any one layer. Alternatively, it might be possible that the eggs of individuals most likely to survive high salinities were no longer present in the sediments because individuals had hatched and entered the water column on previous occasions. This idea fits with the idea that the egg bank represents the "ghost of hatchings past," meaning that the eggs in the bank are those that did not hatch on previous occasions. Lastly, we considered the idea that Moon Lake may never have experienced salinities as high as those estimated by diatom reconstructions. Reconstructions of past conditions using any proxy will be inexact, and even studies of the same lake can produce differing salinity estimates (e.g. Laird et al. 1996 vs. Laird et al. 1998). Unfortunately, it is not possible for us to test this hypothesis, and we do not have the expertise to critically assess past paleolimnological work on Moon Lake.

Our GAM models showed that EC50 values and our salinity-weighted hatching index were significantly related to both diatom-inferred salinity and SPEI values. However, the proportion of variation explained in our models was relatively low, especially for the hatching index models. Despite the low explained variance in our models, we think it is somewhat remarkable that a response to changing salinity levels was detectable for *Ceriodaphnia* hatched from the egg bank. There were many potential sources of error and variability in our study that might have clouded this association. First, for our EC50 values, we were only able to test 3-6 clones from each sediment layer, making it possible that we missed variation in salinity tolerance of individuals in the egg bank. Second, as mentioned in the methods, we were only able to date one core of the seven used for this study, and it is likely that the chronology of the cores did not line up perfectly, even though they were collected in close proximity to one another. Third, we used diatom-inferred estimates from a separate study that obtained estimates of salinity based on dating of diatoms. Estimates of salinity in paleolimnology are made via statistical models called "transfer functions." As with all statistical models, there will be error in predictions when applying the model to a new dataset. For example, salinity levels for Moon Lake during the 20th century provided by Laird et al. (1996) differ from values in Laird et al. (1998) by as much as 10‰ for some years. This is not meant as a criticism of these studies, as the authors conducted several analyses to ensure that they were able to produce reasonable estimates of salinity given their calibration dataset. Instead, it highlights the inherent variability involved in making predictions with biological proxies. We speculate that the detection of a signal showing a relationship between salinity levels and *C. dubia* through all of this potential noise, likely means that salinity levels are an important factor affecting this species.

To evaluate the correlation between salinity/SPEI and zooplankton salinity tolerance, we used cross-correlation analysis and found lags or offsets between the time series (Figures 2.2, 2.4, 2.5, 2.6). Were these misalignments in the time series real, or were they simply artefacts of our analysis and the resolution of our data? There are many methodological reasons to be cautious about interpreting these lags as real manifestations of biological or evolutionary processes. As mentioned previously, there were likely issues with variability in the chronologies of the seven cores we used, and there was likely some error inherent in the diatom-inferred salinity estimates for the lake. Both issues are likely sources of significant variability in our data that could lead to a time mismatch between salinity/SPEI estimates and our laboratory results. In addition, we worked with 1 cm core slices, which are representative of 7-8 years according to our dating. To compare our results with salinity estimates and SPEI, we needed to use predictions from our fitted GAM models. It is easy to imagine how predictions from a GAM model fitted to data with 7-8 year gaps could lead to artificial lags in cross-correlation. If the lags we found in our cross-correlations were real, it would be much more interesting from an ecological or evolutionary standpoint. From an evolutionary standpoint, a time lag might be an indication that salinity levels changed faster than the rate of evolution of osmoregulation in *C. dubia.* This could mean there might be a mismatch between optimal regulation of internal ion concentrations and the salinity of the lake during periods of change. It might also relate to the idea by Hairston et al. (1996) that the rate of evolution in zooplankton can be slowed by resting eggs banks that contribute individuals to present-day populations that are better adapted to past conditions.

Taken together, what do our results mean for zooplankton in Great Plains' lakes as the climate changes? Our projections of the SPEI drought index using the Canadian Regional Climate Model forecast suggested that drought conditions may be extreme during the middle of the 21st century, producing conditions similar to the droughts experienced in the 1930s, but perhaps lasting longer. This increase in aridity for this region has also been predicted in other published studies (Larson 1995; Johnson et al. 2005). Our study provided evidence that a common zooplankter, *Ceriodaphnia dubia*, likely evolved in response to long-term changes in salinity levels in Moon Lake. In addition, we showed that the species was present throughout extreme droughts when salinities would be high, including during the 1930s. Overall, this suggests that a common zooplankton species has the ability to survive fluctuating salinity levels typical of lakes on the Great Plains, and will likely be able to adapt to coming drought conditions. This is a positive result, as it suggests that ecosystem functions provided by this species, including the maintenance of water clarity through filtration (O'Brien and DeNoyelles 1974) and the provisioning of food for invertebrates and juvenile fish (García-Berthou and Moreno-Amich 2000) can be expected to continue during the coming periods of drought. However, since our study focused on a single species, it is not possible for us to determine how other species common to prairie lakes will respond to coming drought conditions. Future studies that examine the limits of salinity tolerance for a variety of a species using distributional data and laboratory evolution experiments would be helpful for understanding which species would be best able to thrive during the drought conditions expected in the middle of this century.

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2.8 Supplementary material

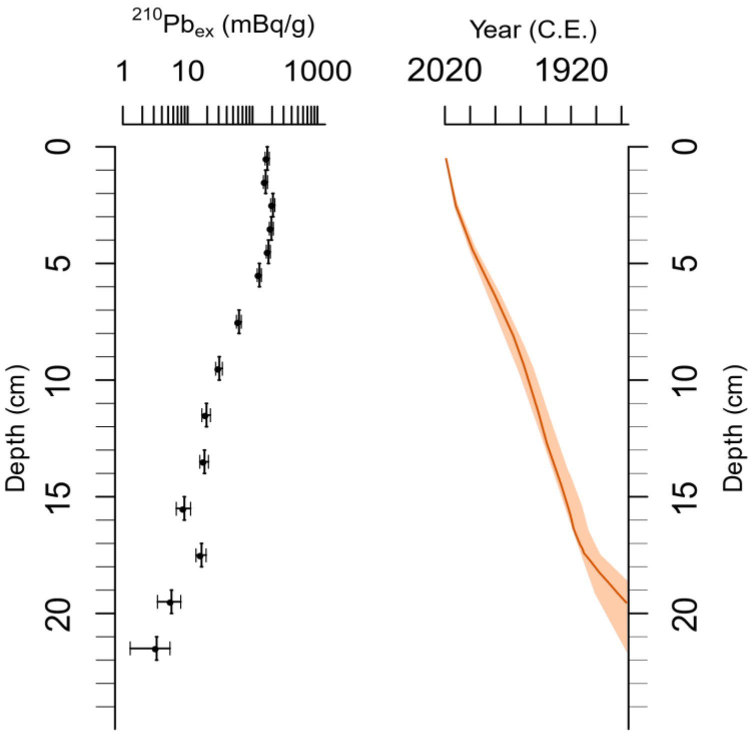

Figure S2.1 Excess 210Pb by depth (left) and age of sediment layers according to the constant rate of supply model (right). The error bars for excess ^{210}Pb correspond with the one sigma uncertainty from the alpha counter (EGG Ortec 476A).

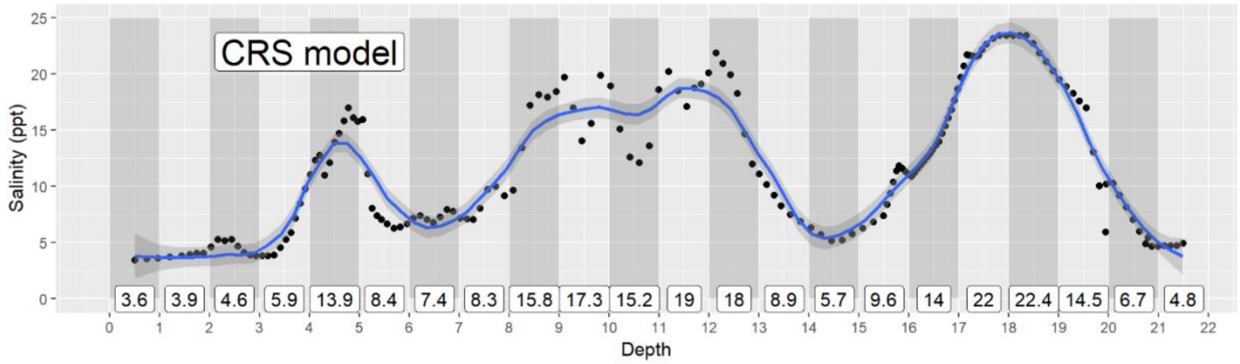

Figure S2.2 Relationship between depth of sediment and salinity of Moon Lake based on data from Laird et al. (1998) and data provided by North Dakota Fish and Game. Values in boxes across the bottom of the plot show the mean salinity of the lake within each depth layer of the sediment core. The match between the salinity levels provided by Laird et al. (1998) and the depth layers in the figure was based on the ^{210}Pb dating and the CRS model used in the current study.

Table S2.1 Vertical profile of conductivity collected from Moon Lake, North Dakota in July 2019. Conductivity values were measured at each vertical meter of depth.

Depth (m)	**Salinity ‰**
0	2.396
1	2.226
2	2.283
3	2.283
4	2.226
5	2.226
6	2.396
7	2.396
8	2.002
9	2.058
10	2.058

Chapter 3: Conclusions

Can zooplankton on the North American Great Plains ‘keep up’ with climate-driven salinity change?

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3.1 Summary

This study used resurrection ecology to examine how a common zooplankter, *Ceriodaphnia dubia*, has responded to long-term salinity change in Moon Lake, North Dakota over the last 150 years. In my experiments to determine the optimum salinity for hatching of *C. dubia*, eggs deposited during periods of drought hatched in higher salinity treatments, while those deposited during periods of high precipitation hatched in lower salinity treatments. In addition, EC50 values in my toxicity experiments had a similar association with fluctuating salinity levels, such that higher EC50 values were found for eggs deposited following periods of drought, and lower EC50 values followed wet periods with low lake salinity levels. This suggests that *C. dubia* can adapt to changes in salinity and might be able to tolerate the coming increases in lake salinity levels in prairie lakes.

Although my results show evidence that *Ceriodaphnia dubia* may have evolved in response to fluctuating lake salinity through time, the ability for individuals to tolerate elevated salinity levels, and for eggs to hatch in elevated salinities, was surprisingly low. In both the hatching experiments and toxicity tests, I found that animals had low tolerance limits and hatching preferences to elevated salinities (<7‰) through time, which was surprising given that some of these eggs were perceived to be deposited during periods of high lake salinities (>20‰) according to diatom reconstruction (Laird et al. 1998). I proposed several possibilities on the potential survival of *C. dubia* through high salinity periods. One explanation is that the lake became chemically stratified and *C. dubia* sought refuge in a layer of freshwater at the surface of the water column. It is difficult to completely discount vertical stratification, however, I think it is unlikely because climate factors such as high winds speeds and prolonged drought are characteristic of the prairies and tend to decrease chemical stratification (Goodrich et al. 1987;

Gillette and Hanson 1989; Valero-Garcés and Kelts 1995). In addition, the vertical profile of conductivity I measured at Moon Lake in 2019 did not show a significant difference in conductivity with depth (Table S1), and surveys of saline lakes on the prairies show that salinity changes little with depth for most lakes (Rawson and Moore 1944). The second possibility is that benthic invertebrates might have redistributed *C. dubia* eggs within the sediment creating a false signal of presence during droughts (Gyllström et al. 2008). One study showed that this process (termed bioturbation) is often limited to the top 2 cm of the sediment (Kearns 1996), however, periods of high and low salinity in Moon Lake were separated by several cm. In addition, the correlations I found between the SPEI drought index and our toxicity findings suggest that the eggs were not mixed enough to remove that signal. Genotypes most fit for surviving in higher salinities also did not hatch during our experiments. Lastly, I considered that reconstructed salinities in Moon Lake were inexact due to the inconsistencies in values found in the same lake (e.g. Laird et al. 1996 vs. Laird et al. 1998). Unfortunately, it was not possible to test this hypothesis, and I do not have the expertise needed to critically assess past paleolimnological evidence in Moon Lake.

To evaluate the correlation between salinity/SPEI and zooplankton salinity tolerance, I used cross-correlation analysis and found lags between the time series. These time lags could mean a number of evolutionary processes or be a misconception from the resolution of my data. For example, since we collected seven cores and but only dated one, this may have caused lags in the time series due the variability within the sediment. However, if the lags are real this could mean that salinity levels changed faster than the rate of evolution of osmoregulation in *C. dubia*.

Nonetheless, my study provided evidence that zooplankton can respond to climate-driven changes in salinity. While these results are positive, the last 120 years have not been characterized by the prolonged droughts I was expecting during the middle of the 21st century, according to the Canadian Regional Climate Model. The closest analogue would be the extreme droughts during the 1930s. Since our study did not address droughts of such length, I am unsure as to whether C. dubia will be able to persist through the droughts expected at mid-century. Likewise, other important variables such as temperature and alkalinity were not tested in combination with salinity, and these variables tend to increase together in this region (Kaushal et al. 2018). In addition, I focused on only one cladoceran species, so it is difficult to predict how the zooplankton community as a whole will weather through the periods of high salinity to come. Future studies should consider using resurrection ecology to examine how copepods and other common zooplankters have responded to long-term changes in salinity. Therefore, fully understanding how zooplankton will cope with climate change will involve further investigating species interactions with variables such as temperature and alkalinity in combination with salinity, and further investigating the response of a range of species to prolonged droughts.

3.2 Contributions to the field

One of the greatest challenges in ecological and environmental research is finding long-term data that can be used to understand how organisms respond to environmental change. Because of this, it is often difficult to propose solutions and provide realistic forecasts detailing how ecological communities might respond to changes in their environment (Smol 2010). My study used 'the power of the past' to examine the effects of fluctuating salinity levels on zooplankton found in Great Plains' lakes. This is important because salinity levels in this region are expected to rise by 2050, and I wanted to gain insight into how zooplankton might respond to these future salinity increases. Since it is infeasible to run experiments in the laboratory over many decades, resurrection ecology is an invaluable approach for understanding how zooplankton responded to past salinity changes, and how they might respond to those same changes in the future. There have been studies looking at how animals have responded to long-term fluctuations in pH, temperature, and eutrophication (Fischer et al. 2001; Frisch et al. 2014; Geerts et al. 2015) however, no study has looked at the evolution of salinity tolerance on the Great Plains over this longer time scale. My study built on previous paleolimnological evidence of changes in lake salinity reconstructed by diatoms (Laird et al. 1998) and tested if a zooplankton species found in the same lake during those salinity changes showed evidence of evolution in response. I also showed that a common drought index, SPEI, is an excellent method for examining relationships between salinity tolerance of organisms in the lake and changes in regional climate and lake salinities. In addition, my results provide a building block for future studies that might examine the response of a wider range of species to changing salinity levels in this region. Finally, my study likely underestimates the total diversity of zooplankton found in the egg bank, so it would be interesting to use genetic analysis to examine the identity of species that did not hatch by looking at the zooplankton eggs that I preserved.

3.3 How this research is integrative

Fundamentally, integrative biology is a disciple that helps resolve many of the complex issues using more than one scientific discipline (Wake 2008). Ecology was the primary discipline in this study, however I used components of other disciplines and subdisciplines such as limnology, paleoecology, evolutionary biology, physiology, chemistry, and toxicology to answer my research questions. For example, evolutionary theory helped me understand how zooplankton might have responded rapidly to changing environments and physiology helped me understand the mechanisms involved in osmoregulation and salinity tolerance. In addition, toxicology tests were used to determine threshold limits to salinity, and this helped me understand that there is an upper salinity level that zooplankton are not able tolerate. Basic chemistry allowed me to measure the constituent ions dissolved in Moon Lake and create a salt recipe to match this composition, as well as to date the sediment core I collected from the lake. Taken together, contributions from many disciplines helped me understand how zooplankton responded to ecological changes that have occurred as a result of climate change.

3.4 Relevance to other research

My research is part of a larger program looking at how zooplankton may respond to future changes in salinity in Great Plains' lakes. My research overlapped with MSc student Mercedes Huynh as she examined community responses to salinity change and the effects of the dispersal of organism among lakes. She recently finished her thesis entitled: "Dispersal as a buffer against zooplankton community change in response to fluctuating salinity levels on the Great Plains." In order to understand and predict how zooplankton communities in lakes on the Great Plains might change in response effects of climate change (i.e. increased salinity), it was important to explore the roles that both dispersal and evolution play in ecosystems responding to current and past environmental changes (Huynh and Gray 2020). While my study focused on examining whether a species that is already living in the lake can gradually adapt to changing salinities, her study focused on the ability of dispersal to mitigate change in salt-stressed zooplankton communities (Huynh and Gray 2020). One assumption of Huynh's work was that variation in salinity tolerance exists within a species, and my study showed that it is possible for salinity tolerance to vary based on the history of salinity levels in a lake. In addition, for decades researchers have been interested on how salinity has changed in freshwater lakes on the Great Plains and how this has affected the organisms living within those lakes. One of the seminal papers on this topic was the research by Laird et al. (1998) on Moon Lake. The marriage of my study with theirs will bring new insights into how living organisms from the past were able to survive through periods of high and low salinity levels in prairie lakes.

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