Investigating the Ecology and Thermal Biology of the Exotic Cladoceran *Daphnia lumholtzi*

By

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DEDICATION

This work is dedicated to my family, my chosen family, and some very supportive women who have together made this journey possible. For my daughter, who is the immeasurable joy and love of my life. You are still and will always be my favorite person and greatest source of inspiration and awe. You have been my co-pilot every day since you were born, on every road-trip and uprooting and moving from one side of the country to the other. You helped me collect plankton from canoes, kayaks, and all manner of boats. Thank you for being you. For my Mother, who is one of the smartest people on the planet, who put aside her own opportunities for the sake of her children. Thank you for the gift of joy in discovery – hunting for spider eyes by flashlight, playing with mud-daubers by the pond, collecting termites to feed the anoles, learning about every plant and why it is wonderful, and the "interconnectedness of all things". For my Dad, who taught us the true meaning of family. I miss you every day. Thank you for the life that you gave us all. For my brothers, who were with me through the worst and the best. Thank you for being my teachers, my protectors, my tribe – "Even one Pompili is quite a few" (Louis L'Amour, 1961, by way of Dad, circa 1990). For Melissa Brown, DiAnne Sharp and Emma Diaz. You lifted me up and changed my perspective, and that has changed everything. Thank you.

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Whatever the reason, you have been my circle of support, laughter, and sisterhood, and I thank you.

ABSTRACT

Daphnia lumholtzi is a microcrustacean with tropical origins in Africa and parts of Asia and Australia. The species was found in US reservoirs approximately thirty years ago where it is thought to have been introduced with fish imported from Africa. *D. lumholtzi* apparently colonized reservoirs by expanding during the summer when water temperatures rise and native *Daphnia* populations decline, suggesting that the species would only thrive in warm reservoirs during the summer months. However, *D. lumholtzi* has spread throughout North America and continues to expand into new areas. *D. lumholtzi* has been studied mostly in reservoirs, and less is known about the species' establishment in other environments. In 2002, *D. lumholtzi* was discovered in the Mobile-Tensaw Delta (MTD), providing an opportunity to study its distribution in a unique environment. In this study, surveys in the MTD and in Weeks Bay National Estuarine Research Reserve (WBNERR) found that *D. lumholtzi* occurred in low abundance in the upper MTD but was not detected in the lower delta or in WBNERR, where salinity levels are higher. In the MTD, *D. lumholtzi* was more likely to be present in the fall compared to native *Daphnia*. Laboratory experiments showed that there is no difference in the upper thermal tolerance limits between *D. lumholtzi* and the native *Daphnia ambigua* collected from the MTD. These studies show that *D. lumholtzi* populations are currently present in freshwater areas of the MTD and have likely been established since at least 2002. There are seasonal differences in the distributions of *D. lumholtzi* and native *Daphnia*, but these do not appear to be solely due to differences in

their thermal tolerances. To better understand *D. lumholtzi* invasion biology in the MTD, laboratory studies are needed to characterize the effects of high and low temperatures on life history traits of *D. lumholtzi* and native *Daphnia* from this area. Field and laboratory studies are needed to determine the effects of salinity on *D. lumholtzi* distribution in coastal environments.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

BRIEF BACKGROUND OF *DAPHNIA LUMHOLTZI* BIOLOGY AND ECOLOGY

Key features of Daphnia Physiology and Life History

Daphnia are small crustacean arthropods in the class Branchiopoda, characterized by a chitinous carapace covering the body (Figure 1.1) and two large biramous antennae used for locomotion (Figure 1.1 and 1.2). The ventral abdomen is open to the environment, where a set of flattened, feather-like appendages (Figure 1.1) agitate the water and provide continuous filtration of algae and other small organisms (Ebert, 2005). Adult *Daphnia* range in size from approximately 1.5 – 3mm, not including head and tail spines found in some species (Swaffar & Obrien, 1996; Ebert, 2005). Defensive structures such as spines and helmets form in many *Daphnia* species in response to the presence of predators such as planktivorous fish (Swaffar & Obrien, 1996). Body growth is indeterminate, with the carapace being shed by molting at each instar. Sexual maturity in females is evidenced by the pronounced appearance of ovaries in the lower abdomen adjacent to the gut, along with structural changes in the dorsal carapace to form a brood chamber. As eggs develop in the ovaries, they are covered with a chitinous membrane and deposited into the brood chamber, where they remain during development and hatching, subsequently being expelled into the environment as fully formed free-swimming offspring. Adult males can be identified by the presence of mating antennules located near the rostrum and extending from the ventral abdomen

(Figure 1.2). *Daphnia* are found globally in a variety of freshwater habitats (Ebert, 2005), where they are ecologically vital as key grazers of algae and primary forage for fish and minnows (e.g., Lampert et al., 1986; Carpenter et al., 1987).

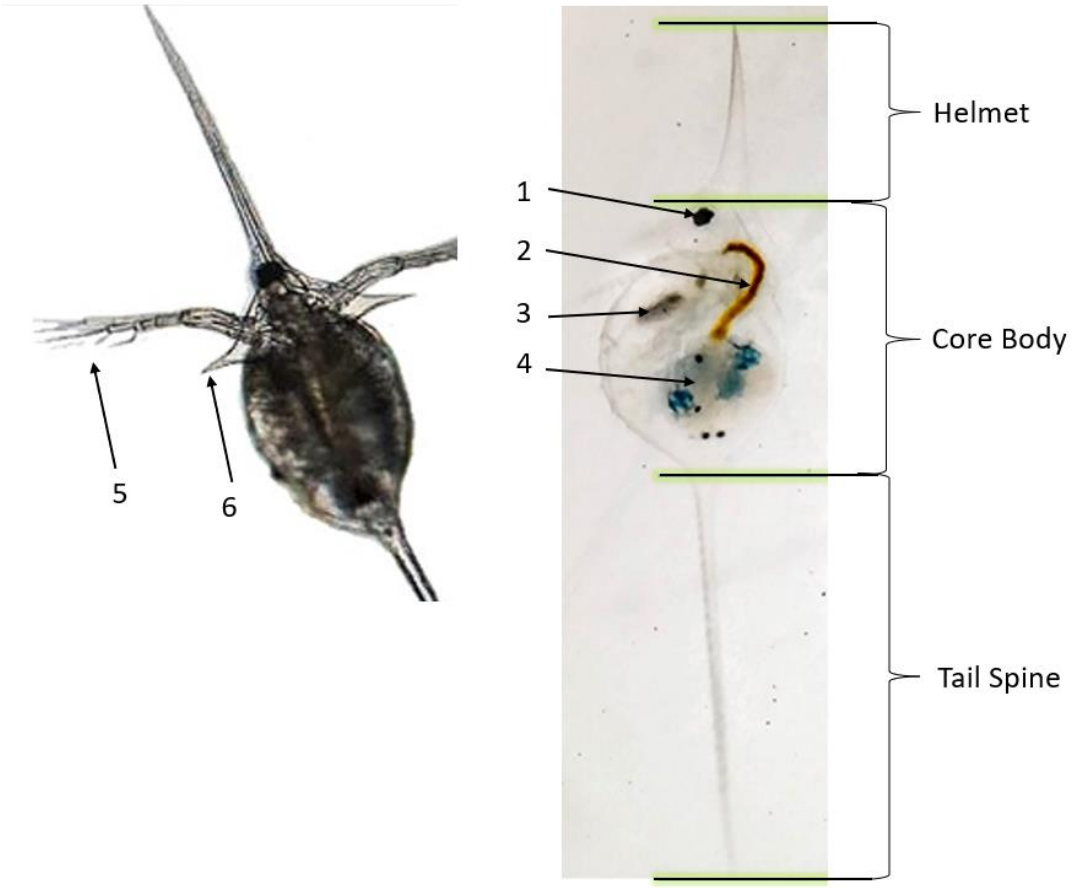

Figure 1.1. Female *D. lumholtzi* anatomy. Markers indicate the 1. compound eye, 2. mid gut, 3. filtering appendages, 4. embryos in the brood chamber, 5. Swimming antenna, and 6. cervical fornix.

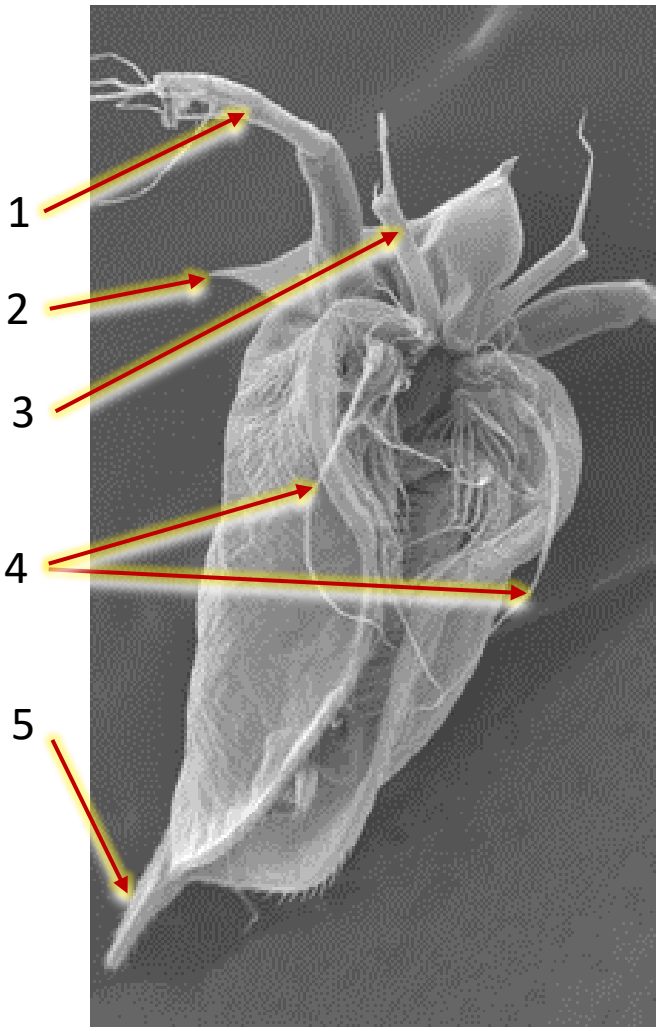

Figure 1.2. Scanning electron micrograph of male *D. lumholtzi*. Markers indicate 1. swimming antenna, 2. cervical fornix, 3. reduced rostrum antenna used in mating, 4. ventral antennae used in mating and 5. tail spine.

As aquatic ectotherms, the life cycles of these species are strongly influenced by temperature. During favorable seasons, asexual females produce clutches of parthenogenetic female offspring every few days, allowing rapid expansion of the population (Figure 1.3). Changes in temperature and other seasonal cues can induce a switch to sexual cycles, in which females produce male offspring and haploid eggs (Ebert, 2005). Upon fertilization by males, haploid eggs are encased within protective ephippia (Figure 1.4) that are shed with the carapace and may then settle into the sediment or be dispersed by flowing water or other animals and human activities (Figure 1.3). Ephippia remain dormant until environmental cues stimulate development and hatching of sedimented eggs, renewing local populations (Ebert 2005) or, in the case of dispersal, providing the opportunity for range expansion and colonization of new habitats (Havel & Shurin, 2004).

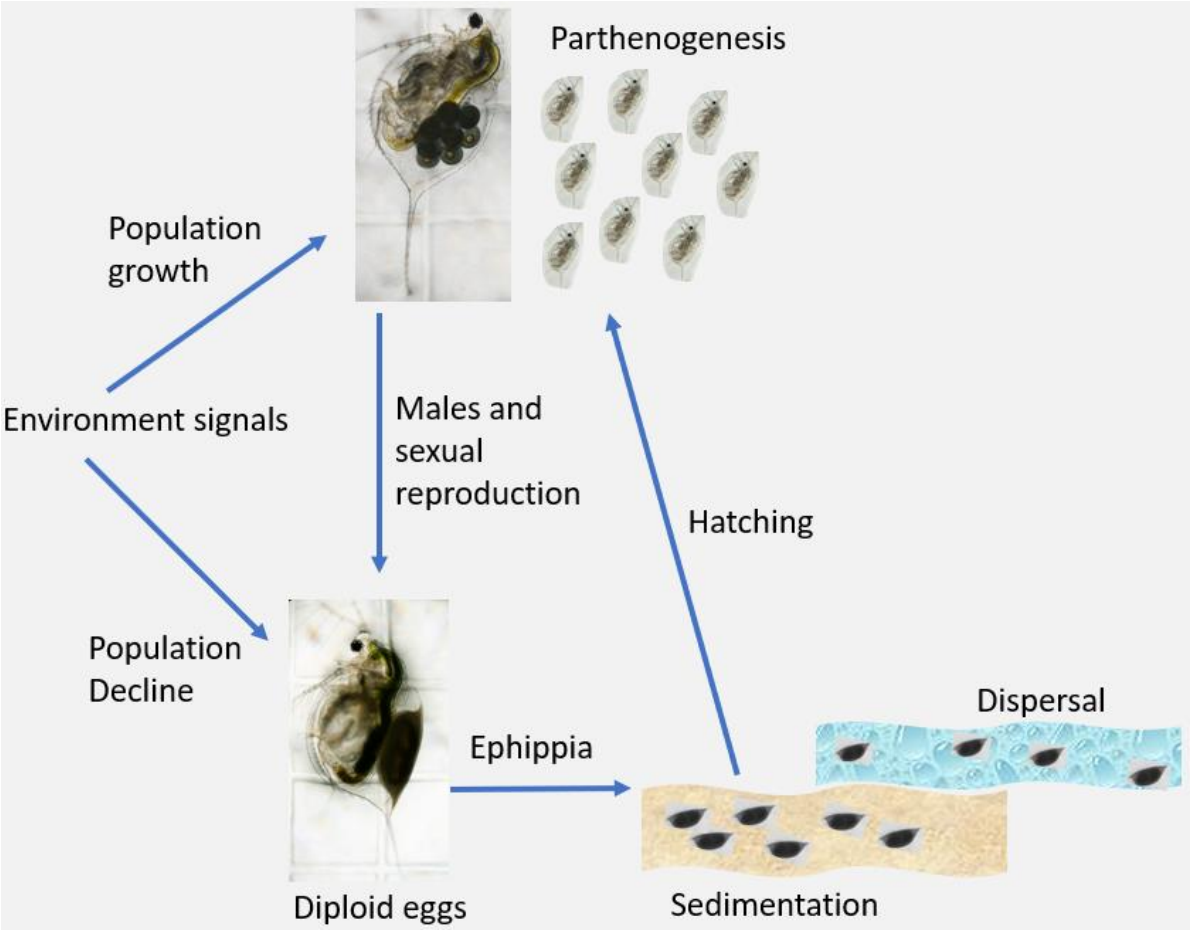

Figure 1.3. *Daphnia* Life History. When the environment signals growth, parthenogenetic females generate frequent and/or large broods of asexual females. When the environment signals decline, sexual reproduction is cued, and asexual females produce males. Fertilized eggs are encased in the ephippium and released into the environment, where they can be dispersed or sedimented to form a dormant "egg bank" that can regenerate the population in suitable environmental conditions.

Figure 1.4. Female *D. lumholtzi* with eggs encased in ephippium formed from an extension of the brood chamber (a) and ephippial eggs released during molting (b).

Daphnia lumholtzi Distribution in Native Range

Daphnia lumholtzi is a species with a broad native range in subtropical and tropical water bodies of Africa, Asia and Australia (Green, 1967; Havel et al., 2000; Mergeay, 2005), where it is found in lakes with temperatures ranging from 14°C to 29°C (Green

1967, 1971; Havel, 2000; Hebert, 1977; King & Greenwood, 1992; Lewis, 1996; Mergeay, 2005). In some large lakes and the limnetic zones of tropical areas, *D. lumholtzi* has been described as "rare" (DuMont & Van De Velde, 1977) or occurring intermittently (Fernando, 1980; Mergeay et al., 2004), yet the species is known to be widespread in major river basins throughout Africa (DuMont & Verheye, 1984). Green (1967) found that *D. lumholtzi* was the predominant zooplankter in areas of Lake Albert (East Africa) with few planktivorous fish, but smaller populations with large head and tail spines were also found in lagoons and near-shore areas of lakes where predators were present. Mergeay and colleagues (2004) reported that *D. lumholtzi* was absent from Lake Naivasha, Kenya, sometimes for long periods, but populations recovered by hatching from the egg bank when predation by fish decreased.

DAPHNIA LUMHOLTZI AS AN EXOTIC SPECIES IN THE UNITED STATES

Introduction to the United States

Exactly when and how *D. lumholtzi* was first introduced to North America is not known, but populations were confirmed to be established in Texas and Missouri reservoirs as early as January of 1991 (Sorensen & Sterner 1992). These populations are thought to have been inadvertently introduced with fish imported from Africa since early detections followed a period during which the Texas Parks and Wildlife Service had conducted trials of stocking Nile perch and tilapia to evaluate their suitability for

recreational fishing activities in (Rutledge & Lyons, 1976; Williams, 2011; US Fish & Wildlife Service, 2014).

Following early reports in Texas and Missouri, multiple studies documented the rapid range expansion of *D. lumholtzi* into reservoirs across the lower half of the U.S. from 1991- 2003 (East et al., 1999; Havel, Colbourne & Hebert 2000; Havel & Graham, 2006; Havel & Hebert, 1993; Lennon et al., 2001; Sorensen & Sterner, 1992; Stoekel et al., 1996, Work & Gophen, 1999) and began over thirty years of studies seeking to explain how the species was able to disperse and become established in new environments throughout the U.S. so rapidly. To date, established populations of *D. lumholtzi* have been detected in 412 counties spread among 26 states from the east to west coast and as far north as Wisconsin (Benson et al., 2023). The species has more recently undergone further range expansion into Mexico and South America (Elias-Gutierrez et al., 2008; Kotov & Taylor 2014; Simoes et al., 2009).

Population Dynamics in Reservoirs and Lakes

Most studies of the zooplankton community structure in U.S. reservoirs and lakes have found *D. lumholtzi* to primarily occur during the warmest months of middle to late summer (Havel & Graham 2006) when temperatures exceed 25°C, with peak abundances reported in summer and early fall at temperatures between 26-31°C (Lennon et al., 2001). In contrast, native *Daphnia* species are generally rare at temperatures above 25°C (Lennon et al., 2001), appearing in samples during winter and

spring (East et al., 1999; Havel & Graham 2006; Havens et al., 2012) and reach peak abundances at water temperatures from 20-25°C (Havel & Graham 2006; Kolar et al., 1997; Lennon et al., 2001).

Because there is apparently little overlap with native *Daphnia* populations, and no evidence of direct impacts on native *Daphnia* community structure, most studies have concluded that in reservoirs, population dynamics between *D. lumholtzi* and native *Daphnia* species are complementary (e.g. Havel & Graham, 2006), with the exotic *D. lumholtzi* undergoing population expansion when native *Daphnia* exhibit seasonal declines (East et al., 1999, 2011; Frisch et al., 2010, Havel & Graham, 2006; Havens et al., 2000, 2012; Kolar et al., 1997; Lennon et al., 2001; Work & Gophen, 1999). In these environments, it appears that tolerance to higher temperatures derived from evolving in the tropics allows *D. lumholtzi* to proliferate when water temperatures exceed the tolerance of temperate-adapted native species (East et al., 1999, 2011; Frisch et al., 2010, Havel & Graham, 2006; Havens et al., 2000, 2012; Kolar et al., 1997; Lennon et al., 2001; Work & Gophen, 1999). While the seasonal dynamics between *D. lumholtzi* and native *Daphnia* populations in reservoirs is well-studied, *D. lumholtzi* has colonized diverse aquatic habitats throughout the US, and less is known about how thermal tolerance may impact interactions of this species with native *Daphnia* in other environments (Mantovano et al., 2019). Southern areas of the US are of particular concern since they more closely resemble the native environment of *D. lumholtzi* and

may therefore be more at risk for significant impacts on native *Daphnia* community structure.

D. lumholtzi was detected in the Mobile-Tensaw Delta, AL by Devries and colleagues (2006), who reported a brief description of the species' occurrence in six locations along the delta from 2002-2005. They found that *D. lumholtzi* occurred both in freshwater areas of the upper delta and in areas of the lower delta that experience increased salinity. Their surveys of dates when *D. lumholtzi* was detected indicated that occurrences increased during the study period. The presence of *D. lumholtzi* in the delta presented a unique opportunity to examine the species' distribution in an estuarine environment. Characterizing distribution patterns in this environment will advance the understanding of *D. lumholtzi* colonization by comparison to what is known in reservoirs and lakes.

SUMMARY OF PROJECTS

In this study, zooplankton community surveys in the Mobile-Tensaw Delta were coupled with laboratory thermal determination assays to:

1. Determine whether *D. lumholtzi* populations still occur in the delta,
2. Determine whether *D. lumholtzi* becomes abundant at higher temperatures when native *Daphnia* abundance declines,

3. Establish the heat death temperatures of *D. lumholtzi* compared to the native species *Daphnia ambigua* collected from the delta,
4. Determine whether *D. lumholtzi* exhibits reduced mortality following acute thermal exposure compared to the native *D. ambigua* collected from the delta.

Field Project

The aims of this project were to determine 1) whether *D. lumholtzi* populations were present in the Mobile -Tensaw Delta and nearby WBNERR, and 2) if distribution patterns were consistent with those in reservoirs, with *D. lumholtzi* being rare during cooler periods when native Daphnia proliferate but becoming abundant at higher temperatures when native *Daphnia* decline. Zooplankton surveys in the Mobile-Tensaw Delta (MTD) were carried out to establish whether *D. lumholtzi* populations were still present because several years had elapsed since they were reported in the MTD (DeVries, 2006), and colonization of novel environments by introduced species is generally unsuccessful (Elton, 1958). Zooplankton survey sites were chosen based on previous observations of *D. lumholtzi* occurrence by Dennis DeVries (2006), who kindly provided maps and Global Position System (GPS) coordinates of sites sampled in his study (Figure 1.5 and Table 1.1), and by accessibility of the sites during our study. One site was chosen based on personal communication with Mr. Chris Mixon, a local guide who provided transport and technical assistance on the MTD, that zooplankton and

other small organisms were frequently present in large numbers near Blakely State Park on the Tensaw River (Chris Mixon, personal communication).

Table 1.1. *Daphnia* survey sites in the Mobile Tensaw Delta.

Survey Site	Latitude (N)	Longitude (W)
D'Olive Bay	30.638250'	-87.921417'
Bay Minette Basin	30.695188'	-87.921649'
Blakely State Park	30.750929'	-87.922372'
Gravine Island	30.802807'	-87.929330'
McReynold's Lake	30.901301'	-87.930634'
Hurricane Landing	30.863267'	-87.895717'
Meaher State Park	30.667253'	-87.936777'

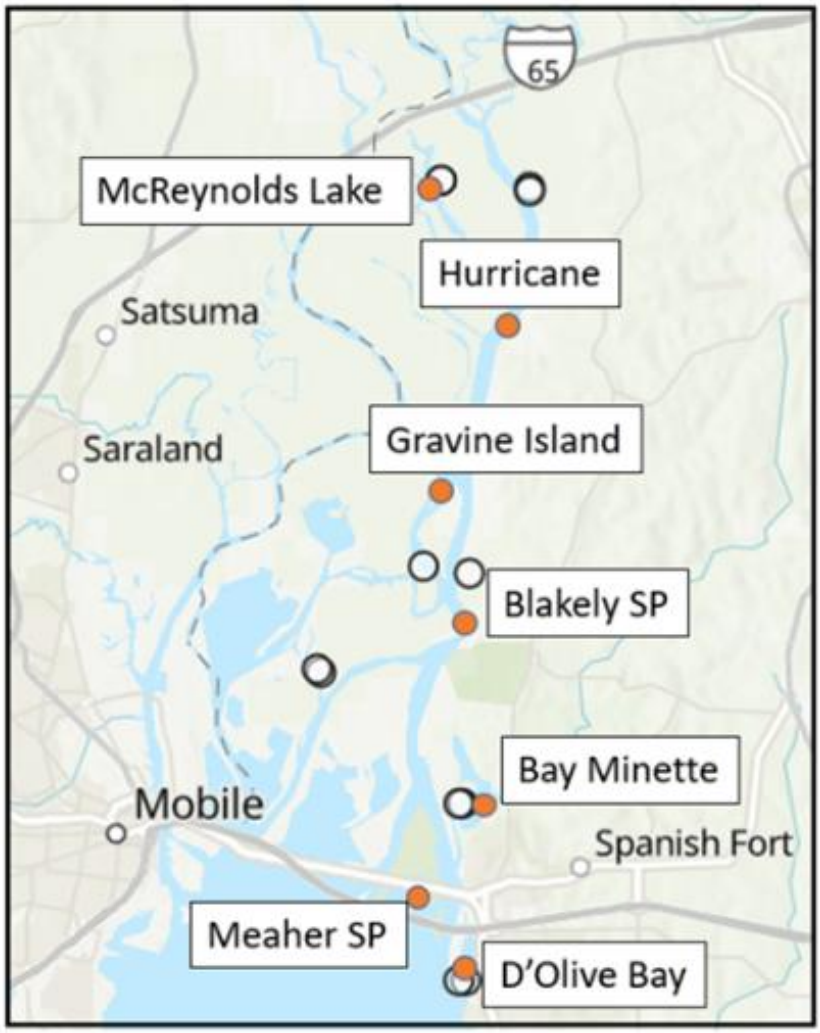

Figure 1.5. Map of *Daphnia* survey sites in the Mobile-Tensaw Delta, Alabama. Red circles show locations where *Daphnia* were surveyed monthly for this study. White circles show locations where *D. lumholtzi* was previously detected from 2002-2005 (DeVries et al., 2006). The straight-line distance from McReynolds Lake to D'Olive Bay is approximately 32 kilometers.

A second series of sites (Table 1.2) was sampled in the nearby Weeks Bay National Estuarine Research Reserve (WBNERR, Figure 1.6) with the generous assistance of Scott Phipps, State Ecologist, Alabama. This estuary is a similar environment to the MTD but receives greater salt influx into the riverine areas (Scott Phipps, personal communication). These sites were surveyed to determine the presence of *D. lumholtzi* beyond the delta since aquatic invertebrates are known to be dispersed by water, human activities, and other animals (Havel & Shurin, 2004), and *D. lumholtzi* has exhibited rapid range expansion since being introduced to the U.S. (Benson et al., 2023)

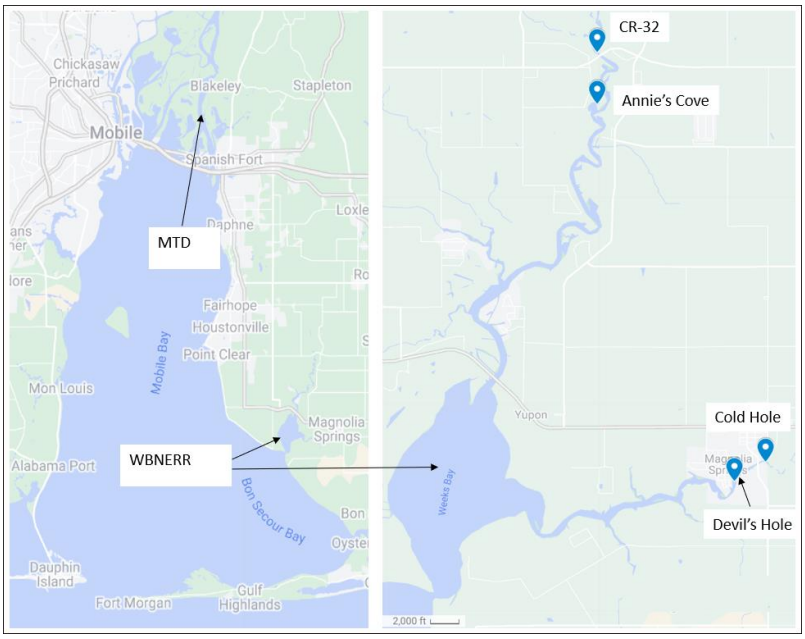

Figure 1.6. Map of Weeks Bay National Estuarine Research Reserve (WBNERR). Labels show proximity to the Mobile-Tensaw Delta (MTD, left panel) and survey sites in the WBNERR where native *Daphnia* were detected (right panel).

Table 1.1 *Daphnia* survey sites in Weeks Bay National Estuarine Research Reserve.

Survey Site	Latitude (N)	Longitude (W)
WBNERR Water Quality Site	30.416944’	-87.822500’
Bay Mouth Water Quality Site	30.380556’	-87.831944’
Fish River Annie's Cove	30.465556’	-87.803333’
Fish River County Road (CR-32)	30.475000’	-87.803333’
Fish River Piney Isles	30.431111’	-87.824444’
Magnolia River Cold Hole	30.400000’	-87.768333’
Magnolia River Devil's Hole	30.396389’	-87.774722’
Magnolia River WQ Site	30.391389’	-87.817222’
Magnolia River Nolte Creek	30.389167’	-87.800833’

Sites were sampled monthly whenever possible based on weather conditions and access to the sites by boat. *Daphnia* were collected by sampling the water column from the near-bottom to the top of the water column using a 153-micron mesh Wisconsin plankton net (Wildco, Yulee, Florida) attached to a 30-meter tow line. To the greatest extent possible given weather and water conditions, samples were taken within a 30-meter radius of each site using a GPS system.

The results of this project confirm that *D. lumholtzi* populations are currently present in the delta. However, neither *D. lumholtzi* nor native *Daphnia* exhibited distinct regular cycles of population abundance and decline. Instead, both groups were generally found only in low numbers. Because *Daphnia* densities were too low to determine

abundances, the presence or absence of *D. lumholtzi* and native *Daphnia* was documented to compare trends. Unlike Devries and colleagues (2006), we did not find *D. lumholtzi* in the brackish sites of the lower delta.

Although *D. lumholtzi* and native *Daphnia* were both present over a wide range of temperatures, there was a significant difference in their occurrence rates seasonally. *D. lumholtzi* was more common in fall while native *Daphnia* were more common in winter. With respect to temperature, 70% of the time that *D. lumholtzi* was present, the water temperature was above 25°C. In contrast, 60% of the time that native *Daphnia* species were present, water temperatures were below 25°C. Thus, patterns reflected what has been observed in reservoirs, but the differences were in winter and fall rather than spring and summer. The occurrence patterns of the two groups were not significantly different in spring and summer, suggesting that at least some native species have thermal tolerance in the same range as *D. lumholtzi*. In contrast to the MTD, *D. lumholtzi* was not detected in any of the sites in the WBNERR. The results and methods of this project are detailed in the publication (submitted) included as Chapter 2 of this dissertation.

Thermal Tolerance Project

Since *D. lumholtzi* distribution in other environments has been linked to having higher thermal tolerance than native species, the aims of the thermal tolerance project were to

establish the heat death temperature and upper thermal limits of *D. lumholtzi* and compare these to native *Daphnia* found in the MTD. It is important to make comparisons among habitat types because colonization success and distribution patterns may vary in environments with different ecological characteristics (Hansen et al., 2020), or within one environment like the MTD, where abiotic conditions like salinity and temperature change rapidly (Ricciardi, 2015). Further, MacIsaac and colleagues (1985) found that thermal tolerance differences varied among species based on the maximum temperatures experienced in their natural environment. Animals were therefore collected from the MTD and acclimated to laboratory culture for these experiments. Since heat death is a measure of maximum physiological tolerance, this project first measured the heat death of *D. lumholtzi* and compared it to the heat death of *D. ambigua* collected from the MTD. After the heat death was determined, thermal tolerance trials were conducted to determine the upper thermal limits of *D. lumholtzi* between the heat death temperature and the maximum temperature measured in field collection sites. Results from these trials were also used to derive the median lethal temperature dose (LD50) measured 48 hours after recovery from heat exposures. These were compared between *D. lumholtzi* and *D. ambigua* to test whether *D. lumholtzi* has greater upper limits of thermal tolerance compared to *D. ambigua* collected from the MTD.

Daphnia Laboratory Cultures

Individual Daphnia were isolated in 2-liter beakers to establish clonal lineages of each species. Laboratory cultures were maintained between 22-23°C (ambient) with a 16-hour photoperiod in modified lake water (Figure 1.7) with gentle aeration provided by tubing connected to aquarium pumps. Lake water was collected in clean carboys and aerated overnight in the laboratory. Daphnia culture water was prepared by filtering lake water over G6 glass-fiber filters (Fisher Scientific, USA), adjusting conductivity to 400-600 micro-Siemens/centimeter and pH to 7.2 – 7.4. Water was filtered over a sterile 0.22- micron filter (Millipore, USA) before use to prevent contamination and growth of microorganisms. Daphnia were fed ad libitum with the algae Scenedesmus (Carolina Biological Supply), Nannochloropsis (Reed Mariculture, USA), or a mixture of both.

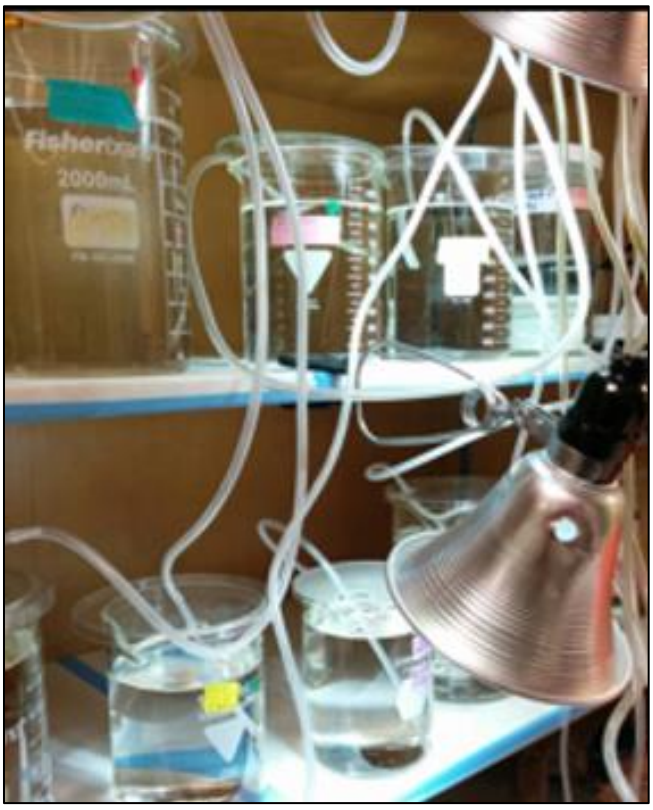

Figure 1.7. Laboratory cultures of *Daphnia* clones. Single female *Daphnia* collected from field sites were isolated in individual beakers to establish clonal lineages for use in laboratory studies.

Species Identification

Daphnia lumholtzi was identified morphologically by the unique helmet and sharply pointed neck fornices present in this species (Figure 1.1). Native *Daphnia* were initially separated and identified morphologically using digital image-based anatomy keys kindly provided by Paul Hebert, University of Guelph (Hebert 2005) and image-based morphological keys (Haney et al., 2013) published online at the University of New Hampshire Center for Freshwater Biology. Where there was ambiguity, *Daphnia* morphological identifications were confirmed by DNA barcoding using detailed protocols kindly provided by Dr. Manuel Elias-Gutierrez, University of Guelph. DNA was extracted from tissues using the HotSHOT technique developed specifically for isolation of DNA from crustacean zooplankton (Montero-Pau et al., 2008). In some cases, the HotSHOT technique was unsuccessful and commercially available kits were used (QIAamp DNA Micro Kit, Qiagen). Zooplankton-specific primers (Prosser et al., 2013) were used for the polymerase chain reaction (PCR) to amplify isolated DNA from a single egg or antenna. The PCR reaction mixture and amplification profile are provided in Appendix I. PCR products were separated and visualized on 2% agarose gels using GelGreen DNA stain (Biotium, Inc). Samples with positive PCR products were sequenced by a commercial vendor (Eurofins, Lancaster, PA.; MC Lab, San Francisco, CA.) and compared to known sequences published in Genbank (https://www.ncbi.nlm.nih.gov/genbank/).

Four native *Daphnia* species were found to occur in samples collected from the MTD. These included *D. ambigua*, *D. magniceps*, *D. obtusa* and *D. parvula*. *D. magniceps* is found in ponds and swamps of the central U.S. and potentially the southeastern U.S. (Taylor et al., 1998). *D. obtusa* is common in shallow ponds in the south-central and eastern U.S. (Gillooly & Dodson, 2000; Hebert and Grewe, 1985; Hebert et al., 1989). *D. parvula* is a common species in south-central U.S. reservoirs (Johnson & Havel, 2001). The most common species found in both the MTD and WBNERR was *D. ambigua*, a temperate species with a native geographic range that spans from southern Canada to central Argentina (Hebert et al., 2003).

Thermal Experiments

Establishing the heat death temperature is important because it identifies the boundaries between the highest temperature to which an animal can become acclimated and the critical thermal maximum (Jobling 1981). Between these boundaries, the exposure time to a given temperature will have a strong impact on survival, but above the critical thermal maximum, heat death occurs (Jobling 1981). The heat death temperature can therefore be used to determine which species have greater capacity for thermal acclimation (Jobling, 1981). The heat death can be measured by placing *Daphnia* in a small volume of water and heating them at a constant rate until swimming ceases, and recording this temperature (Kivivuori et al., 1996). and the animal is monitored to ensure it does not recover after removal from the heat. Any animals that

recover are removed from the analysis and discarded after the monitoring period is complete.

Another common method to characterize differences in upper thermal tolerance limits is to expose animals to a sublethal temperature and then monitor their survival or mortality during recovery (Kivivuori et al., 1996). This assay can therefore be used to compare species' capacity to recover from thermal stress. In this study, the data from these trials was also used to estimate the median lethal temperature dose (LD50) that resulted in 50% mortality (LD50) after a given recovery period. Specifically, 48-hour mortality was used to compare responses between *D. lumholtzi* and native *Daphnia* since this is a commonly used reference measure for aquatic ectotherms (Kivivuori et al., 1996). The 48-hour assessment time is ecologically relevant since *Daphnia* can generate eggs and release free-swimming offspring within two days (Obreshkove 1940, Orcutt & Porter 1984, Yurista 2004).

Summer surface water temperatures can reach 32- 33°C in the MTD, indicating that this is the maximum temperature range that *Daphnia* are exposed to in the field. In heat death trials, Daphniids began to show signs of swimming cessation near 39°C. To compare differences in mortality following exposure to sublethal temperatures, therefore, experimental animals were heated to target temperatures of 32°C - 39°C.

The results of this project showed that there was no significant difference in the heat death temperature of *D. lumholtzi* compared to the native species *D. ambigua*. This indicates that these species have similar physiological capacity for heat tolerance and thermal acclimation. 48-hour mortality was not significantly different between *D. lumholtzi* and *D. ambigua* at any temperature, nor was there a difference in their estimated LD50 temperatures. This indicates that *D. lumholtzi* populations that occur in the delta do not have higher thermal tolerance than *D. ambigua* found in the delta. The detailed results methods of this project are provided in the publication (submitted) included as Chapter 3 of this dissertation.

CHAPTER 2: Occurrence Patterns of *Daphnia lumholtzi* in the Mobile-Tensaw Delta Ten Years after Detection

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ABSTRACT

Characterizing the factors that influence exotic species distribution in novel habitats is important to advance the understanding of invasion biology and predict impacts. *Daphnia lumholtzi* is an exotic zooplankter with tropical origins that has colonized water bodies throughout North America. In reservoirs, high thermal tolerance allows *D. lumholtzi* populations to expand during summer when native *Daphnia* are seasonally rare, whereas native *Daphnia* populations peak during cooler periods when *D. lumholtzi* is rare or absent. Less is known about *D. lumholtzi* distribution patterns in other environments, but in 2002-2005, *D. lumholtzi* populations were found in the Mobile-Tensaw Delta, Alabama (MTD), sometimes occurring in sites with elevated salinity. Since few studies have documented *D. lumholtzi* colonization of coastal environments, we sought to determine whether the species has persisted in the MTD, and if population patterns were consistent with those observed in reservoirs. We surveyed the MTD from 2011-2013 to monitor the occurrence of *D. lumholtzi* and native *Daphnia* in relation to temperature and season. Occurrence patterns were significantly different (χ^2 = 17.333, df = 1, $p < 0.001$) , with *D. lumholtzi* more common in autumn and native *Daphni*a more common in winter. However, both groups occurred at temperatures from 12 - 31°C and their occurrence rates in summer were similar, suggesting that other factors also impact their distribution. Our results show that *D. lumholtzi* populations have likely been present in the MTD for at least ten years, and the species overlaps with native *Daphnia*

in the summer. However, the species was not found in brackish water sites, suggesting that salinity levels may limit *D. lumholtzi* distribution in the delta.

INTRODUCTION

The transport and introduction of species into areas outside of their native range became commonplace with the advent of global shipping of goods, the exotic pet trade, and intentional importation of animals for human use (Havel et al., 2015; Pimentel et al., 2005; Strayer, 2010). While most introduced species may fail to colonize new habitats, and a relatively small proportion exert deleterious effects (Ehrlich, 1986; Elton, 1958), there has been a significant increase in the incidence of transplanted species becoming invasive, causing significant harm in their introduced environments (Simberloff, 2013; Strayer, 2010). Impacts may include biodiversity loss, species extinctions, reduced ecosystem services (Simberloff, 2013; Strayer, 2010), and substantial economic costs to industry (Pimentel et al., 2005). Aquatic ecosystems are at particular risk due to international shipping, a primary vector for exotic species introductions, largely through the exchange of ballast water (National Research Council, 1996). The interconnectedness of waterways allows greater dispersal of introduced organisms and their propagules (Havel & Medley, 2006; Simões et al., 2009), complicating mitigation efforts. Aquatic species are often introduced deliberately or inadvertently through intentional stocking (Cohen & Carlton, 1998; Sorensen & Sterner, 1992) or by escape or release from the aquaculture industry (Courtenay Jr. & Williams, 1992) and aquarium trade (Padilla & Williams, 2004). Once established, invasive aquatic species are difficult to eradicate (e.g., Andradi-Brown, 2019), and subsequent dispersal can occur actively through migration (Andradi-Brown, 2019), passively in flowing water

(Havel & Shurin, 2004), by transport on vessels and equipment (Havel & Stelzleni-Schwent, 2000) or by other animals (Frisch et al., 2007, Taylor-Jarnagin et al., 2000). Since aquatic species introductions are not likely to abate (Seebens et al., 2021), it is important to understand the factors underlying their colonization and distribution in novel environments to predict areas at risk for invasive impacts and identify factors limiting their expansion.

Compared to exotic vertebrates, less is known about introduced zooplankton, but there are notable examples of invasive effects (reviewed in Dexter & Bollens, 2020), particularly among microcrustaceans like the predatory cladoceran *Bythotrephes longimanus* (Dexter & Bollens, 2020). *Bythotrephes* impacts native zooplankton communities by direct predation (Kerfoot et al., 2016) and by inducing behavioral and life history changes that impact population growth (Pangle & Peacor, 2006), which can subsequently impact fish populations (Hansen et al., 2020). *Bythotrephes* and other cladocerans exhibit life history traits associated with successful colonizers (Havel et al., 2015), including parthenogenetic life cycles. Female *Daphnia*, for example, can reproduce asexually every few days, potentially allowing a single live female to colonize an introduced habitat (Gerritsen, 1980). *Daphnia* also release environmentally rugged ephippia containing viable fertilized eggs that can regenerate local populations from sedimented egg banks, even after prolonged periods of dormancy (Frisch et al., 2013). The establishment, distribution, and impacts of aquatic invertebrates can vary among introduced ecosystems that have differing environmental characteristics (Hansen et al.,

2020), or within a single ecosystem that exhibits environmental gradients or instability, such as estuaries (Ricciardi, 2015). One introduced species that has demonstrated an exceptional capacity for colonization of new habitats is the zooplankton *Daphnia lumholtzi.* The native range of this cladoceran includes a broad distribution in Africa, southwestern Asia, and Australia (Green, 1967; Havel & Hebert, 1993). First detected in Texas and Missouri reservoirs in 1991 (Havel & Hebert, 1993; Sorensen & Sterner, 1992), the species has colonized habitats across the Americas (Benson, et al., 2022; Kotov & Taylor, 2014), from the Great Lakes of the U.S. and Canada (Muzinic, Benson, et al., 2022) to Brazil and Argentina (Kotov & Taylor, 2014; Nunes et al., 2018). A risk assessment by the U.S. Fish and Wildlife Service (2016) concluded that *D. lumholtzi* poses a high invasive risk in the U.S. based on the potential for continued range expansion, competition for resources with native *Daphnia*, and being associated with reduced populations of native zooplankton in some habitats (Kolar et al., 1997). Besides parthenogenesis, the species exhibits other traits associated with successful aquatic invaders (Bates et al., 2013), such as higher thermal tolerance compared to native *Daphnia* (Lennon et al., 2001).

Surveys in reservoirs and lakes colonized by *D. lumholtzi* found the species is rarely detected during cooler months but occurs at higher densities when water temperatures rise above 25°C (Havel & Graham, 2006; Kolar et al., 1997; Work & Gophen, 1999). *D. lumholtzi* becomes common in reservoirs primarily during the inherent seasonal decline

of native *Daphnia* populations (Lennon et al., 2001; Work & Gophen, 1999). Alternating seasonal peaks between the exotic and native species' populations suggests that *D. lumholtzi* may colonize these habitats through thermal niche segregation, proliferating during conditions too warm for temperate native *Daphnia* (Dzialowski et al., 2000; East et al., 1999; Havens et al., 2000; Kolar et al., 1997; Lennon et al., 2001). Less is known about the establishment and distribution of *D. lumholtzi* in other environments. However, *D. lumholtzi* was detected in the Mobile-Tensaw Delta (MTD), Alabama from 2002-2005 (DeVries et al., 2006), a coastal environment where abiotic conditions change rapidly due to tidal influences and freshwater inflow, and there is less seasonal temperature variation than inland areas. Comparing occurrence patterns in the MTD to reservoirs and lakes can provide valuable insights into the factors influencing *D. lumholtzi* establishment and distribution in novel environments. Therefore, we investigated the occurrence of *D. lumholtzi* in the MTD to determine 1) whether *D. lumholtzi* populations are still present in the MTD and 2) if occurrence patterns are consistent with those in reservoirs, with *D. lumholtzi* proliferating at higher temperatures when native *Daphnia* are rare.

MATERIALS AND METHODS

Study Area

The Mobile-Tensaw Delta (MTD, Figure 2.1) is a southern coastal wetland containing diverse interconnected aquatic habitats. The head of the delta is delineated by the union of the

Alabama and Tombigbee Rivers forming the Mobile River 80 km north of Mobile Bay (Atkins, 1998). Approximately ten kilometers downstream, the Mobile River diverges into the Tensaw and Mobile Rivers (Atkins, 1998). Freshwater inflow and tidal patterns result in overall decreased salinity levels moving upstream from D'Olive Bay to McReynolds Lake (DeVries et al., 2006, Valentine et al., 2004).

Field Surveys

Daphnia were collected at seven sites along the MTD bounded by the latitude and longitude coordinates -87.992496, 30.597344 and -87.841434, 30.936203 (Figure 2.1). Sites were selected based on previous reports of *D. lumholtzi* presence (DeVries *et al*., 2006) and site accessibility. A subset of sites was sampled in March and April of 2011, and then each site was sampled monthly from June 2011 to October 2013 to capture each season based on summer/winter solstice and spring/autumn equinox. Samples were collected from within a 30m radius of each site using an onboard GPS system. Three vertical hauls were made at approximately 1m/s through the water column (near-bottom to surface) with a 153µm Wisconsin plankton net (Wildco, Yulee, FL.) attached to a 30-meter tow line. These were combined and kept on top of ice in a cooler until transferred to cold 95% ethanol. For comparison of relative densities between *D. lumholtzi* and native *Daphnia*, samples collected at the Blakely location from June 2011 - May 2012 were enumerated as the number of animals per liter based on sampled depth and net volume, assuming 100% sampling efficiency. Flow rate through the net was not measured. Since *Daphnia* were too scarce to permit abundance determinations,

occurrence (presence or absence) was recorded at each site for analysis of seasonal and thermal patterns. Abiotic parameters were measured in the top meter of the water column. Measuring temperature near the surface was deemed to be adequate given the shallow depths of most sites and mixing due to water currents in the river. Temperature, electrical conductivity, dissolved oxygen, and salinity were measured with a calibrated YSI Pro2030 multimeter (YSI Inc., Yellow Springs, OH). Water transparency was determined with a standard 200mm Secchi disk (Wildco, Yulee, FL). When measurements could not be made directly, mean seasonal values from the nearest sampling site or the nearest water quality monitoring station (available from the Environmental Protection Agency Water Quality Monitoring Portal, https://www.epa.gov/waterdata/water-quality-data) were used for upper delta sites. For the lower delta area, mean values were obtained from the Dauphin Island Sea Lab monitoring station near Meaher State Park (https://arcos.disl.org/download-data/). In some cases, salinity values were determined from measured electrical conductivity values.

Survey Sites

A map of the MTD showing the survey sites is shown in figure 2.1. The uppermost site, McReynolds Lake (MCR, 30.901301, -87.930634) is a lentic habitat indirectly connected to the main river about twenty-nine kilometers north of the most downstream site, D'Olive Bay (DOB, 30.638250, -87.921417), in straight-line distance. The Hurricane Landing site (HRC, 30.863267, -87.895717), directly on the Tensaw River downstream of

MCR, is a shallow site that was accessed by a fishing dock. Gravine Island (GRI, 30.802807, -87.929330) and Blakely State Park (BLK, 30.750929, -87.922372) are both on the Tensaw River, with GRI on the west side of Gravine Island, and BLK just downstream on the east side. The sites approaching Mobile Bay, DOB, Meaher State Park (MSP, 30.667253, -87.936777) and Bay Minette Basin (BMB, 30.695188, -87.921649) are influenced more by salinity than the riverine BLK and GRI, while the most upstream sites HRC and MCR are tidal freshwater habitats.

Species Identification

Daphnia lumholtzi was identified by microscopy based on its unique neck fornices and distinct helmet. Native *Daphnia* were identified morphologically using keys by Hebert (1995) and Haney et al. (2013). Some native *Daphnia* identifications were confirmed by DNA barcoding using zooplankton-specific primers and polymerase chain reaction (PCR) protocols developed by Prosser and colleagues (2013). DNA was extracted from tissues using the HotSHOT technique (Montero-Pau et al. 2008) or commercially available kits (QIAamp DNA Micro Kit, Qiagen). Samples with positive PCR products were sequenced by a commercial vendor (Eurofins, Lancaster, PA.; MC Lab, San Francisco, CA.) and compared to known sequences published in Genbank (https://www.ncbi.nlm.nih.gov/genbank/). Only one species, *D. magniceps*, was morphologically ambiguous. Morphological identificatins and DNA barcoding gave the same identifications for the other native species found in the delta: *D. ambigua*, *D. obtusa* and *D. parvula.*

Data Analysis

Differences in the means of environmental variables among sites were evaluated using one-way analysis of variance (ANOVA) with an alpha level of 0.05. Salinity levels could not be normalized using standard transformations and therefore were normalized prior to ANOVA with a two-step algorithm following the method of Templeton (2011). Procedures were performed using SPSS statistical computing software (Version: 28.0.1.1 (15), IBM, New York, USA).

Differences in surface water temperatures among seasons and sites were evaluated by two-way ANOVA on untransformed data. Seasons were delineated by summer/winter solstices and spring/autumn equinox. The *p*-value and confidence intervals were adjusted for making multiple comparisons. Confidence intervals were corrected using the Tukey method. Analyses were performed using JASP statistical computing software (Version 0.16, University of Amsterdam).

Significant differences between mean water temperatures and salinity at which species occurred were assessed by *t*-tests performed using SPSS statistical computing software. The McNemar chi-square test for paired binomial data with continuity correction was used to test whether there were significant differences in the occurrence patterns by date between *D. lumholtzi* and native *Daphnia* from April 2011 to October of 2013 (Smith & Ruxton 2020).

RESULTS

Daphnia lumholtzi Establishment in the Delta

D. lumholtzi was detected in 17 of 150 monthly samples collected from 2011 -2013, resulting in an overall presence of 11%. However, the species was not found in the three downstream sites (BMB, MSP and DOB). In the upstream sites (BLK, GRI, HRC, MCR), both *D. lumholtzi* and native *Daphnia* were found year-round, and we did not find large seasonal changes in population size. Instead, both groups were sparse on most dates and determination of abundances was not possible. Therefore, presence or absence was used to evaluate *Daphnia* occurrence patterns.

There were no significant differences in abiotic factors (surface water temperature, salinity, dissolved oxygen, and Secchi disk depth) among the four upstream sites (BLK, GRI, HRC, MCR), so these were grouped for analysis as the upper delta (UD). There were also no significant differences among the downstream sites (BMB, MSP, DOB), which were grouped as the lower delta (LD). Annual means of abiotic variables are given in Table 2.1.

Occurrence and Co-occurrence of Daphnia by Site in the Upper Delta

Since *D. lumholtzi* was absent from LD samples, we compared co-occurrence with native *Daphnia* in the UD only. *D. lumholtzi* was present in 17% of all samples collected from

the UD (Table 2.2). Of these, 65% were from BLK on the Tensaw River. Like *D. lumholtzi*, native *Daphnia* occurred most frequently at BLK (Table 2.2) but was found four times as often as *D. lumholtzi* at both GRI and MCR (Table 2.2). Native *Daphnia* were detected nearly twice as frequently as *D. lumholtzi* in the UD, occurring in 38% of samples**.** Native species also occurred in 16% of samples collected from the LD.

D. lumholtzi co-occurred with native *Daphnia* 11 of the 17 times that the species was detected (Table 2.3). Most co-occurrences were at BLK, accounting for 37% of samples when any species was present at this site (Table 2.3). The two groups occurred together two of the three times that *D. lumholtzi* was detected at MCR. Similarly, *D. lumholtzi* was detected only twice at GRI, co-occurring with native *Daphnia* on both dates. Both groups were rare at the near-shore HCR and did not co-occur. Species found with *D. lumholtzi* at BLK included *D. ambigua*, *D. obtusa* and *D. parvula*.

Occurrence of Daphnia lumholtzi in Relation to Season and Temperature

Minimum temperatures occurred December - February, ranging from 12°C to 17°C. Maxima occurred late June - September, ranging from 25°C to 33°C (Table 2.4). Minimum temperature variation occurred in summer, with 5.5 degrees difference between the lowest and highest temperatures, and the greatest variation occurred in autumn with approximately 11 degrees difference. Mean temperatures were significantly different among the seasons ($F(3, 97) = 121.4$, $p < 0.001$), but not among sampling sites ($p = 0.934$, Figure 2.2). Summer was significantly warmer than the other

seasons while winter was significantly cooler than the other seasons. Autumn and spring temperatures were not significantly different.

While densities were generally too low to allow determination of abundances (< 0.1 animals/L), they were estimated from a subset of surveys at BLK for comparison of trends (Figure 2.3). *D. lumholtzi* densities at BLK were highest in June and September 2011, accounting for 74-90% of all *Daphnia* detected. Native *Daphnia* were less than 0.01 animals/L on these dates. Only *D. lumholtzi* was present November - December 2011, but densities were below 0.01 animals/L in both months. *Daphnia lumholtzi* was present in January of 2012, making up 20% of *Daphnia* detected, but was absent February - March 2012, reappearing in May 2012 in equal proportion to native species. Native *Daphnia* occurred at their highest densities in January – March 2012, although numbers were below 0.02 animals/L. When the groups co-occurred, *D. lumholtzi* densities were lower than natives only in January 2012, and only *D. lumholtzi* densities exceeded 0.02 animals/L. *D. lumholtzi* occurred most frequently at BLK, including the only occasion that the species was detected in winter. Occurrence frequency was highest in spring and autumn, followed by summer (Table 2.5). Native *Daphnia* were present in all winter and spring surveys at BLK and were detected with equal frequency to *D. lumholtzi* in summer but were not detected in autumn.

In the UD overall, *D. lumholtzi* was present from 13°C in winter to 32°C in summer, occurring with similar frequency in all seasons except winter (Table 2.6). Native *Daphnia* were present in the UD from 12°C in winter to 32°C in summer. During the single autumn survey in which native species were detected, the water temperature was 15°C. Winter and spring together accounted for over 75% of all native *Daphnia* occurrences (Table 2.6) and detection probability was lower in summer followed by autumn. Thus, in the UD, *D. lumholtzi* occurrence patterns (Autumn>Spring>Summer>Winter) compared to native *Daphnia* (Winter>Spring>Summer>Autumn) were similar in spring and summer but were opposite in autumn and winter, resulting in a significantly different occurrence rate overall (χ^2 = 17.333, df = 1, p = 3.136e-05).

In relation to water temperatures, *D. lumholtzi* and native *Daphnia* co-occurred at temperatures from 12.8°C to 31.1°C, most often at BLK. They also occurred together at GRI and MCR, but *D. lumholtzi* was only found at temperatures above 25°C, while natives were present at temperatures from 12.2°C to 31.6°C. Overall, *D. lumholtzi* occurred above 25°C 71% of the time, while native *Daphnia* occurred above 25°C 39% of the time (Figure 2.4). However, there was not a significant difference in the mean temperature when *D. lumholtzi* were present and the mean temperature when native *Daphnia* were present (p = 0.06). Similarly, the difference between the mean temperatures when *D. lumholtzi* was absent compared to when native species were absent was not statistically significant (p = 0.10, Table 2.7). Within each group, there

was not a significant difference in surface water temperatures when *D. lumholtzi* was present or absent (p = 0.41, Table 2.8). However, mean temperatures when native *Daphnia* were absent were significantly higher than when they were present (p = 0.003, Table 2.8).

Variation in Presence of Daphnia with Respect to Salinity

D. lumholtzi was not detected in the LD. Since the only difference between abiotic factors in the UD and the LD was salinity levels, we compared mean salinity when each group was present or absent. In the UD, salinity was in the freshwater range (< 0.5 parts per thousand, ppt), although it was periodically elevated during summer and autumn (Figure 2.5). Mean seasonal salinity was significantly different between the LD and the UD in all seasons except winter ($F(3, 142) = 5.599$, p = 0.001). Salinity was above 0.5 ppt twice as often at GRI as BLK, resulting in slightly higher annual means for GRI (Table 2.1). HRC and MCR were below 0.5 ppt.

Although salinity in the UD was over 3.0 ppt on multiple occasions, the highest salinity that *D. lumholtzi* occurred was 0.8 ppt in autumn at BLK. This was the only time that *D. lumholtzi* was detected above freshwater salinity levels. There was a significant difference (p = 0.053) in the mean salinity when *D. lumholtzi* was present (0.2 ppt) compared to when they were absent (0.6 ppt, Table 2.9). Although native *Daphnia* were found in both the LD and UD, they were detected during low salinity levels. Except for a single occurrence at BMB when salinity was 0.6 ppt, salinity was below 0.5 ppt when

natives were found in the LD (17% of all detections). Similarly, 83% of native *Daphnia* detections in the UD occurred at salinity below 0.5 ppt. The mean salinity when native *Daphnia* were present was 0.1 ppt (Table 2.9), which was significantly lower than when they were absent (0.8 ppt, $p < 0.001$).

DISCUSSION

Occurrence Patterns in Relation to Temperature

Previous studies have found that *Daphnia lumholtzi* exhibits a complementary, rather than competitive, colonization pattern in lakes and by potentially filling a vacant temporal or thermal niche when water temperatures exceed 25°C in reservoirs (Havel & Graham, 2006; Lennon et al., 2001). In our study, overall trends in detection showed similar seasonal and temperature differences between the two groups as observed in reservoirs. *D. lumholtzi* was detected more frequently when temperatures were above 25°C, while native *Daphnia* were detected more frequently when temperatures were below 25°C, and there was a significant difference in their seasonal occurrences in winter and autumn. However, there were also trends that indicate *D. lumholtzi* overlaps regularly with native species in the delta, including the broad range of temperatures at which *D. lumholtzi* and native species co-occurred in summer and late autumn.

In relation to thermal tolerance, *D. lumholtzi* and native *Daphnia* occurred together regularly in the UD at temperatures from 13°C - 32°C and there was no significant difference in mean water temperatures of either presence or absence between *D. lumholtzi* and native species. Although there was a significant difference in mean temperatures when natives were present compared to when they were absent, there was no significant difference in mean temperatures when *D. lumholtzi* was present or absent. This indicates that *D. lumholtzi* can occupy a broad thermal range in the MTD.

Seasonal frequencies showed that detection probability for *D. lumholtzi* was highest in autumn while native *Daphnia* were detected in autumn only once. Similarly, detection probability was highest for natives in winter, while *D. lumholtzi* was detected only once in winter. While this pattern may be complementary, it does not appear to be based simply on thermal differences. During autumn, water temperatures ranged from 12°C - 27°C, but *D. lumholtzi* occurred most often below 19°C in autumn. Although water temperatures ranged from 12°C - 23°C in winter, the temperature was near minimum on the occasion that *D. lumholtzi* was detected. Further, *D. lumholtzi* and native *Daphnia* exhibited overlapping occurrence patterns in spring and summer over a broad thermal range. While the species may tolerate elevated temperatures better than native *Daphnia* (Work & Gophen, 1999), *D. lumholtzi* populations were present during both the coolest and warmest periods in our study, consistent with observations made from 2002-2005 (DeVries et al., 2006). Native *Daphnia* were more likely to be present during

winter at water temperatures below 23°C, but they were also more likely to be absent in autumn, when temperatures were also predominantly below 25°C. Notably, the autumn rarity in native *Daphnia* coincided with the highest *D. lumholtzi* occurrence frequency. While direct competitive effects between *D. lumholtzi* and native *Daphnia* have not been shown in natural populations to our knowledge, it is possible that in the delta, *D. lumholtzi* impacts the occurrence of some native *Daphnia* during autumn. Alternatively, native species may be more heavily impacted by a seasonal predator compared to *D. lumholtzi* (Celik et al., 2002; Engel et al., 2014; Swaffar & O'Brien, 1996).

Although *D. lumholtzi* was detected only once in winter, the species occurred at temperatures below 15°C approximately 18% of the time. This is consistent with earlier surveys (DeVries et al., 2006), wherein *D. lumholtzi* was present in 16% of surveys conducted from December to February of 2005. The similarity in these observations spaced ten years apart suggests that *D. lumholtzi* winter populations occur regularly in the MTD. This idea is supported by reports of occasional late autumn and winter *D. lumholtzi* populations in other environments (East et al., 1999; Havel & Graham, 2006; Havens et al., 2012; Sorensen & Sterner, 1992; Stoeckel & Charlebois, 1999) as well as regular winter occurrences of patchy *D. lumholtzi* populations in reservoirs and lakes (Beaver et al., 2018; Beyer & Hambright, 2019; East et al., 1999; Frisch & Weider, 2010; Kannan & Lenca, 2013). In both reservoirs and the MTD, studies are needed to characterize winter *D. lumholtzi* populations. It is not clear whether there are few *D.*

lumholtzi present in late autumn and winter in the MTD, or whether they are spatially localized in areas that require an alternate sampling strategy, such as near-shore areas.

We previously observed a dense congregation of *D. lumholtzi* in the benthic zone of Lake Texoma, Texas, in mid-December (unpublished observation) that contained males swarming with ephippial females, indicating sexual reproduction. This swarm was detected visually while inspecting the water surface from the dock. To detect a population like this in the delta would require a sampling strategy and gear suitable for sampling near the riverbanks. Other reports of dense *D. lumholtzi* congregations during cool weather in reservoirs and lakes (Beaver et al., 2018; Havel & Graham, 2006; Havens et al., 2012; Kannan & Lenca, 2013) have also noted high densities of males and ephippia-bearing females (Beaver et al., 2018; Kannan & Lenca, 2013). Seasonal swarming behavior in other *Daphnia* (Colebrook, 1960; Kvam & Kleiven, 1995; Young, 1978) has been hypothesized to facilitate sexual reproduction (Gerritsen, 1980; Young, 1978) because fertilization success is dependent on population density (Gerritsen, 1980).

Daphnia overwinter both by parthenogenesis of asexual clones in the open water (Rellstab and Spaak, 2009) and by the production of diapausing ephippial eggs in autumn that overwinter in sediments (Kleiven et al., 1992). Overwintering asexual clones may be advantageous in rapidly changing ecotones like the MTD, because neither the persistence nor the growth rate of the population is limited by the need to produce

males or encounter mates (Gerritsen, 1980), allowing comparatively few females to maintain viable populations during conditions that limit expansion to higher densities (Gerritsen, 1980). *D. lumholtzi* may use both reproductive strategies in fluctuating environments like the delta, since egg-banking can reduce the risk of local extinctions by allowing the population to regenerate in favorable conditions (Kleiven et al., 1992; Pietrzak & Slusarczyk 2006). In Lake Naivasha, Kenya, for example, *D. lumholtzi* populations driven to extinction by intense predation reappeared decades later, hatching from ephippia released from disturbed sediments (Mergeay et al., 2004).

Decreasing photoperiod and dropping temperatures are both effective cues that induce sexual reproduction cycles (Kleiven et al., 1992), and sexual reproduction at the end of a growing season means that females are more likely to have sufficient energy stores to generate and provision ephippial eggs (Gerritsen, 1980). It is possible that the late autumn/early winter *D. lumholtzi* populations observed in the MTD are localized sexually reproducing congregations. In support of this idea, Havel and Graham (2006) also observed a late autumn peak of *D. lumholtzi* in Stockton Lake, Missouri. Males and ephippial females were intermittently present and one of these populations was detectable into December. Similarly, Burdis and Hirsch (2005) observed localized high-density *D. lumholtzi* males and ephippial females together in Lake Pepin (Mississippi River).

Over-wintering *D. lumholtzi* may be difficult to detect because they are confined to areas with specific habitat features. More intensive sampling with greater spatial coverage is needed in the MTD during the winter to determine whether *D. lumholtzi* populations are overwintering by parthenogenesis, engaging in late-season sexual reproduction cycles, or both. Additionally, comparisons of thermal tolerance and life history studies of *D. lumholtzi* survival and reproduction under both winter and summer thermal regimes will be useful to characterize these processes.

Establishment in the Estuary

The presence of *D. lumholtzi* in the estuary over the two years of this study shows that this species is an established component of the zooplankton community in the MTD. Together with an earlier report documenting *D. lumholtzi* in the same area from 2002-2005 (DeVries et al., 2006), our study shows that permanent populations of *D. lumholtzi* have likely persisted in the estuary for at least thirteen years. Like the earlier report, we found *D. lumholtzi* to occur at low density during all seasons, although occasional higher abundance populations were present in the Tensaw River.

Other studies have reported similar patterns of intermittently present low-density populations (Dzialowski et al., 2000; East et al., 1999) and sparse but long-term occurrence punctuated by patchy higher-density populations during short periods (Havel et al., 1995; Havel & Graham, 2006; Havens et al., 2012). In a study of Lake

Okeechobee, Florida, from 2000 – 2009, Havens and colleagues (2012) noted periods of *D. lumholtzi* absence followed by brief occurrences of low-density populations, while an earlier study of the same lake from 1995-1996 detected *D. lumholtzi* during every sample collection period (East et al., 1999). Similarly, in Kentucky Lake, Kentucky, *D. lumholtzi* population density reached 27 animals/L in 1991, but in two subsequent periods 7-15 years later, the species was not detected above 1 animal/L (Levine & White, 2009). Despite low densities, *D. lumholtzi* is now a permanent component of the Kentucky Lake cladoceran community (Levine et al., 2013).

In the MTD, a different sampling strategy may be needed to identify habitats where higher density populations occur. We found *D. lumholtzi* most frequently at sites on the Tensaw River, and the species is known to maintain high abundance summer populations in other rivers (Soeken-Gittinger et al., 2009). In the Atchafalaya River Basin, Louisiana, however, *D. lumholtzi* densities were highest in shallow areas and river branches (Davidson Jr. & Kelso, 1997; Kelso et al., 2003). Expanding sampling around the MTD to include a variety of habitats will be useful to determine whether there is variation in *D. lumholtzi* occurrence or population density in these areas.

Our findings differed from Devries and colleagues (2006) in both *D. lumholtzi* detection frequency and occurrence in the UD versus LD areas. *D. lumholtzi* frequency increased during their study, with the species occurring in approximately 58 percent of UD samples from 2002-2005. In contrast, we detected *D. lumholtzi* most frequently in 2011,

and occurrences were three times lower overall compared to the earlier study. These findings may indicate that *D. lumholtzi* populations have decreased in the MTD since 2005, or they have undergone local extinctions with reintroductions or regeneration from the egg bank.

Daphnia abundance and distribution is impacted by physicochemical parameters like salinity, which can change unpredictably over brief time scales in coastal ecosystems. In the MTD, water quality monitoring has shown differential changes in dissolved oxygen and salinity occurring among sites over the course of a few days, even within the same season (Valentine, et al., 2004). Among the variables we measured, the only significant difference in the occurrence of *D. lumholtzi* in the UD was in relation to salinity.

Salinity can influence *Daphnia* based on physiological tolerance and impacts on life history traits. Seasonal hatching success of ephippia from the sediment is dependent upon suitable abiotic conditions, both to initiate development of eggs (Ebert, 2005) and for survival of juveniles following hatching. While *Daphnia* may tolerate elevated salinity levels (DeVries et al., 2006; Frisch & Weider, 2010; Work & Gophen, 1999), species differ in their tolerance (Gonçalves et al., 2007) and juveniles of some species are more sensitive to salinity than adults (Hall & Burns, 2002). Salinity has been shown empirically to decrease hatching rates of ephippia (Bailey et al., 2004), an effect that was not reversible for eggs initially developing under increased salinity levels, even after the salt exposure was removed (Bailey et al., 2004).

In the UD, *D. lumholtzi* occurred most frequently in the BLK site on the Tensaw River, including the only detection of the species at salinity above freshwater levels. *D. lumholtzi* is known to colonize large rivers (Mantovano et *al*., 2018), with high-abundance populations found during summer and late autumn (Soeken-Gittinger et al., 2009). Although we found *D. lumholtzi* year-round at BLK, densities were low, even in summer and autumn. Since salinity is highest during these seasons, this suggests that the timing of increased salinity may inhibit high-abundance *D. lumholtzi* populations in the Tensaw River. Similarly, decreasing saltwater influence in late autumn through winter may facilitate overwintering *D. lumholtzi* populations in the Tensaw River.

DeVries and colleagues (2006) found *D. lumholtzi* in 25% of samples from the brackish LD sites BMB and DOB from 2002 - 2005. In contrast, we did not detect the species in the LD during our study. Among the factors we measured, the LD and UD differed only in mean salinity levels, which were significantly higher in the LD in summer and autumn. Since this coincides with periods when *D. lumholtzi* occurred most frequently in the UD, it is unlikely that the species would not also occur in the LD unless conditions were unsuitable. If salinity levels in the LD inhibit the hatching success of *D. lumholtzi* ephippia or exceed the physiological tolerance of juveniles, this could result in death rates that exceed asexual reproduction rates, leading to population deterioration. Although more in-depth studies of *Daphnia* occurrence in relation to salinity in the MTD is needed, our results suggest that both the extent and timing of elevated salinity levels may impact *D. lumholtzi* colonization and distribution in this environment.

CONCLUSIONS

Few studies have characterized *Daphnia lumholtzi* occurrence in estuarine environments as compared to other colonized habitats. In the MTD *D. lumholtzi* populations do not appear to be thermally limited, but distribution may be constrained by salinity. The maintenance of permanent populations may be prohibited in mesohaline sites, and in upstream areas that experience salinity flux. This is valuable information for understanding factors that influence *D. lumholtzi* colonization and distribution in introduced habitats. More intensive sampling to examine *D. lumholtzi* occurrence with salinity variation is needed to confirm these findings and further characterize the impact of salinity on *D. lumholtzi* persistence.

D. lumholtzi and native *Daphnia* have different occurrence patterns in autumn and winter in the MTD, yet they co-occur during late autumn and summer, suggesting the difference is not solely related to thermal tolerance. Additional laboratory studies are needed to clarify differences in thermal tolerance between native *Daphnia* and *D. lumholtzi* populations found in the MTD. Considering the winter populations found here and the increasing reports of sexually reproducing *D. lumholtzi* during winter, life history studies under broad thermal regimes should be a priority and will be particularly useful.

D. lumholtzi has been present in the MTD since at least 2002, and occurrences were apparently increasing from 2002-2005. In our study, they occurred somewhat sporadically at low abundance with periodic higher-density patches occurring in late autumn and early winter from 2011-2014. Notwithstanding low abundances, *D. lumholtzi* persists in colonized habitats despite fluctuations in population size and periods of absence. Continued monitoring is needed to determine if the observed low occurrence frequency is due to declining presence of *D. lumholtzi* in the MTD, naturally occurring population fluctuations, local extinctions with reintroduction, or whether more spatially intensive sampling is needed to better characterize *D. lumholtzi* distribution. Genetic studies are needed to establish whether re-introductions are another source of propagules contributing to *D. lumholtzi* occurrence. Persistence in the MTD may be enabled by the diversity of habitats, some of which permit suitable conditions for maintenance of stable populations. The results of this study suggest that those habitats include predominantly freshwater riverine sites. Further studies are needed to clarify the effect of salinity on *D. lumholtzi* found in the MTD.

TABLES

Table 2.1. Annual water quality measurements for sample collection sites in the Mobile-Tensaw Delta. The means ± SE for temperature (T), salinity, dissolved oxygen concentration (DO), and Secchi disk depth (SDD), are given with ranges shown in parentheses.

Site	T (°C)	Salinity (ppt)	DO (mg/L)	SDD (m)
McReynolds Lake (MCR)	24.5 ± 1.2 (13.10 - 31.9)	0.4 ± 0.1 (0.1 - 2.5)	6.6 ± 0.3 (4.1 - 10.0)	0.69 ± 0.04 (0.30 - 0.95)
Hurricane (HRC)	24.6 ± 1.85 (12.1 - 32.0)	0.2 ± 0.1 (0.1 - 0.8)	6.7 ± 0.5 (4.2 - 10.2)	0.56 ± 0.06 (0.25 - 0.96)
Gravine Island (GRI)	23.6 ± 1.3 (12.0 - 33.0)	0.9 ± 0.2 (0.0 - 3.8)	6.7 ± 0.3 (4.3 - 10.6)	0.59 ± 0.03 (0.15 - 0.90)
Blakely State Park (BLK)	23.8 ± 1.3 (12.1 - 33.1)	0.6 ± 0.2 (0.0 - 4.4)	6.6 ± 0.3 (3.4 - 10.8)	0.62 ± 0.03 (0.25 - 1.00)
Bay Minette (BMB)	23.1 ± 1.4 (11.2 - 31.1)	1.6 ± 0.4 (0.0 - 5.7)	6.8 ± 0.5 (3.9 - 10.5)	0.50 ± 0.03 (0.20 - 0.90)
Meaher State Park (MSP)	22.6 ± 2.3 (12.9 - 29.9)	1.1 ± 0.5 (0.1 - 3.8)	7.0 ± 0.6 (5.4 - 9.6)	0.54 ± 0.07 (0.25 - 0.80)
D'Olive Bay (DOB)	22.5 ± 1.5 (12.1 - 32.6)	2.8 ± 0.7 (0.1 - 8.8)	7.5 ± 0.4 (4.2 - 10.5)	0.45 ± 0.04 (0.15 - 0.80)

Table 2.2. *Daphnia* occurrence at each site in the upper and lower delta. The last column gives the percentage (%) of samples in which each group was present in all sites combined. The number of months that each site was sampled from 2011-2013 is given in parentheses.

	Upper Delta					Lower Delta				
	MCR (28)	HRC (16)	GRI (29)	BLK (28)	Upper Overall (101)	BMB (21)	MSP (8)	DOB (20)	Lower Overall (49)	Occurrence All Sites (150)
Daphnia lumholtzi	11	6	7	39	17	—	—	—	—	11
Native *Daphnia*	43	13	31	54	38	24	13	10	16	31

Upper delta sites: McReynolds Lake (MCR), Hurricane Landing (HRC), Gravine Island (GRI), Blakely State Park (BLK). Lower sites: Bay Minette Basin (BMB), Meaher State Park (MSP), D'Olive Bay (DOB).

Table 2.3. Co-occurrence of *Daphnia lumholtzi* and native *Daphnia* in the upper delta. Percent co-occurrence is the fraction of samples in which the two groups occurred together (Both Present) among all samples that contained any *Daphnia* (Any Species Present).

Site	Total Surveys	Exotic Only Present	Native Only Present	Any Species Present	Both Present	Percent Co-occurrence
†MCR	28	1	10	13	2	15%
‡HRC	16	1	2	3	0	0%
§GRI	29	0	7	9	2	22%
¶BLK	28	4	8	19	7	37%
Total	101	6	27	44	11	25%

†McReynolds Lake, ‡Hurricane Landing, §Gravine Island, ¶Blakely State Park

Table 2.4. Seasonal surface water temperature (°C) ranges among upper delta sites measured at the time of *Daphnia* collection in the top meter of the water column.

	†MCR	‡HRC	§GRI	¶BLK	Overall
Winter	13.1 - 22.5	12.1 - 21.0	12.2 - 19.2	12.1 - 19.2	12.1 - 22.5
Spring	22.2 - 31.0	23.5 - 30.0	19.2 - 29.4	18.3 - 29.0	18.3 - 31.0
Summer	26.4 - 31.9	29.5 - 32.0	26.0 - 33.0	25.5 - 33.1	25.5 - 33.1
Autumn	14.7 - 25.8	14.7 - 21.7	12.0 - 27.3	13.8 - 26.3	12.0 - 27.3

†McReynolds Lake; ‡Hurricane Landing; §Gravine Island; ¶Blakely State Park

Table 2.5. *Daphnia* occurrence at the Blakely State Park site. Detection probability is the proportion of surveys in which *Daphnia lumholtzi* (Exotic), or native *Daphnia* (Native) occurred among the total number of surveys in each season or overall.

	Exotic			Native			Total
	Present	Absent	Detection Probability	Present	Absent	Detection Probability	
Winter	1	4	0.20	5	0	1	5
Spring	3	3	0.50	6	0	1	6
Summer	4	7	0.36	4	7	0.36	11
Autumn	3	3	0.50	0	6	0	6
Total	11	17	0.39	15	13	0.54	28

Table 2.6. *Daphnia* occurrence in the upper delta. Detection probability is the proportion of months that *Daphnia lumholtzi* (Exotic) or native *Daphnia* (Native) occurred among the total number of months sampled each season or overall.

	Exotic			Native			Total
	Present	Absent	Detection Probability	Present	Absent	Detection Probability	
Winter	1	19	0.05	15	5	0.75	20
Spring	5	18	0.22	14	9	0.61	23
Summer	6	32	0.16	8	30	0.21	38
Autumn	5	15	0.25	1	19	0.05	20
Total	17	84	0.17	38	63	0.38	101

Table 2.7. Comparison of mean occurrence temperature (°C) between groups in the upper delta. Exotic (*Daphnia lumholtzi*): N = 101, 17 present, 84 absent. Native *Daphnia*: N = 101, 38 present, 63 not present. Temperatures were measured in the top meter of the water column at the site and time of *Daphnia* surveys.

	Temperature Present	SD	*t*	df	*P*
Exotic	25.3	6.44	1.885	53	0.06
Native	21.6	7.02			
	Temperature Absent				
Exotic	23.8	6.84	-1.618	145	0.10
Native	25.6	6.18			

Table 2.8. Comparison within each group of mean surface water temperatures when *Daphnia* were present or absent in the upper delta. Exotic (*Daphnia lumholtzi*): N= 101, 17 present, 84 not present; Native *Daphnia*: N = 101, 38 present, 63 not present.

		Present		Absent		Means Test		
		Mean	SD	Mean	SD	*t*	df	*P*
Temperature (°C)	Exotic	25.3	6.44	23.8	6.84	0.835	99	0.41
	Native	21.6	7.02	25.6	6.18	-3.015	99	0.003

Table 2.9. Comparison of mean salinity levels when *Daphnia lumholtzi* (exotic) or native *Daphnia* (native) were present or absent in the delta.

		Present		Absent		Means Test		
		Mean	SD	Mean	SD	*t*	df	*P*
[†]Salinity (ppt)	Exotic	0.2	0.18	0.6	0.89	-1.960	98	0.05
	Native	0.1	0.11	0.8	0.96	-5.655	98	<0.001

[†]Salinity in parts per thousand

FIGURES

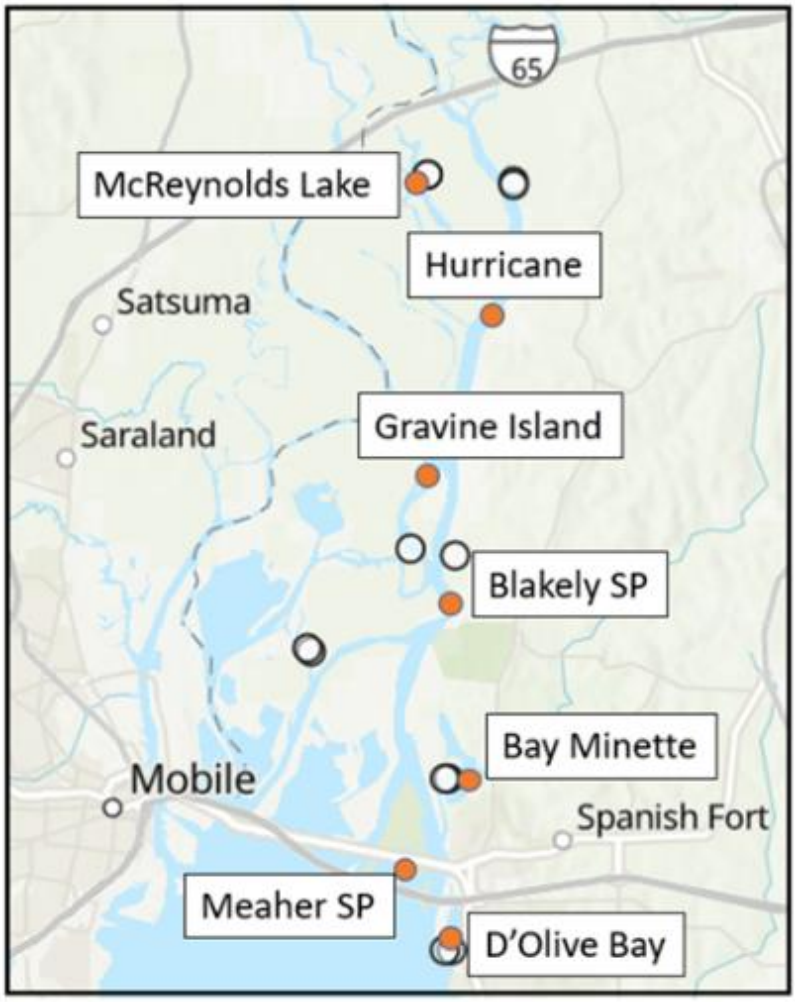


Figure 2.1. Map of the Mobile-Tensaw Delta, Alabama. Red circles show sites sampled for *Daphnia* monthly from 2011-2013. GPS coordinates for each site are given in the text. White circles show sites where *Daphnia lumholtzi* were detected by DeVries and colleagues (2006) from 2002-2005.

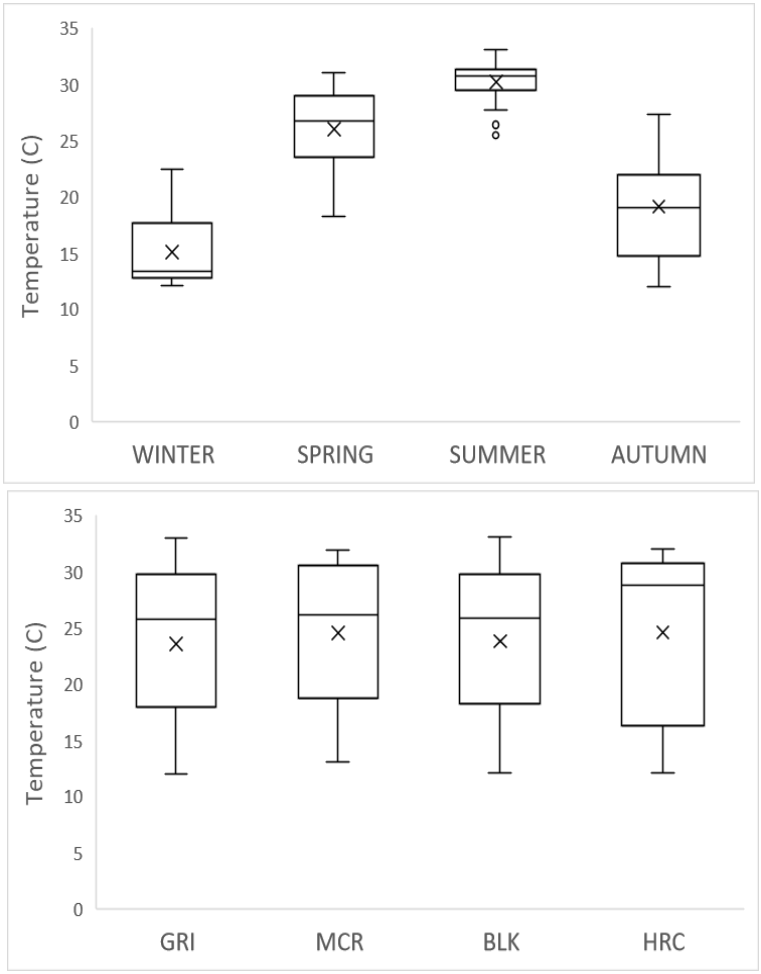


Figure 2.2. Variation in surface water temperatures in the upper delta (UD) by season (top) and site (bottom). Measurements were taken during monthly *Daphnia* surveys. Solid bars inside boxes show median values, X symbols show means, and circles show outliers. Mean temperatures were significantly different between all seasons except fall and spring but were not significantly different among sites (p = 0.934).

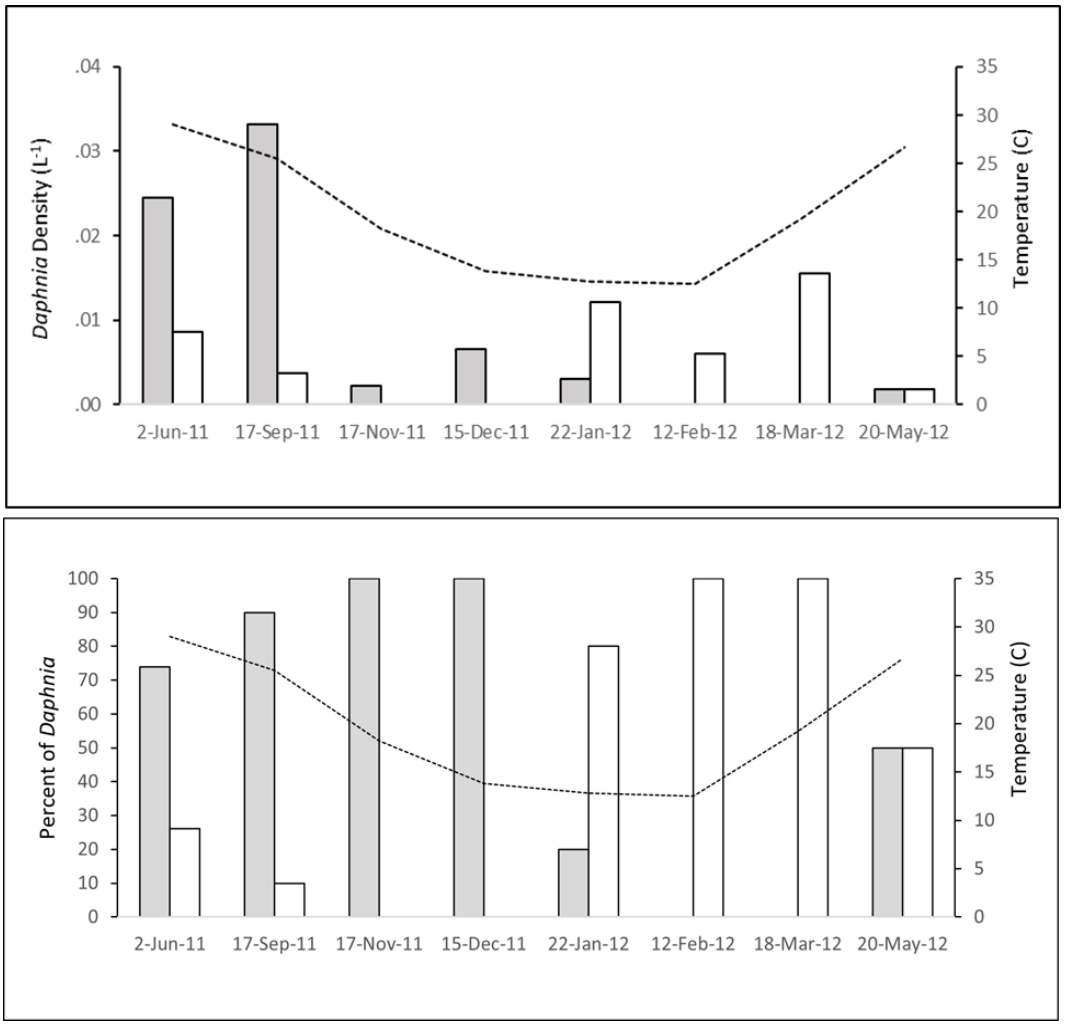
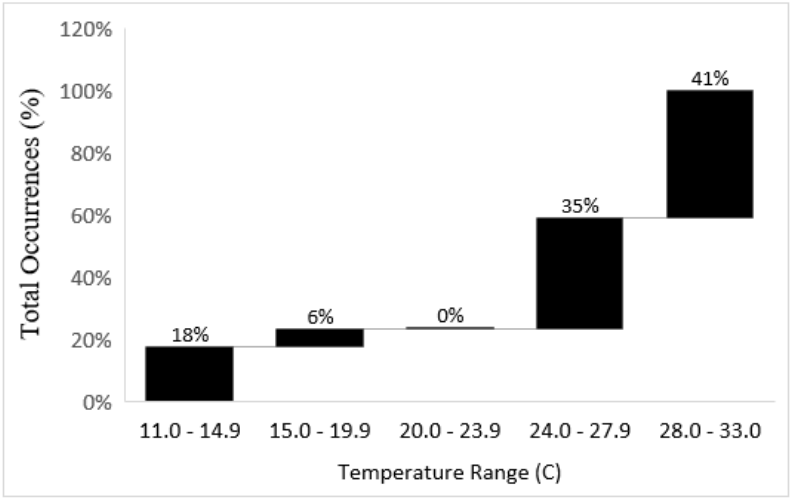


Figure 2.3. Estimated densities (top) and percent of total *Daphnia* (bottom) for *D. lumholtzi* (gray bars) and native *Daphnia* (white bars) at Blakely State Park (BLK) from June 2011 – May 2012. Values are daphnids per liter of water filtered during 1 m/s vertical hauls of a 153 µm plankton net through the water column from near-bottom to the surface. Dotted line shows surface water temperatures.



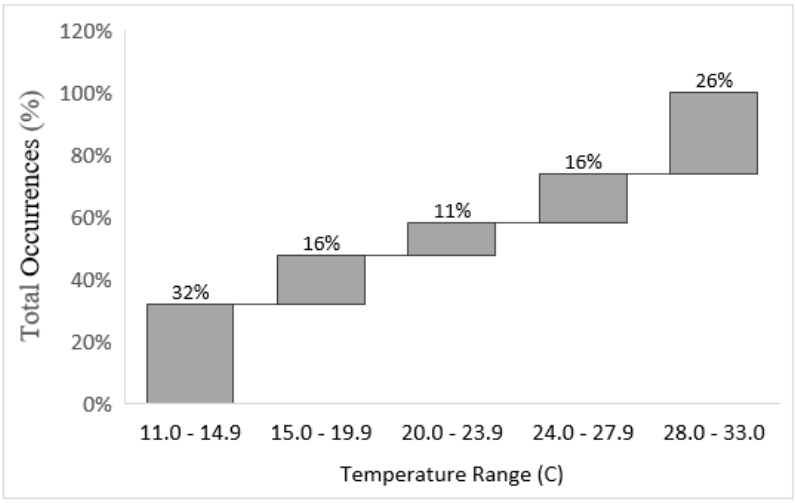


Figure 2.4. Percent of *D. lumholtzi* (top) and native *Daphnia* (bottom) occurrences in the upper delta (UD) that fell within the given temperature ranges. Cumulative total is 100%. *D. lumholtzi* were detected in 17/101 surveys; Native *Daphnia* were detected in 38/101 surveys.

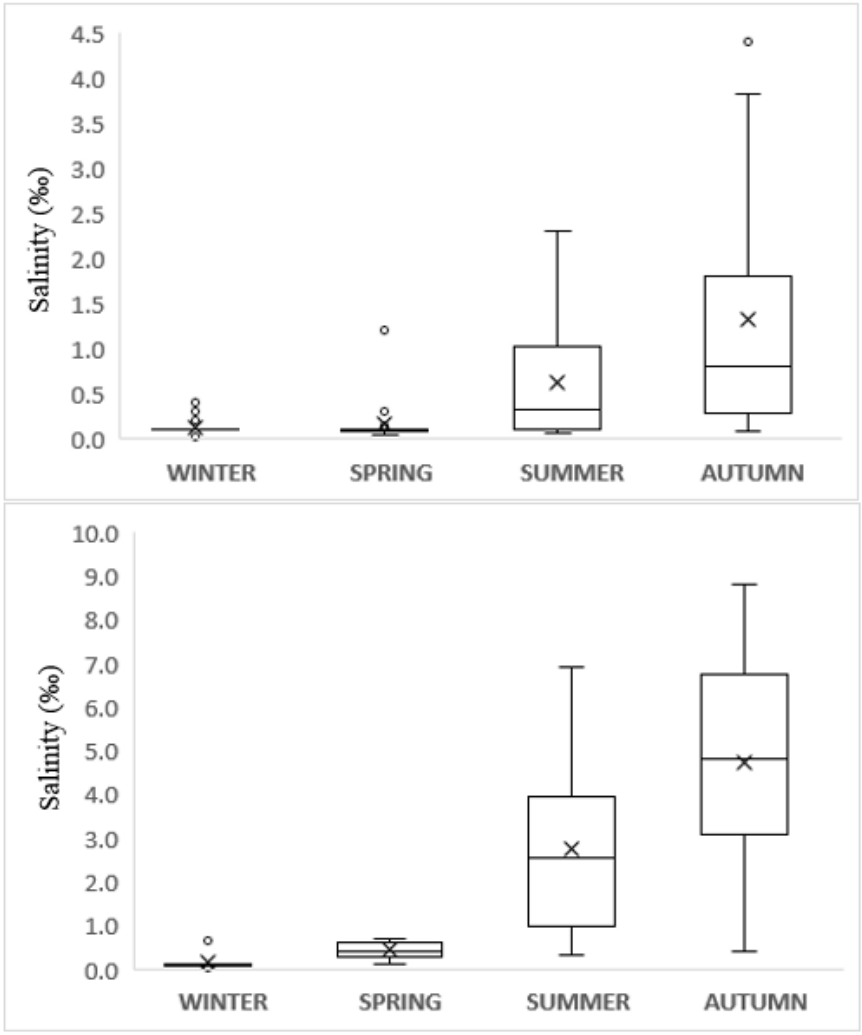


Figure 2.5. Variation in seasonal salinity in the upper (top) and lower (bottom) delta. Measurements were taken during *Daphnia* surveys. Solid bars inside boxes show median values, X symbols show means and circles show outliers. Note different y-axis scales. Winter and spring salinity levels were significantly lower than summer and autumn. Salinity (‰) in parts per thousand.

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AUTHOR CONTRIBUTION STATEMENT

Author contribution statement: Conceptualization, Data Analysis: MP, RF. Developing Methods, Conducting the research, Data interpretation, Writing: MP, RF, JW. Preparation of Figures and Tables: MP, RF.

CHAPTER 2 REFERENCES

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Andradi-Brown, *D.* A. (2019). Invasive lionfish (Pterois volitans and P. miles): distribution, impact, and management. In *Mesophotic coral ecosystems* (pp. 931-941). Springer, Cham.

Appel, D. S., Gerrish, G. A., Fisher, E. J., & Fritts, M. W. (2020). Zooplankton sampling in large riverine systems: A gear comparison. River Research and Applications, 36(1), 102-114.

Atkins, J. B. (1998). *National water-quality assessment program: Mobile River basin* (No. 100-98). US Dept. of the Interior, US Geological Survey.

Bailey, S. A., Duggan, I. C., van Overdijk, C. *D.*, Johengen, T. H., Reid, *D.* F., & MacIsaac, H. J. (2004). Salinity tolerance of diapausing eggs of freshwater zooplankton. *Freshwater Biology*, *49*(3), 286-295.

Bates, A. E., McKelvie, C. M., Sorte, C. J., Morley, S. A., Jones, N. A., Mondon, J. A., ... & Quinn, G. (2013). Geographical range, heat tolerance and invasion success in aquatic species. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1772), 20131958.

Beaver, J. R., Renicker, T. R., Tausz, C. E., Young, J. L., Thomason, J. C., Wolf, Z. L., ... & Koenig, *D.* T. (2018). Winter swarming behavior by the exotic cladoceran *Daphnia lumholtzi* Sars, 1885 in a Kentucky (USA) reservoir. *BioInvasions Rec*, *7*, 43-50.

Benson, A., Maynard, *D.*, Raikow, J., Larson, TH., & Fusaro, A. (2022). A waterflea (*Daphnia lumholtzi*)—Species Profile. USGS Nonindigenous Aquatic Species Database. https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=164

Beyer, J. E., & Hambright, K. *D.* (2019). The niche and morphology of the invasive *Daphnia lumholtzi* in a subtropical reservoir, 20 years after invasion. *Journal of Plankton Research*, *41*(2), 90-100.

Burdis, R. M., & Hirsch, J. K. (2005). Establishment of a viable population of *Daphnia lumholtzi* in Lake Pepin, Upper Mississippi River.

Çelik, K., Schindler, J. E., Foris, W. J., & Knight, J. C. (2002). Predator-mediated coexistence of exotic and native crustaceans in a freshwater lake?. *Biological Invasions*, *4*(4), 451-454.

Cohen, A. N., & Carlton, J. T. (1998). Accelerating invasion rate in a highly invaded estuary. *Science*, *279*(5350), 555-558.

Colebrook, J. M. (1960). Some observations of zooplankton swarms in Windermere. *Journal of Animal Ecology*, *29*(2), 241-242.

Courtenay Jr, W. R., & Williams, J. *D.* (1992). Dispersal of exotic species from aquaculture sources, with emphasis on freshwater fishes. *Dispersal of Living Organisms Into Aquatic Ecosystems, Maryland Sea Grant Program, College Park, MD, USA*, 49-82.

Davidson Jr, N. L., & Kelso, W. E. (1997). The exotic daphnid, *Daphnia lumholtzi*, in a Louisiana river-swamp. *Journal of Freshwater Ecology*, *12*(3), 431-435.

DeVries, *D.* R., Wright, R. A., & DeVries, T. S. (2006). *Daphnia lumholtzi* in the Mobile River drainage, USA: Invasion of a habitat that experiences salinity.

Dexter, E., & Bollens, S. M. (2020). Zooplankton invasions in the early 21st century: a global survey of recent studies and recommendations for future research. *Hydrobiologia*, *847*(1), 309-319.

Dzialowski, A. R., O'Brien, W. J., & Swaffar, S. M. (2000). Range expansion and potential dispersal mechanisms of the exotic cladoceran *Daphnia lumholtzi*. *Journal of Plankton Research*, *22*(12), 2205-2223.

East, T. L., Havens, K. E., Rodusky, A. J., & Brady, M. A. (1999). *Daphnia lumholtzi* and *Daphnia ambigua*: population comparisons of an exotica and a native cladoceran in Lake Okeechobee, Florida. *Journal of Plankton Research*, *21*(8), 1537-1551.

Ebert *D.* (2005). Ecology, Epidemiology, and Evolution of Parasitism in *Daphnia* [Internet]. Bethesda (MD): National Center for Biotechnology Information (US). Chapter 2, Introduction to *Daphnia* Biology. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2042/

Ehrlich, P. R. (1986). Which animal will invade?. In *Ecology of biological invasions of North America and Hawaii* (pp. 79-95). Springer, New York, NY.

Elton, C. S. (2020). *The ecology of invasions by animals and plants*. Springer Nature.

Engel, K., Schreder, T., & Tollrian, R. (2014). Morphological defences of invasive *Daphnia lumholtzi* protect against vertebrate and invertebrate predators. *Journal of plankton research*, *36*(4), 1140-1145.

Frisch, *D.*, Green, A. J., & Figuerola, J. (2007). High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquatic Sciences*, *69*(4), 568-574.

Frisch, *D.*, Havel, J. E., & Weider, L. J. (2013). The invasion history of the exotic freshwater zooplankter *Daphnia lumholtzi* (Cladocera, Crustacea) in North America: a genetic analysis. *Biological Invasions*, *15*(4), 817-828.

Frisch, *D.*, & Weider, L. J. (2010). Seasonal shifts in genotype frequencies in the invasive cladoceran *Daphnia lumholtzi* in Lake Texoma, USA. *Freshwater Biology*, *55*(6), 1327-1336.

Gerritsen, J. (1980). Sex and parthenogenesis in sparse populations. *The American Naturalist*, *115*(5), 718-742.

Gonçalves, A. M. M., Castro, B. B., Pardal, M. A., & Gonçalves, F. (2007). Salinity effects on survival and life history of two freshwater cladocerans (*Daphnia* magna and *Daphnia* longispina). In *Annales De Limnologie-International Journal of Limnology* (Vol. 43, No. 1, pp. 13-20). EDP Sciences.

Green, J. (1967). The distribution and variation of *Daphnia lumholtzi* (Crustacea: Cladocera) in relation to fish predation in Lake Albert, East Africa. *Journal of Zoology*, *151*(1), 181-197.

Hall, C. J., & Burns, C. W. (2002). Mortality and growth responses of *Daphnia* carinata to increases in temperature and salinity. *Freshwater Biology*, *47*(3), 451-458.

Haney, J. F., Aliberti, M. A., Allan, E., Allard, S., Bauer, *D.* J., Beagen, W., ... & Doan, U. (2013). An image-based key to the zooplankton of North America. *University of New Hampshire Center for Freshwater Biology*.

Hansen, G. J., Ahrenstorff, T. *D.*, Bethke, B. J., Dumke, J. *D.*, Hirsch, J., Kovalenko, K. E., ... & Wagner, T. (2020). Walleye growth declines following zebra mussel and *Bythotrephes* invasion. *Biological Invasions*, *22*(4), 1481-1495.

Havel, J. E., & Graham, J. L. (2006). Complementary population dynamics of exotic and native *Daphnia* in North American reservoir communities. *Archiv fur Hydrobiologie*, *167*(1-4), 245-264.

Havel, J. E., & Hebert, P. *D.* (1993). *Daphnia lumholtzi* in North America: another exotic zooplankter. *Limnology and Oceanography*, *38*(8), 1823-1827.

Havel, J. E., Kovalenko, K. E., Thomaz, S. M., Amalfitano, S., & Kats, L. B. (2015). Aquatic invasive species: challenges for the future. Hydrobiologia, 750(1), 147-170.

Havel, J. E., Mabee, W. R., & Jones, J. R. (1995). Invasion of the exotic cladoceran *Daphnia lumholtzi* into North American reservoirs. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*(1), 151-160.

Havel, J. E., Medley, K. A., Dickerson, K. *D.*, Angradi, T. R., Bolgrien, *D.* W., Bukaveckas, P. A., & Jicha, T. M. (2009). Effect of main-stem dams on zooplankton communities of the Missouri River (USA). *Hydrobiologia*, *628*(1), 121-135.

Havel, J. E., & Shurin, J. B. (2004). Mechanisms, effects, and scales of dispersal in freshwater zooplankton. *Limnology and Oceanography*, *49*(4part2), 1229-1238.

Havel, J. E., & Stelzleni-Schwent, J. (2000). Zooplankton community structure: the role of dispersal. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *27*(5), 3264-3268.

Havens, K. E., Beaver, J. R., East, T. L., Work, K., Phlips, E. J., Cichra, M. F., ... & Rosati, T. C. (2012). The outcome of the invasion of Florida lakes by *Daphnia lumholtzi*. *Freshwater Biology*, *57*(3), 552-562.

Havens, K. E., East, T. L., Marcus, J., Essex, P., Bolan, B., Raymond, S., & Beaver, J. R. (2000). Dynamics of the exotic *Daphnia lumholtzii* and native macro-zooplankton in a subtropical chain-of-lakes in Florida, USA. *Freshwater Biology*, *45*(1), 21-32.

Hebert, P. *D.* N. (1995). The *Daphnia* of North America: an illustrated fauna. CD-ROM, University of Guelph. Distributed by the author.

Kannan, MS., & Lenca, N. (2013). Field guide to algae and other “scums” in ponds, lakes, streams and rivers. Second Edition, 19. Northern Kentucky University.

Kelso, W. E., Rutherford, *D.* A., & Davidson Jr, N. L. (2003). Diel vertical migration of cladocerans and copepods in the Atchafalaya River Basin floodplain. Journal of Freshwater Ecology, 18(2), 259-268.

Kerfoot, W. C., Hobmeier, M. M., Yousef, F., Lafrancois, B. M., Maki, R. P., & Hirsch, J. K. (2016). A plague of waterfleas (*Bythotrephes*): impacts on microcrustacean community structure, seasonal biomass, and secondary production in a large inland-lake complex. Biological Invasions, 18(4), 1121-1145.

Kleiven, O. T., Larsson, P., & Hobæk, A. (1992). Sexual reproduction in *Daphnia magna* requires three stimuli. Oikos, 197-206.

Kolar, C. S., Boase, J. C., Clapp, *D.* F., & Wahl, *D.* H. (1997). Potential Effect of Invasion by an Exotic Zooplankter, *Daphnia Iumholtzi*. *Journal of Freshwater Ecology*, *12*(4), 521-530.

Kotov, A. A., & Taylor, *D.* J. (2014). *Daphnia lumholtzi* Sars, 1885 (Cladocera: Daphniidae) invades Argentina. *Journal of Limnology*, *73*(2).

Kvam, O. V., & Kleiven, O. T. (1995). Diel horizontal migration and swarm formation in *Daphnia* in response to *Chaoborus*. In *Cladocera as model organisms in biology* (pp. 177-184). Springer, Dordrecht.

Lennon, J. T., Smith, V. H., & Williams, K. (2001). Influence of temperature on exotic *Daphnia lumholtzi* and implications for invasion success. *Journal of Plankton Research*, *23*(4), 425-433.

Levine, T. *D.*, Sickel, J. B., & White, *D.* S. (2013). Colonization, trends and status of two non-native species in Kentucky Lake, *Plectomerus dombeyanus* and *Daphnia lumholtzi*. *Proceedings of the 14th Symposium on the Natural History of Lower Tennessee and Cumberland River Valleys*, 79.

Levine, T. *D.*, & White, *D.* A. (2009). Spatial and temporal patterns of zooplankton densities with emphasis on *Daphnia* in a large reservoir, Kentucky Lake. In 94th Annual Ecological Society of America Meeting, Albuquerque, New Mexico, USA (Vol. 2).

Mantovano, T., Schwind, L. T., Braghin, L. *D.* S. M., Arrieira, R. L., Tiburcio, V. G., Nascimento, K. C., ... & Lansac-Tôha, F. A. (2018). An analysis of publications on *Daphnia lumholtzi* in freshwater ecosystems. *Limnetica*, *37*(2), 199-208.

McDonald, JH (2014). Handbook of Biological Statistics (3rd ed.). Sparky House Publishing. https://www.biostathandbook.com

Mergeay, J., Verschuren, *D.*, Kerckhoven, L. V., & Meester, L. D. (2004). Two hundred years of a diverse *Daphnia* community in Lake Naivasha (Kenya): effects of natural and human-induced environmental changes. Freshwater biology, 49(8), 998-1013.

Montero-Pau, J., Gómez, A., & Muñoz, J. (2008). Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography: Methods*, *6*(6), 218-222.

Muzinic, C. J. (2000). First record of *Daphnia lumholtzi* Sars in the Great Lakes. *Journal of Great Lakes Research*, *26*(3), 352-354.

National Research Council. (1996). Stemming the tide: Controlling introductions of nonindigenous species by ships' ballast water. National Academies Press.

Nunes, A. H., Miracle, M. R., Dias, J. *D.*, Fabrin, T. M., Braghin, L. S., & Bonecker, C. C. (2018). First genetic characterization of non-native *Daphnia lumholtzi* Sars, 1885 in Brazil confirms North American origin. International Review of Hydrobiology, 103(3-4), 48-53.

Padilla, *D.* K., & Williams, S. L. (2004). Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Frontiers in Ecology and the Environment, 2(3), 131-138.

Pangle, K. L., & Peacor, S. *D.* (2006). Non-lethal effect of the invasive predator *Bythotrephes longimanus* on *Daphnia mendotae*. Freshwater Biology, 51(6), 1070-1078.

Pembury Smith, M. Q., & Ruxton, G. D. (2020). Effective use of the McNemar test. Behavioral Ecology and Sociobiology, 74, 1-9.

Pietrzak, B., & Slusarczyk, M. (2006). The fate of the ephippia-*Daphnia* dispersal in time and space. Polish Journal of Ecology, 54(4), 709-714.

Pimentel, *D.*, Zuniga, R., & Morrison, *D.* (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological economics, 52(3), 273-288.

Prosser, S., Martínez-Arce, A., & Elías-Gutiérrez, M. (2013). A new set of primers for COI amplification from freshwater microcrustaceans. Molecular ecology resources, 13(6), 1151-1155.

Rellstab, C., & Spaak, P. (2009). Lake origin determines *Daphnia* population growth under winter conditions. Journal of Plankton Research, 31(3), 261-271.

Ricciardi, A. (2015). Ecology of invasive alien invertebrates. In Thorp and Covich's freshwater invertebrates (pp. 83-91). Academic Press.

Seebens, H., Bacher, S., Blackburn, T. M., Capinha, C., Dawson, W., Dullinger, S., ... & Essl, F. (2021). Projecting the continental accumulation of alien species through to 2050. Global Change Biology, 27(5), 970-982.

Simberloff, *D.* (2013). *Invasive species: what everyone needs to know*. Oxford University Press.

Simões, N. R., Robertson, B. A., Lansac-Tôha, F. A., Takahashi, E. M., Bonecker, C. C., Velho, L. F. M., & Joko, C. Y. (2009). Exotic species of zooplankton in the upper Paraná river floodplain, *Daphnia lumholtzi* Sars, 1885 (Crustacea: Branchiopoda). *Brazilian Journal of Biology*, *69*, 551-558.

Soeken-Gittinger, L. A., Stoeckel, J. A., & Havel, J. E. (2009). Differing effects of suspended sediments on the performance of native and exotic *Daphnia*. *Freshwater Biology*, *54*(3), 495-504.

Sorensen, K. H., & Sterner, R. W. (1992). Extreme cyclomorphosis in *Daphnia lumholtzi*. *Freshwater Biology*, *28*(2), 257-262.

Stoeckel, J. A., & Charlebois, P. M. (1999). *Daphnia lumholtzi*: The Next Great Lakes Exotic?. *Illinois–Indiana Sea Grant College Program at Purdue University and the University of Illinois Urbana-Champaign, West Lafayette, Indiana and Champaign, Illinois.*

Strayer, *D.* L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater biology*, *55*, 152-174.

Swaffar, S. M., & O'Brien, W. J. (1996). Spines of *Daphnia lumholtzi* create feeding difficulties for juvenile bluegill sunfish (Lepomis macrochirus). *Journal of Plankton Research*, *18*(6), 1055-1061.

Taylor Jarnagin, S., Swan, B. K., & Charles Kerfoot, W. (2000). Fish as vectors in the dispersal of Bythotrephes cederstroemi: diapausing eggs survive passage through the gut. *Freshwater Biology*, *43*(4), 579-589.

Templeton, G. F. (2011). A two-step approach for transforming continuous variables to normal: implications and recommendations for IS research. *Communications of the association for information systems*, *28*(1), 4.

US Fish & Wildlife Service (2016). Ecological Risk Screening Summary - Water Flea *(Daphnia lumholtzi*) - High Risk | FWS.gov [Internet]. https://fws.gov/media/ecological-risk-screening-summary-water-flea-*Daphnia-lumholtzi*-high-risk.

Valentine, J., Sklenar, S., & Goecker, M. (2004). Mobile-Tensaw Delta hydrological modifications impact study. *Final report prepared for the Mobile Bay National Estuary Program. Dauphin Island Sea Lab, Dauphin Island, AL*.

Work, K., & Gophen, M. (1999). Environmental variability and the population dynamics of the exotic *Daphnia lumholtzi* and native zooplankton in Lake Texoma, USA. *Hydrobiologia*, *405*, 11-23.

Young, J. P. W. (1978). Sexual swarms in *Daphnia magna*, a cyclic parthenogen. *Freshwater Biology*, *8*(3), 279-281.

CHAPTER 3: THERMAL TOLERANCES OF EXOTIC AND NATIVE *DAPHNIA* FROM AN ALABAMA ESTUARY

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ABSTRACT

The exotic zooplankton *Daphnia lumholtzi* has rapidly colonized reservoirs throughout North America by proliferating during summer when water temperatures exceed the tolerance of native species. We investigated differences in heat death and upper thermal tolerance limits between the tropical *D. lumholtzi* and the temperate native species *Daphnia ambigua* in laboratory experiments. Measurements from a standard heat death assay showed that the mean heat death temperatures of *D. lumholtzi* (39.90 ± .13°C) and *D. ambigua* (40.04 ± .16°C) were not significantly different (p = 0.512). Upper thermal limits were compared by assessing the 48-hour mortality following heating to sublethal temperatures from 32-39°C. Mortality during the 3-day recovery period increased similarly in both species at all temperatures tested. There were few deaths in experimental animals of either species during recovery from heating to 32°C. The 48-hour mortality for each species was modeled separately as a function of temperature in a generalized linear model (GLM) to compare mortality curves between the species. Based on overlap in their confidence intervals (CI), the median lethal temperatures (LD50) calculated from the fitted regression models for *D. lumholtzi* (36.5°C, 95% CI 35.03 – 38.03) and *D. ambigua* (35.3°C, 95% CI 34.37 – 36.96) were not significantly different. Fitted mortality curves with an interaction between temperature and species showed that there was not a significant difference in the mortality of the two species at any temperature (estimate = 5.63, S.E. = 19.20, p = 0.77). These results show that clones of *D. lumholtzi* collected from the Mobile-Tensaw delta do not have

higher upper thermal tolerance limits compared to clones of the native *D. ambigua* collected from the same environment.

INTRODUCTION

The introductions and establishment of exotic species in the U.S. has been increasing with the growth of global trade, and rates of introductions are expected to continue rising (Finch et al., 2021). This is a significant concern because some introduced species can become invasive, impacting native species by direct predation, high competition for food or other resources, and by causing changes that degrade native species habitat (reviewed in Finch et al., 2021). Reflecting the magnitude of these impacts on native community stability, the US Department of Agriculture National Invasive Species Information Center lists invasive exotic species as one of the four primary reasons for decreasing global biodiversity (Finch et al., 2021). Although global waters and inland waterways play a key role in the transport and introduction of exotic species (Pimentel et al., 2005), studies of aquatic ecosystems are underrepresented in the primary literature covering invasion biology (Thomaz et al., 2015).

It is often noted that most introduced species are incapable of colonizing when transplanted into novel environments (Thomaz et al., 2015 and references therein), and there are no distinct predictors of which will persist and become invasive (Gallien et al., 2010). However, among abiotic factors, temperature is a critical parameter impacting

exotic species colonization and distribution, particularly among invertebrates. The exotic *Daphnia lumholtzi*, for example, has rapidly spread to water bodies throughout the U.S. since the species was first reported in 1992 (Sorenson and Sterner, 1992). *D. lumholtzi* is a tropical zooplankter native to Africa, Asia and Australia (Green, 1967, 1971; Havel et al., 2000). In Africa, it has been reported in lakes with temperatures ranging from 14°C to 29°C (Green, 1967, 1971; Havel et al., 2000; Hebert, 1977; King & Greenwood, 1992; Lewis, 1996; Mergeay 2005). The traits responsible for the rapid expansion of this species have not been fully characterized, but most field studies have indicated that *D. lumholtzi* presence is associated with high water temperatures. For example, studies of the zooplankton community in colonized reservoirs found that *D. lumholtzi* is primarily present in samples collected when water temperatures exceed 25°C, with peak abundances in summer and early fall at temperatures between 26-31°C (Havel & Graham, 2006; Kolar et al., 1997; Work & Gophen, 1999, Yurista et al., 2000). This contrasts with native *Daphnia*, which are abundant from spring through early summer at water temperatures ranging from 20-25°C but are generally scarce at temperatures above 25°C (Havel & Graham, 2006; Kolar et al., 1997; Work & Gophen, 1999, Yurista et al., 2000). In many reservoirs, therefore, higher thermal tolerance derived from tropical origins apparently allows *D. lumholtzi* to thrive during warmer months when water temperatures exceed the tolerance of native species (Burdis and Hirsch, 2005; East et al., 1999; Frisch et al., 2010; ; Havel & Graham, 2006; Havens et al., 2000, 2012; Kolar et al., 1997; Lennon et al., 2001; Work & Gophen, 1999).

Most studies of *D. lumholtzi* outside of their native range have focused on the invasion ecology of the species in reservoirs and lakes (Mantovano et al., 2018), and there are comparatively few experimental laboratory studies, especially using *D. lumholtzi* clones from other environments. However, results from a variety of laboratory experiments have supported that *D. lumholtzi* invasion success is related to temperature or the combined effect of temperature and other factors. For example, higher temperatures were shown to induce substantial elongation of unique defensive spines in *D. lumholtzi* (Sorensen and Sterner, 1992; Yurista, 2001), providing greater protection from fish predation compared to other *Daphnia* (Engel and Tollrian, 2009; Fey & Herren, 2014), enabling *D. lumholtzi* to out-compete natives at warmer temperatures in mesocosm experiments (Engel & Tollrian, 2009; Fey & Herren, 2014). Lennon and colleagues (2001) investigated variation in life history responses of *D. lumholtzi* collected from Clinton Reservoir, Kansas and cultured in the laboratory. In the reservoir, *D. lumholtzi* were only present at temperatures between 25°C to 31°C, which coincided with the decline of native species. However, in life-table experiments, *D. lumholtzi* survival time declined above 20°C. Nevertheless, the population growth rate increased at temperatures up to 25°C, apparently due to faster growth and earlier asexual reproduction. Similarly, Work and Gophen (1999) performed laboratory experiments with *D. lumholtzi* collected from Lake Texoma and used directly (without acclimation) in life history studies under varying abiotic conditions at temperatures from 15-29°C. *D. lumholtzi* survival time was significantly lower at 29°C compared to 15°C or 22°C. They did not observe changes in

asexual reproduction with temperature, but asexual eggs developed faster at 22°C and 29°C and offspring reached sexual maturity twice as fast at 29°C compared to 15°C. Overall, temperature had the greatest effect on *D. lumholtzi* survival and reproduction compared to variations in electrical conductivity and turbidity. Wejnerowski et al. (2020) investigated the combined effects of diet quality (green algae vs. cyanobacteria) and temperature on three clones of *D. lumholtzi* collected from three lakes in the south-central US. Compared to animals fed green algae, population growth rates for animals fed cyanobacteria were reduced at 20°C due to malformation of eggs and neonates. This effect was reversed at 26°C such that there was no difference in the release of live neonates at 26°C in the presence of either a low- or high-quality diet. Regardless of diet type, *D. lumholtzi* produced more asexual eggs at 26°C compared to 20°C. Engel and Tollrian (2012) compared population growth in laboratory experiments between *D. lumholtzi* and the native species *D. pulicaria* cultured separately or together to evaluate the effect of temperature on competitive ability at 20°C, 24°C and 28°C. Both species had similar population growth patterns at all three temperatures when grown as individual species, and both had higher population growth individually compared to when they were grown in competition. However, when grown together, the native species achieved higher abundance compared to *D. lumholtzi* at 20°C and 24°C, while *D. lumholtzi* was the dominant competitor at 28°C.

There are few studies investigating the thermal physiology of *D. lumholtzi* from introduced habitats, but Yurista (2004) characterized the effects of temperature on food assimilation rates and respiratory functions of *D. lumholtzi* collected from Kentucky Lake, USA. In laboratory experiments, *D. lumholtzi* was found to have a higher optimal cellular respiration temperature compared to temperate *Daphnia* species, even though both groups had similar breadth of thermal tolerance. The energy available for *D. lumholtzi* growth and reproduction (based on ingestion rates adjusted for respiratory activity) increased with increasing temperature up to 31°C, the highest temperature tested. In comparison, assimilation efficiency in temperate *Daphnia* species peaks at approximately 20°C (Yurista 2004 and references therein).

These studies demonstrate the influence of temperature on *D. lumholtzi* population dynamics, ecological interactions, and physiological processes. When *D. lumholtzi* has an advantage compared to native species regarding survival, population growth and biotic challenges like predation risk and poor food quality, it appears to be at higher temperatures. However, there are few experimental studies that have specifically measured the upper thermal limits of *D. lumholtzi*, yet characterizing these limits is fundamental to characterizing how temperature influences *D. lumholtzi* establishment and distribution in colonized environments.

Two of the most common methods for determining the upper thermal tolerance limits of aquatic invertebrates are by measuring the heat death temperature, and by determining the lethal temperature range that results in the death of 50% (LD50) of the animals within a given exposure time (Kivivuori and Lahdes, 1996 and references therein). The thermal limits of *Daphnia* and other cladocerans are distinctly related to where they occur geographically and when they occur within a given environment, with species that occur in warmer areas and warmer seasons having comparatively higher lethal temperatures (Brown, 1929).

The heat death temperature is obtained by heating animals at a constant rate until a response indicating lethality is observed, such as cessation of swimming. Evaluating the heat death temperature is important because it establishes the boundaries between the highest temperature to which an animal can acclimate, and the critical thermal maximum (Jobling, 1981). Between these upper thermal limits, the exposure time to a given temperature will have a strong impact on survival, but above the critical thermal maximum, heat death occurs (Jobling, 1981). Kivivuori and Lahdes (1996) have pointed out that assessing mortality over several days during recovery from sublethal heat exposures provides a way to both characterize the upper thermal limits and to assess the potential for reproduction in *Daphnia* following thermal stress (Kivivuori and Lahdes, 1996).

Here, we investigated the upper limits of heat tolerance in *D. lumholtzi* collected from the Mobile-Tensaw Delta (MTD), Alabama by determining the heat death temperature and by assessing mortality during recovery following heating to sublethal temperatures. Mortality following experimental heating was modeled as a function of exposure temperature to estimate the LD50 for *D. lumholtzi*. We additionally compared *D. lumholtzi* thermal responses to those of the temperate native species *Daphnia ambigua* collected from the same environment.

MATERIALS AND METHODS

Daphnia Culture

Animals were collected from field sites along the Mobile-Tensaw River, Alabama between 2011 and 2014. Single-female clonal lineages were established in the laboratory and acclimated for two months before use in experiments. *Daphnia lumholtzi* was identified by the unique neck fornices and sharp helmeted carapace of this species. Native species were identified morphologically using keys available online (Haney, et al., 2013) and published on CD-ROM (Hebert, 1995). Some *Daphnia* identifications can be difficult to resolve morphologically (Elias-Gutierrez et al., 2018; Taylor et al., 1998), such as the *Daphnia laevis* species complex, which is known to be common the southeastern U.S. (Taylor et al., 1998). Therefore, some identifications were confirmed using DNA barcoding of the mitochondrial COI gene (Eurofins Genomics, KY., USA; MC Lab, San Francisco, CA., USA). DNA barcoding results from clonal lineages established in the

laboratory generally confirmed morphological identifications. However, identification of *D. magniceps,* which is a member of the *D. laevis* species complex, was ambiguous morphologically but was confirmed by DNA barcoding. Laboratory cultures were maintained at 22-23°C with a 16-hour photoperiod in modified lake water. Modified lake water was prepared by adjusting aerated, glass-fiber filtered lake water (G6 filter, Fisher Scientific) to electrical conductivity in the range of 400-600µS/cm, and pH 7.2-7.4. Culture water was filtered over a sterile 0.22-micron filter (Millipore) and stored in autoclaved glass media bottles before use to prevent contamination. *Daphnia* cultures were fed ad libitum with either *Scenedesmus*, *Nannochloropsis* or mixture of both obtained commercially (Carolina Biological Supply, North Carolina, USA; Reed Mariculture, Campbell, CA., USA).

Heat Death Trials

The heat death temperature was determined following the method of Kivivuori and Lahdes (1996): 1-2 daphnids were placed into glass tubes (25 x 150mm) containing 10mLs of culture water at ambient room temperature (22-23°C). The test tubes were suspended in a 2L bath that was placed on a stirring hot plate to keep the water in the 2L bath mixing throughout the experiment. The temperature of the hot plate was adjusted to maintain a heating rate of one degree Celsius every two minutes. The end point of the experiment was defined as the temperature at which the animals ceased swimming movements. Some animals sank to the bottom of the tube, and some animals

were found to float on the surface rather than sinking, but in both cases, no swimming movements were observable. When the endpoint was reached, tubes were immediately transferred to an unheated bath and allowed to return to ambient temperature. Animals were checked after four hours to ensure that none recovered, confirming that the end point was reached. Any animal that recovered was deemed to not have reached the endpoint and was removed from the determination and not used in other trials. This occurred in two trials with D*. lumholtzi* but did not occur in any trials with *D. ambigua*.

Heating to Sublethal Temperatures at Constant Heating Rate

Mortality during recovery from heating to 35, 37, 38 or 39°C was assessed following the method of Kivivuori and Lahdes (1996) test III: 2-10 adult animals (determined by the presence of a brood chamber) were placed into glass tubes in 10mL of culture water and heated from ambient temperature (22-23°C) to the target temperature at a rate of one degree Celsius every two minutes. The temperature in each test tube was monitored with a multi- channel thermocouple data logger (TC-08, Pico Technology, Cambridgeshire, UK) set to monitor the maximum number of readings possible each second and log the average temperature in each tube every 30 seconds. The end point of each trial was determined to be the time at which the average temperature in the glass tube reached target temperature. Tubes were removed from the heat bath immediately upon reaching the target temperature and allowed to cool to room temperature in an unheated bath for four hours. After four hours, the numbers of

animals dead or surviving in each tube was assessed. Animals that remained alive after four hours were transferred to 150mL culture vessels, fed green algae, and monitored for mortality each day for at least two days and generally for three days given sufficient survival of the heated animals. A set of control tubes was included for each species and handled the same except that they were kept in an unheated bath during the experiment. Additional trials were carried out at 32°C since this was close to the highest temperature that *D. lumholtzi* was found to occur in the Mobile-Tensaw Delta. Mortality of the controls was also assessed. There were occasional deaths in control groups, but these amounted to less than 5 percent when pooled across all experiments.

Data Analysis

Heat Death

Significant differences in heat death were determined by an independent samples *t*-test (pooled equal variances) with significance set at an alpha level of 0.05. For *Daphnia lumholtzi*, one trial endpoint at 30°C was a suspected outlier presumably due to handling effects. It was confirmed to be a statistical outlier using the Tukey's fences method with k=3 for detecting extreme outliers and was therefore removed from the comparison. Two trial endpoints at 41.0°C were statistical outliers but there was not a methodological reason to remove them. There were no deaths in control animals, which were handled similarly except that they were not heated. *T*-tests were performed using SPSS statistical software (SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM

Corp). Outlier detection was performed using the R statistical computing environment (R Core Team, 2022).

Mortality Following Exposure to Sublethal Temperatures

Trials with *D. lumholtzi* were carried out at 32, 25, 37, 38 and 39°C and *D. ambigua* trials were carried out at the same temperatures, except for 35°C. Mortality was analyzed at 48 hours since *Daphnia lumholtzi* can produce eggs or offspring within 2-3 days based on observations of our laboratory cultures. No adjustment was applied to the observed mortality since the control mortality was less than 6%. Exposure temperatures were log-10 transformed for analysis. 48- hour mortality (the number of animals dead after 48-hours) was modeled as a function of exposure temperature using a generalized linear model (GLM) with a probit link (Finney 1952; Faraway 2006). Possible over-dispersion was indicated by dispersion parameters greater than 1 (*D. lumholtzi* 1.79, *D. ambigua* 2.9). The model was adjusted for over-dispersion using a quasibinomial error parameter (Faraway 2006). Separate models were fit for *D. lumholtzi* and *D. ambigua* and resulting mortality curves were compared with an interaction between temperature and species. The fitted regression model was used to calculate the median lethal temperature (LD50) from the probit link function (Φ^{-1}) and the fitted intercept (β_0) and slope (β_1) using the equation:

$$LD_{50} = 10^{(\Phi^{-1}(0.5)-\beta_0)/\beta_1}$$

To calculate the 95% confidence interval (CI) of the LD50, bootstrapping was performed with random sampling-with-replacement of the original data 10,000 times. The CI was then calculated from the resulting distribution of LD50 estimates. All analyses were performed using the R statistical computing environment (R Core Team, 2022).

RESULTS

Heat Death Temperature

Daphnia lumholtzi test animals stopped swimming between 39.0°C (minimum) and 41.0°C (maximum). Two trial endpoints at 41°C were statistically outliers, but there was no justification to remove these. The most frequent endpoint was 40.0°C and this was also the median endpoint (Figure 3.1).

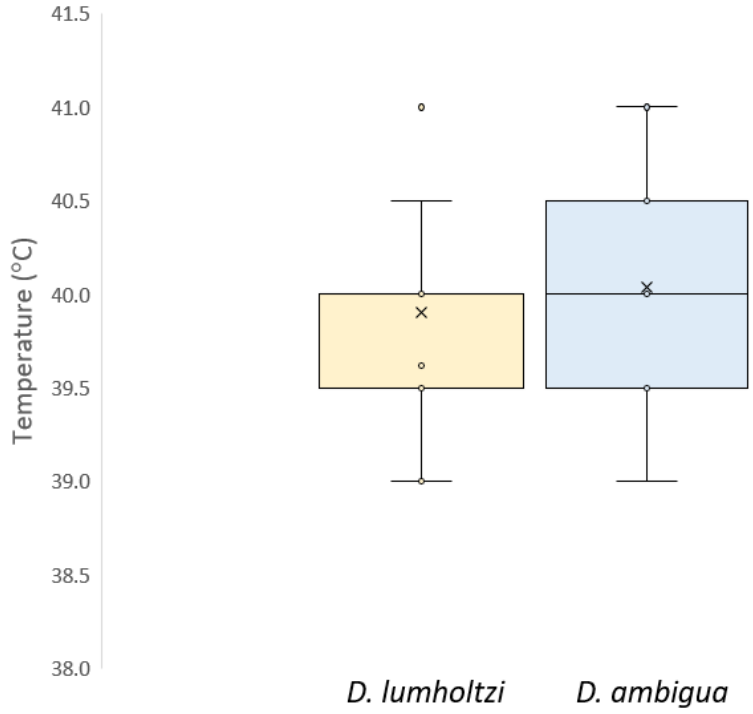

Figure 3.1. Boxplot of heat death temperatures of *D. lumholtzi* (39.90 ± 0.13°C , n= 19) and *D. ambigua* (40.04 ± 0.16°C, n= 13). Means are shown by the x markers. Note that the y-axis starts at 38°C.

D. lumholtzi mean heat death temperature (±SEM) was 39.90 ± 0.13°C. *Daphnia ambigua* also stopped swimming between 39.0°C (minimum) and 41.0°C (maximum). The mean heat death temperature (±SEM) of *D. ambigua* was 40.04 ± 0.16°C (Table 3.1) and the median and the mode were both 40.00°C. There was no significant difference in the mean heat death temperatures between the two species (p = 0.512).

Table 3.1. Mean heat death temperatures and independent samples *t*-test results for *Daphnia.*

	Temperature (°C)	N	SD	*t*	df	*P*
Daphnia lumholtzi	39.9	19	0.563	-0.663	30	0.512
Daphnia ambigua	40.0	13	0.594			

Mortality Following Heating to Sublethal Temperatures

There were 33 experimental trials carried out with *Daphnia lumholtzi* in total. There was a single death (1.3%) among all *D. lumholtzi* unheated control groups. *D. lumholtzi* mortality increased with recovery time at all temperatures and this effect was more pronounced above 35°C (Fig. 3.2). Four trials at 32°C included a total of 14 experimental animals, and 12 trials at 35°C included a total of 30 experimental animals. 48-hour mortality during recovery was less than 10% for trials performed at both temperatures (Fig. 3.2) and there was no discernable difference in mortality between these two temperatures at any time point. In the middle temperature range, there were 9 trials at

37°C with a total of 45 experimental animals. Mortality at this temperature was 2% after 4 hours of recovery but increased significantly to 31% after 24 hours and 87% after 48 hours. Mortality increased between 48 and 72 hours (91.5%), but the change was not significant. There were 11 trials with 26 total experimental animals at 38°C and at 39°C there was 1 trial with 6 experimental animals. Mortality was significantly higher in these trials compared to the lower temperatures, increasing to over 65% by 24 hours and to 100% by 48-hours at both temperatures (Fig 3.2). There were 22 experimental trials carried out with *Daphnia ambigua* in total. Among all trials, there were four total deaths (5.7%) in the *D. ambigua* control groups. Mortality increased with recovery time at all temperatures (Fig. 3.3). There were 4 trials at 32°C that included a total of 16 experimental animals. Among these trials, there were no deaths from 4-48 hours and 72-hour mortality was 13%. There were three trials at 37°C that tested a total of 24 animals and 11 trials at 38°C that tested a total of 40 animals. 4-hour mortality among experimental animals heated to 37°C was lower than those heated to 38°C (7% vs. 27% respectively), but mortality increased to 50% by 24 hours at both temperatures. 48-hour mortality was higher for animals heated to 37°C (80%) than those heated to 38°C (56%), but the differences were not statistically distinguishable (Fig 3.3). There was not a significant change in mortality between 48 and 72 hours at either temperature. Mortality was 100% by 24 hours in the 4 trials (13 total animals) at 39°C.

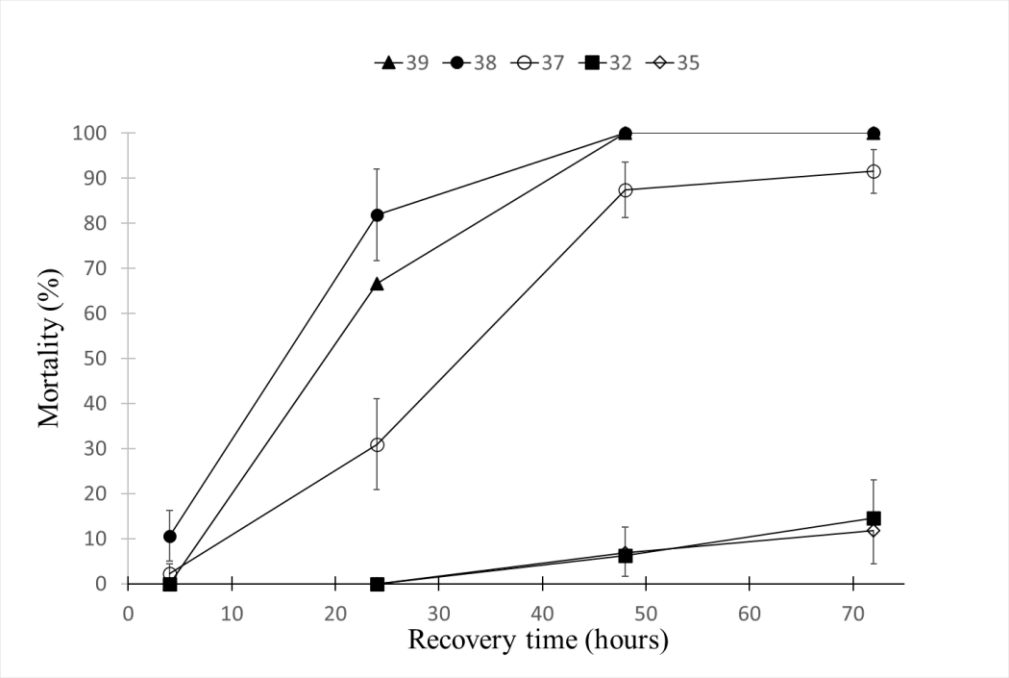

Figure 3.2. Mortality of *D. lumholtzi* following exposure to 32-39°C. Animals were heated at a constant rate of 0.5°C/min up to the target temperature and allowed to cool to room temperature in an unheated bath. Each experiment included a set of unheated controls. Daphniids were transferred to culture vessels after 4 hours and mortality was assessed for 3 days. One trial is shown at 39°C that included 6 animals. Otherwise, markers are means of percentage mortality from a series of at least 4 trials at each temperature that included 3-6 animals per trial. Error bars indicate ± S.E.M. among trials.

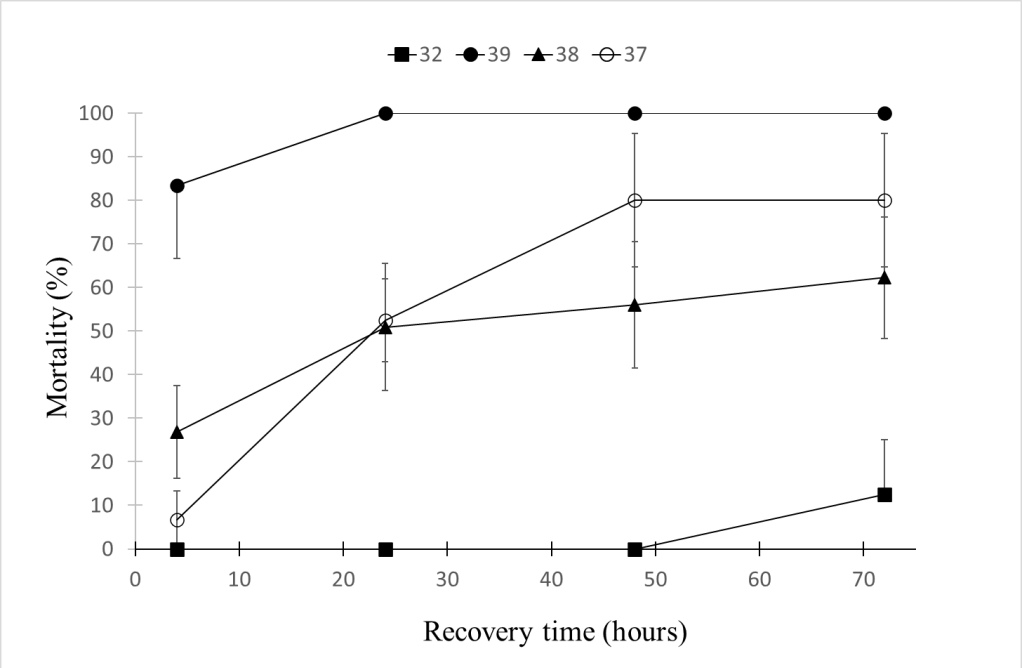

Figure 3.3. Mortality of *D. ambigua* following exposure to 32-39°C. Animals were heated at a constant rate of 0.5°C/min up to the target temperature and allowed to cool to room temperature in an unheated bath. Each experiment included a set of unheated controls. Daphniids were transferred to culture vessels after 4 hours and mortality was assessed for 3 days. Markers are the means of percentage mortality from a series of at least 3 trials at each temperature that included 4-8 animals per trial. Error bars indicate ± S.E.M. among trials.

Fitted GLM curves reflecting the observed mortality as a function of exposure temperature for each species are shown in Figure 3.4. Trials for which 48-hour mortality was indeterminate were removed from the analysis (but are shown in Figures 3.2 and 3.3).

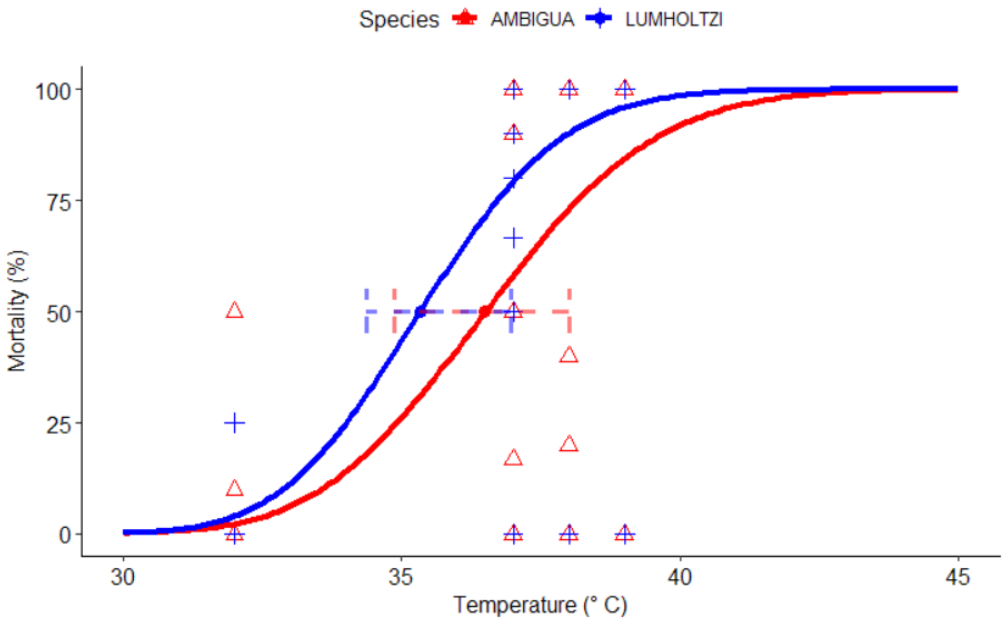

Figure 3.4. Mortality as a function of exposure temperature for *D. lumholtzi* (blue line and crosses) and *D. ambigua* (red line and triangles). Curves show fitted values from generalized linear models for each species. The LD50 values (circles) with 95% bootstrap confidence intervals (horizontal error bars) calculated from the corresponding regression models for each species are also shown.

The estimated LD50 calculated from the fitted regression model for *D. lumholtzi* was 35.3°C (Table 3.2, Figure 3.4), which was slightly lower than the calculated LD50 for *D.*

ambigua (36.5°C). However, the difference was not significant given that the 95% confidence intervals overlap (Table3.2, Figure 3.4).

Table 3.2. Summary of fitted generalized linear models for *Daphnia lumholtzi* (N = 28) and *Daphnia ambigua* (N = 20). Asterisks indicate significance: [§]: $P < 0.01$ *: $P < 0.05$. SE indicates standard error.

Species	Intercept (y-axis)			Slope (log10(temp))			LD50 (°C)		
	Estimate	SE	*P*	Estimate	SE	*P*	Estimate	95% CI Lower	95% CI Upper
Daphnia lumholtzi	-55.01	24.37	0.0366*	35.21	15.49	0.0355*	36.5	35.03	38.03
Daphnia ambigua	-63.23	18.92	0.0025[§]	40.84	12.08	0.0022[§]	35.3	34.37	36.96

The slope of probit-mortality vs. log-10 transformed temperature was estimated to be 40.84 ± 12.08 (mean ± SE, N=28) for *D. lumholtzi* (Table 3.2). In comparison, the slope of probit-mortality vs. log-10 transformed temperature for *D. ambigua* was 35.21 ± 15.49 (N = 20). However, when the mortality curves between the two species were compared, there was not a statistically significant difference in mortality at any temperature since neither the intercept nor the slope of the species-interaction terms were significant (Table 3.3).

Table 3.3. Summary of fitted generalized linear model with an interaction between exposure temperature and species. The dispersion parameter for the quasibinomial was estimated as 2.24. N = 48. Asterisks indicate significance: *: P < 0.05.

Model Parameter	Estimate	Standard Error	*P*-value
Intercept	-55.01	21.43	0.01*
Slope (log10(temp))	35.21	13.62	0.01*
Intercept [species *D. lumholtzi*]	-8.23	30.14	0.79 n.s.
Interaction (log10(dose) species *D. lumholtzi*]	5.63	19.2	0.77 n.s.

DISCUSSION

Ultimately, the occurrence of *Daphnia* geographically and seasonally is constrained by the upper and lower thermal limits that they can tolerate (Brown, 1929). The heat death temperature is a commonly used metric that identifies differences in the maximum temperature a species can withstand (Jobling 1981). The LD50 provides a means for comparing mortality or survival following thermal stress (Kivivuori & Lahdes 1996). Characterizing thermal tolerance limits is important for understanding the colonization success of the exotic *Daphnia lumholtzi* because tolerance of warmer temperatures has been linked to this species' colonization success and distribution (Burdis & Hirsch, 2005; East et al., 1999, Engel & Tollrian, 2012; Frisch et al., 2010; Havel & Graham, 2006; Havens et al., 2000; Havens et al., 2012; Kolar et al., 1997; Lennon et al., 2001; Work & Gophen, 1999; Yurista et al, 2000).

Our results show that *D. lumholtzi* and the native species *Daphnia ambigua* have similar tolerances to high temperatures. Their heat death temperatures and LD50 estimates were comparable, indicating that they have similar upper thermal limits and acclimation capacity (Jobling 1981). The maximum water temperatures measured in the MTD were 32- 33°C, and *D. lumholtzi* was detected at temperatures up to 31.6°C. In thermal trials shown here, there were few deaths in either species during three days of recovery following exposure to 32°C. Further, the LD50 estimates for both species were higher than the maximum temperatures experienced in the environment. This suggests that *D.*

lumholtzi and *D. ambigua* should be similarly able to tolerate the maximum water temperatures experienced in the MTD.

The high heat death temperature for *D. lumholtzi* is not surprising considering the tropical and semi-tropical origins of this species (Benson et al., 2022) and its broad geographic native range distribution (Green, 1967). Species that have broad distributions with peak abundances during periods of maximum temperatures are known to have higher lethal limits compared to temperate *Daphnia* species (Brown, 1929). Based on our results, *D. ambigua* from the MTD has a similar heat death temperature to *D. lumholtzi*. This may reflect that both species were collected from the same environment since thermal tolerance limits among species are correlated with the temperature ranges experienced in their habitat (MacIsaac et al., 1985).

We assessed mortality following acute heat exposures at 48 hours since this is long enough for surviving *Daphnia* to produce asexual eggs or release free-swimming offspring (Obreshkove, 1940, Orcutt & Porter, 1984, Yurista, 2004). Mortality increased each day of the recovery period in a similar manner for *D. lumholtzi* and *D. ambigua*, indicating that their capacities to recover following thermal stress are similar. While we did not measure reproductive output during this study, egg production was noted in experimental groups of *D. lumholtzi* on several occasions in 37°C trials and in *D. ambigua* experimental groups in 38°C trials. Extending the monitoring period in these trials to 7-8 days (as in Kivivouri & Lahdes, 1996) would add an additional measure for

comparing responses to heat stress. It may also help clarify why some native *Daphnia* species seem to have the thermal tolerance capacity to co-occur with *D. lumholtzi,* yet in natural populations, they generally do not overlap in their temporal or spatial distribution (Burdis & Hirsch, 2005; East et al., 1999, Frisch et al., 2010; Havel & Graham, 2006; Havens et al., 2000, 2012; Kolar et al., 1997; Lennon et al., 2001; Work & Gophen, 1999).

Our findings support the idea that temperature is integral to *D. lumholtzi* invasion success (Engel & Tollrian 2012, Havel & Graham 2006, Lennon et al., 2001, Work & Gophen 1999) in the MTD. Given that the native species *D. ambigua* exhibited similar tolerances to *D. lumholtzi*, these results also reinforce the idea that in some environments, *D. lumholtzi* invasion success may result from the interaction of temperature tolerance and biotic factors (Engel & Tollrian, 2009; Fey & Herren, 2014; Lennon et al., 2001; Wejnerowski et al., 2020; Work & Gophen, 1999). Temperature may also impact the effects of abiotic factors such as salinity (Chen & Stillman, 2012) and turbidity (Work & Gophen, 1999) on *D. lumholtzi* distribution.

Overall, our results show that *Daphnia lumholtzi* from the MTD does not have higher upper thermal tolerance compared to *D. ambigua*, the native species most common in field samples collected from the MTD. More studies are needed to characterize the heat death and upper thermal limits of other native *Daphnia* found in this environment to clarify how *D. lumholtzi* presence in the MTD may impact these species. There are few

laboratory studies characterizing the direct effects of temperature and upper thermal tolerance limits in *D. lumholtzi* compared to native species collected from colonized habitats (but see Lennon et al., e.g.). Since *D. lumholtzi* invasion biology has been studied most often in reservoirs (Mantovano et al., 2018), characterizing the upper thermal limits of *D. lumholtzi* and native *Daphnia* clones from reservoirs would be useful for making comparisons among colonized environments.

Although few direct impacts of *D. lumholtzi* on native *Daphnia* species have so far been identified, it is possible that this species' distribution and interactions with native *Daphnia* could be altered by warming water temperatures in response to climate change or increases in thermal pollution (e.g., Engel at al., 2011; Fey & Cottingham, 2011; Mantovano et al., 2021; Nowakowski & Slugocki, 2021). As water temperatures rise, the temporal and spatial distribution of *D. lumholtzi* may expand. Characterizing the thermal limits of *Daphnia* species in colonized environments can help predict which areas and species are at greatest risk and identify likely routes of *D. lumholtzi* range expansion.

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AUTHOR CONTRIBUTIONS

Author contribution statement: Conceptualization, Data Analysis: MP, RF. Developing Methods, Conducting the research, Data interpretation, Writing: MP, RF, JW, CV, LH. Preparation of Figures and Tables: MP, RF.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

CHAPTER 3 REFERENCES

Benson, A., E. Maynard, D. Raikow, J. Larson, T.H. Makled, and A. Fusaro. Daphnia lumholtzi G.O. Sars, 1885: U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, and NOAA Great Lakes Aquatic Nonindigenous Species Information System, Ann Arbor, MI, 2019. https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?Species_ID=164, Revision Date: 9/12/2019, Access Date: 4/1/2022.

Brown, L. A. (1929). The natural history of cladocerans in relation to temperature. I. Distribution and the temperature limits for vital activities. The American Naturalist, 63(686), 248-264.

Burdis, Robert M., and Jodene K. Hirsch. "Establishment of a Viable Population of Daphnia Lumholtzi in Lake Pepin, Upper Mississippi River." Journal of Freshwater Ecology, vol. 20, no. 1, Mar. 2005, pp. 205–07. DOI.org (Crossref), https://doi.org/10.1080/02705060.2005.9664956.

Chen X, Stillman JH. Multigenerational analysis of temperature and salinity variability affects on metabolic rate, generation time, and acute thermal and salinity tolerance in Daphnia pulex. Journal of Thermal Biology. 2012 Apr 1;37(3):185-94.

Davidson, Norman L., and William E. Kelso. "The Exotic Daphnid, Daphnia Lumholtzi , in a Louisiana River-Swamp." Journal of Freshwater Ecology, vol. 12, no. 3, Sept. 1997, pp. 431–35. DOI.org (Crossref), https://doi.org/10.1080/02705060.1997.9663553.

DeVries, Dennis R., et al. "Daphnia Lumholtzi in the Mobile River Drainage, USA: Invasion of a Habitat That Experiences Salinity." Journal of Freshwater Ecology, vol. 21, no. 3, Sept. 2006, pp. 527–30. DOI.org (Crossref), https://doi.org/10.1080/02705060.2006.9665031.

Dzialowski, A. R. "Range Expansion and Potential Dispersal Mechanisms of the Exotic Cladoceran Daphnia Lumholtzi." Journal of Plankton Research, vol. 22, no. 12, Dec. 2000, pp. 2205–23. DOI.org (Crossref), https://doi.org/10.1093/plankt/22.12.2205.

East, T. “Daphnia Lumholtzi and Daphnia Ambigua: Population Comparisons of an Exotica and a Native Cladoceran in Lake Okeechobee, Florida.” Journal of Plankton Research, vol. 21, no. 8, Aug. 1999, pp. 1537–51. DOI.org (Crossref), https://doi.org/10.1093/plankt/21.8.1537.

Elías-Gutiérrez, Manuel, et al. "Improved protocols to accelerate the assembly of DNA barcode reference libraries for freshwater zooplankton." Ecology and evolution 8.5 (2018): 3002-3018.
Engel K, Tollrian R. Inducible defences as key adaptations for the successful invasion of Daphnia lumholtzi in North America? Proceedings of the Royal Society B: Biological Sciences. 2009 May 22;276(1663):1865-73. https://doi.org/10.1098/rspb.2008.1861.

Finch, Deborah M., et al. “Effects of Climate Change on Invasive Species.” Invasive Species in Forests and Rangelands of the United States, edited by Therese M. Poland et al., Springer International Publishing, 2021, pp. 57–83. DOI.org (Crossref), https://doi.org/10.1007/978-3-030-45367-1_4.

Frisch, Dagmar, and Lawrence J. Weider. “Seasonal Shifts in Genotype Frequencies in the Invasive Cladoceran Daphnia Lumholtzi in Lake Texoma, U.S.A.: Seasonal Genotype Frequency Shifts of an Invasive Cladoceran.” Freshwater Biology, vol. 55, no. 6, June 2010, pp. 1327–36. DOI.org (Crossref), https://doi.org/10.1111/j.1365-2427.2009.02356.x.

Gallien, Laure, et al. “Predicting Potential Distributions of Invasive Species: Where to Go from Here?: Predicting Potential Distributions of Invasive Species.” Diversity and Distributions, vol. 16, no. 3, Apr. 2010, pp. 331–42. DOI.org (Crossref), https://doi.org/10.1111/j.1472-4642.2010.00652.x.

Gillooly, James F., and Stanley I. Dodson. “Latitudinal Patterns in the Size Distribution and Seasonal Dynamics of New World, Freshwater Cladocerans.” Limnology and Oceanography, vol. 45, no. 1, Jan. 2000, pp. 22–30. DOI.org (Crossref), https://doi.org/10.4319/lo.2000.45.1.0022.
Green, J. “The Distribution and Variation of Daphnia Lumholtzi (Crustacea: Cladocera) in Relation to Fish Predation in Lake Albert, East Africa.” Journal of Zoology, vol. 151, no. 1, Jan. 1967, pp. 181–97. DOI.org (Crossref), https://doi.org/10.1111/j.1469-7998.1967.tb02872.x.

Hall, Catherine J., and Carolyn W. Burns. “Mortality and Growth Responses of Daphnia Carinata to Increases in Temperature and Salinity: Mortality and Growth of D. Carinata.” Freshwater Biology, vol. 47, no. 3, Mar. 2002, pp. 451–58. DOI.org (Crossref), https://doi.org/10.1046/j.1365-2427.2002.00815.x.

Hanazato T. Effects of a Chaoborus-released chemical on Daphnia ambigua: Reduction in the tolerance of the Daphnia to summer water temperature. Limnology and Oceanography. 1991 Jan;36(1):165-71.

Haney, J. F., et al. “An Image-Based Key to the Zooplankton of North America.” An Image-Based Key To The Zooplankton Of North America, University of New Hampshire Center for Biological Diversity, http://cfb.unh.edu/cfbkey/html/. Accessed 19 Dec. 2022.

Havel, John E., and Jennifer L. Graham. “Complementary Population Dynamics of Exotic and Native Daphnia in North American Reservoir Communities.” Archiv Für Hydrobiologie, vol. 167, no. 1–4, Oct. 2006, pp. 245–64. DOI.org (Crossref), https://doi.org/10.1127/0003-9136/2006/0167-0245.

Havel, John E., and Jennifer Stelzleni-Schwent. “Zooplankton Community Structure: The Role of Dispersal.” SIL Proceedings, 1922-2010, vol. 27, no. 5, Dec. 2000, pp. 3264–68. DOI.org (Crossref), https://doi.org/10.1080/03680770.1998.11898285.

Havens, Karl E., Therese L. East, et al. “Dynamics of the Exotic Daphnia Lumholtzii and Native Macro-Zooplankton in a Subtropical Chain-of-Lakes in Florida, U.S.A.: Zooplankton Dynamics in a Subtropical Chain-of-Lakes.” Freshwater Biology, vol. 45, no. 1, Sept. 2000, pp. 21–32. DOI.org (Crossref), https://doi.org/10.1046/j.1365-2427.2000.00614.x.

Havens, Karl E., John R. Beaver, et al. “The Outcome of the Invasion of Florida Lakes by Daphnia Lumholtzi: D. Lumholtzi in Florida Lakes.” Freshwater Biology, vol. 57, no. 3, Mar. 2012, pp. 552–62. DOI.org (Crossref), https://doi.org/10.1111/j.1365-2427.2011.02723.x.

Hebert, P. D. N. The Daphnia of North America: An Illustrated Fauna. CD-ROM, University of Guelph: Department of Zoology, 1995.

Hebert, Paul D. N., et al. “Geographical Patterns in Genetic Diversity and Parthenogenesis within the Daphnia Pulex Group from the Southern United States.” American Midland Naturalist, vol. 122, no. 1, July 1989, p. 59. DOI.org (Crossref), https://doi.org/10.2307/2425683.

Hebert, Paul D. N., and Peter M. Grewe. “Chaoborus -Induced Shifts in the Morphology of Daphnia Ambigua 1: Chemomorphosis in D. Ambigua.” Limnology and Oceanography, vol. 30, no. 6, Nov. 1985, pp. 1291–97. DOI.org (Crossref), https://doi.org/10.4319/lo.1985.30.6.1291.

Hebert, Paul D. N., “A Revision of the Taxonomy of the Gennus Daphnia (Crustacea : Daphnidae) in South-Eastern Australia.” Australian Journal of Zoology, vol. 25, no. 2, 1977, p. 371. DOI.org (Crossref), https://doi.org/10.1071/ZO9770371.

Jobling, M. “Temperature Tolerance and the Final Preferendum-Rapid Methods for the Assessment of Optimum Growth Temperatures.” Journal of Fish Biology, vol. 19, no. 4, Oct. 1981, pp. 439–55. DOI.org (Crossref), https://doi.org/10.1111/j.1095-8649.1981.tb05847.x.

Johnson, J. L., and J. E. Havel. “Competition Between Native and Exotic Daphnia: In Situ Experiments.” Journal of Plankton Research, vol. 23, no. 4, Apr. 2001, pp. 373–87. DOI.org (Crossref), https://doi.org/10.1093/plankt/23.4.373.

King, C. R., and J. G. Greenwood. “The Productivity and Carbon Budget of a Natural Population of Daphnia Lumholtzi Sars.” Hydrobiologia, vol. 231, no. 3, Apr. 1992, pp. 197–207. DOI.org (Crossref), https://doi.org/10.1007/BF00018203.

Kivivuori, Liisa A., and Eila O. Lahdes. “How to Measure the Thermal Death of Daphnia? A Comparison of Different Heat Tests and Effects of Heat Injury.” Journal of Thermal Biology, vol.

21, no. 5–6, Oct. 1996, pp. 305–11. DOI.org (Crossref), https://doi.org/10.1016/S0306-4565(96)00014-9.

Kolar, Cynthia S., et al. “Potential Effect of Invasion by an Exotic Zooplankter, Daphnia Iumholtzi.” Journal of Freshwater Ecology, vol. 12, no. 4, Dec. 1997, pp. 521–30. DOI.org (Crossref), https://doi.org/10.1080/02705060.1997.9663566.

Kolar CS, Boase JC, Clapp DF, Wahl DH. Potential effect of invasion by an exotic zooplankter, daphnia iumholtzi. Journal of Freshwater Ecology. 1997 Dec 1;12(4):521-30.

Lennon, J. T., et al. “Influence of Temperature on Exotic Daphnia Lumholtzi and Implications for Invasion Success.” Journal of Plankton Research, vol. 23, no. 4, Apr. 2001, pp. 425–33. DOI.org (Crossref), https://doi.org/10.1093/plankt/23.4.425.

Lewis Jr, W. M. “Tropical Lakes: How Latitude Makes a Difference.” Perspectives in Tropical Limnology, vol. 4364, 1996, pp. 43–64, http://agustincapriati.com/wp-content/uploads/2015/09/Lewis-1996.pdf.

MacIsaac, Hugh J., et al. “Inter- and Intraspecific Variation in Acute Thermal Tolerance of Daphnia.” Physiological Zoology, vol. 58, no. 3, May 1985, pp. 350–55. DOI.org (Crossref), https://doi.org/10.1086/physzool.58.3.30156006.

Mallin, Michael A., and Willard E. Partin. “Thermal Tolerances of Common Cladocera.” Journal of Freshwater Ecology, vol. 5, no. 1, June 1989, pp. 45–51. DOI.org (Crossref), https://doi.org/10.1080/02705060.1989.9665212.

Mantovano, Tatiane, et al. “A Global Analysis of the Susceptibility of River Basins to Invasion of a Freshwater Zooplankton (Daphnia Lumholtzi).” Freshwater Biology, vol. 66, no. 4, Apr. 2021, pp. 683–98. DOI.org (Crossref), https://doi.org/10.1111/fwb.13670.

Mergeay, Joachim, et al. “Cryptic Invasion and Dispersal of an American Daphnia in East Africa.” Limnology and Oceanography, vol. 50, no. 4, July 2005, pp. 1278–83. DOI.org (Crossref), https://doi.org/10.4319/lo.2005.50.4.1278.

Muzinic, Christopher J. “First Record of Daphnia Lumholtzi Sars in the Great Lakes.” Journal of Great Lakes Research, vol. 26, no. 3, Jan. 2000, pp. 352–54. DOI.org (Crossref), https://doi.org/10.1016/S0380-1330(00)70698-8.

Nowakowski, K., Sługocki, Ł. Short-term heat shock perturbation affects populations of Daphnia magna and Eurytemora carolleeae: a warning to the water thermal pollution. Sci Rep 11, 16909 (2021). https://doi.org/10.1038/s41598-021-96464-8.

Obreshkove, Vasil, and Alan W. Fraser. “GROWTH AND DIFFERENTIATION OF DAPHNIA MAGNA EGGS IN VITRO.” The Biological Bulletin, vol. 78, no. 3, June 1940, pp. 428–36. DOI.org (Crossref), https://doi.org/10.2307/1537664.

Orcutt, John D., and Karen G. Porter. “The Synergistic Effects of Temperature and Food Concentration of Life History Parameters of Daphnia.” Oecologia, vol. 63, no. 3, Aug. 1984, pp. 300–06. DOI.org (Crossref), https://doi.org/10.1007/BF00390657.

Pattinson, K. R., et al. “Invasibility of a Reservoir to Exotic Daphnia Lumholtzi : Experimental Assessment of Diet Selection and Life History Responses to Cyanobacteria: Algal Diet and Daphnia Life History.” Freshwater Biology, vol. 48, no. 2, Feb. 2003, pp. 233–46. DOI.org (Crossref), https://doi.org/10.1046/j.1365-2427.2003.00987.x.

Pimentel, David, et al. “Update on the Environmental and Economic Costs Associated with Alien-Invasive Species in the United States.” Ecological Economics, vol. 52, no. 3, Feb. 2005, pp. 273–88. DOI.org (Crossref), https://doi.org/10.1016/j.ecolecon.2004.10.002.

R Core Team. R: A language and environment for statistical computing, 2022. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Soeken-Gittinger, Lori A., et al. “Differing Effects of Suspended Sediments on the Performance of Native and Exotic Daphnia.” Freshwater Biology, vol. 54, no. 3, Mar. 2009, pp. 495–504. DOI.org (Crossref), https://doi.org/10.1111/j.1365-2427.2008.02126.x.

Sorensen, Kathryn H., and Robert W. Sterner. “Extreme Cyclomorphosis in Daphnia Lumholtzi.” Freshwater Biology, vol. 28, no. 2, Oct. 1992, pp. 257–62. DOI.org (Crossref), https://doi.org/10.1111/j.1365-2427.1992.tb00582.x.

Stoeckel, James A., et al. “Establishment of Daphnia Lumholtzi (an Exotic Zooplankter) in the Illinois River.” Journal of Freshwater Ecology, vol. 11, no. 3, Sept. 1996, pp. 377–79. DOI.org (Crossref), https://doi.org/10.1080/02705060.1996.9664461.

Taylor, Derek J., et al. “BIOGEOGRAPHY OF A WIDESPREAD FRESHWATER CRUSTACEAN: PSEUDOCONGRUENCE AND CRYPTIC ENDEMISM IN THE NORTH AMERICAN DAPHNIA LAEVIS COMPLEX.” Evolution, vol. 52, no. 6, Dec. 1998, pp. 1648–70. DOI.org (Crossref), https://doi.org/10.1111/j.1558-5646.1998.tb02245.x.

Thomaz, Sidinei M., et al. “Aquatic Invasive Species: General Trends in the Literature and Introduction to the Special Issue.” Hydrobiologia, vol. 746, no. 1, Mar. 2015, pp. 1–12. DOI.org (Crossref), https://doi.org/10.1007/s10750-014-2150-8.

Walsh, Jake R., et al. “Comparing Models Using Air and Water Temperature to Forecast an Aquatic Invasive Species Response to Climate Change.” Ecosphere, vol. 11, no. 7, July 2020. DOI.org (Crossref), https://doi.org/10.1002/ecs2.3137.

Work, Kirsten A., and Moshe Gophen. “Factors Which Affect the Abundance of an Invasive Cladoceran, Daphnia Lumholtzi , in U.S. Reservoirs: Analysis of a Reservoir Invader.” Freshwater Biology, vol. 42, no. 1, Aug. 1999, pp. 1–10. DOI.org (Crossref), https://doi.org/10.1046/j.1365-2427.1999.00449.x.

Yurista PM. Bioenergetics of a Semi-Tropical Cladoceran, Daphnia lumholtzi. Journal of Freshwater Ecology. 2004 Dec 1;19(4):681-94.

Yurista, Peder M. “Cyclomorphosis in Daphnia Lumholtzi Induced by Temperature.” Freshwater Biology, vol. 43, no. 2, Feb. 2000, pp. 207–13. DOI.org (Crossref), https://doi.org/10.1046/j.1365-2427.2000.00543.x.

Yurista PM, Rice GT, White DS. Long-term establishment of Daphnia lumholtzi Sars in Kentucky Lake, USA. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen. 2000 Dec 1;27(5):3102-6.

CHAPTER 4: CONCLUSIONS AND FUTURE WORK

CONCLUSIONS

The studies presented here expand our understanding of the factors influencing *Daphnia lumholtzi* establishment and distribution in novel environments. In the MTD, which is a less predictable environment than reservoirs, *Daphnia lumholtzi* populations are present across a broad range of temperatures, although at apparently lower densities than reported in reservoirs, generally overlapping in seasonal occurrence with native *Daphnia*. As observed in reservoirs, there is a difference in their seasonal distributions, with *D. lumholtzi* occurring in autumn exclusive of native *Daphnia*, while native *Daphnia* dominated in winter. The two groups overlapped in spring and summer, suggesting that population dynamics are not as distinctly separate as they are in reservoirs. *D. lumholtzi* was present during both the coldest and warmest periods, suggesting other factors may be responsible for the observed occurrence patterns since the species does not seem to be limited by temperature in the delta. For example, salinity may limit the long-term establishment of *D. lumholtzi* in some areas of the estuary. During this study, *D. lumholtzi* was not found in the lower delta sites that are frequently in the range of brackish water salinity levels, nor was the species found in the upper delta during periods of elevated salinity. Since the species was found during periods of elevated salinity in an earlier study from 2002-2005 (DeVries et al., 2006), it is possible that *D. lumholtzi* is not able to maintain long term populations in areas that

regularly experience elevated salinity. Interestingly, the native *Daphnia ambigua* was found in the lower sites of the MTD and was also the only species detected in any sites of the WBNERR, while *D. lumholtzi* was not detected in either area. WBNERR is known to experience greater salinity intrusion compared to the MTD. Together, these observations support the idea that salinity tolerance may play a role in limiting *D. lumholtzi* distribution. More expansive surveys of habitats within and around the MTD and WBNERR can provide important contrasts to what is currently known about *D. lumholtzi* distribution in reservoirs and inland freshwater environments to better understand the invasion ecology of this species.

The results of thermal tolerance trials show that *D. lumholtzi and D. ambigua* from the MTD have similar upper thermal limits as measured by 48-hour mortality following acute heat exposure from 32-39°C. This supports the idea that both are euryhaline species able to tolerate the warmest temperatures experienced in the delta. There was no difference in the 48-hour mortality rate following heating to 32°C between *D. lumholtzi* and any of the native species tested. This indicates that each of these species has similar capacity to tolerate the maximum water temperatures in the MTD (MacIsaac et al., 1985). Together, the results of these projects support the idea that thermal tolerance is not the delimiting factor for *D. lumholtzi* community patterns in the MTD. An important next step will be to investigate the effects of longer-term exposure to

32°C and other seasonally relevant temperatures on mortality and reproduction in *D. lumholtzi* compared to native *Daphnia* from the MTD.

FUTURE DIRECTIONS

To better understand the invasion ecology of *Daphnia lumholtzi*, future field work will entail studies designed to answer three questions related to *D. lumholtzi* distribution: 1) where does *D. lumholtzi* go in the winter, 2) what is the effect of salinity on *D. lumholtzi* survival and reproduction, and related to this, 3) why is *D. lumholtzi* not found in WBNERR?

Daphnia lumholtzi Absence in Weeks Bay

WBNERR is an estuary that is similar climatically and to the MT*D. D. lumholtzi* dispersal can happen in several ways, including dispersal of daphniids and ephippia in flowing water and inadvertent transport by other animals and by human activities like boating and other watercraft. Since WBNERR is geographically close to the MTD, is a popular recreational area and is fed by two rivers, it seems likely that *D. lumholtzi* propagules would sometimes be introduced to the area. This suggests that the species may not be able to become established in WBNERR, providing a unique opportunity to study potentially opposing outcomes in the species' invasion ecology, in particular with regard to salinity tolerance, given that salinity appears to influence *D. lumholtzi* distribution in the MTD, and that salinity influx is higher in WBNERR.

Expanded surveys around the MTD and WBNERR will also provide important information regarding which native species occur in the area and their status. Considering the dearth of recent information available about native *Daphnia* found in the lower coastal plain and their habitat use, updated community surveys are needed. *Daphnia magniceps*, for example, has not been previously reported in the MTD to my knowledge, although the species is known to inhabit southern swamps (Taylor et al., 1998). *D. magniceps* is a member of the *Daphnia laevis* species complex, and a "*D. laevis*-like species" is reportedly common in the southeast (Hebert et al., 1989, Taylor et al., 1998).

Winter Populations of Daphnia lumholtzi

D. lumholtzi was present during late fall and winter when water temperatures were below 15C in the delta during this study, consistent with observations by DeVries and colleagues (2006), and with several reports of winter populations in other studies (reviewed in Chapter 2 of this dissertation). I also found the species swarming in the shallows of Lake Texoma in December during an unrelated sampling event, and another *D. lumholtzi* winter swarm has also been recently reported (Beaver et al., 2018) in Lake Kentucky. In both cases, the congregation contained both males and ephippial females, consistent with the association of *Daphnia* swarms with sexual reproduction (Gerritsen, 1980; Young, 1978). More spatially extensive and temporally frequent sampling is needed near sites where *D. lumholtzi* is found in winter in order to clarify overwintering in this species in the delta. For example, Frisch & Weider (2010) found winter

populations of *Daphnia lumholtzi* are generally restricted to specific areas of Lake Texoma. Data comparing *D. lumholtzi* occurrence in winter compared to summer is lacking in general (Mantovano et al., 2019), so these studies are important to understanding the role of cold tolerance in the species' invasion ecology.

Effects of Temperature on Life History Traits of Daphnia lumholtzi

A critical complement to field studies will be life history studies using laboratory clones of *D. lumholtzi* collected from summer and winter populations. These studies are important to characterize the relationship between temperature and other traits that affect the distribution and colonization capacity in this species, yet of the 109 published studies of *Daphnia lumholtzi* from 1976 to 2016, only 12 were controlled laboratory experimental studies (Mantovano et al., 2019). It is widely understood that aquatic invertebrates like *Daphnia* possess several life history traits associated with successful invaders (Havel et al., 2015), and for aquatic ectotherms, temperature often controls the timing and rate of life history processes (Schwartz, 1984). Among these traits, short generation times and alternating reproductive strategies are particularly advantageous. Asexually reproducing females can release clutches of offspring every 2-3 days, increasing the likelihood that a single female can found a sustainable population in an introduced environment (Gerritsen, 1980). Alternatively, fertilized eggs in protective ephippia can regenerate the population from sedimented egg banks or provide dispersal to potentially suitable environments (Mergeay et al., 2004). These processes are unclear in natural populations of *D. lumholtzi*, but preliminary data from a pilot study provides

compelling evidence that, in conjunction with the broad thermal range demonstrated in the field study, variability in LH traits impacts the colonization capacity seen in *D. lumholtzi*. (Table 4.1 and 4.2). The preliminary results indicate that two genetically distinct clones from the MTD and one clone from Normandy Dam reservoir in Tennessee exhibit life history trait plasticity with temperature (Table 4.1 and Table 4.2). The age of first reproduction was 1-3 days earlier in animals cultured at 30°C compared to those cultured at 23°C, allowing earlier reproduction to offset lower survival at the higher temperature. Both asexual and sexual reproduction (as measured by ephippial egg formation) were higher at the lower temperature.

Table 4.1. Life history responses of *Daphnia lumholtzi* laboratory clones cultured at 23°C

Trait	MTD Clone 1	MTD Clone 2	NDR Clone 1
Median Lifespan	24.0	22.5	18.0
Age of First Reproduction (Days)	9	12	7
Lifetime Parthenogenetic Potential	48.5	35.4	27.7
Per Capita Sexual Reproduction	1.3	1.1	2.7

Table 4.2. Life history responses of *Daphnia lumholtzi* laboratory clones cultured at 30°C

Trait	MTD Clone 1	MTD Clone 2	NDR Clone 1
Median Lifespan	13	13	7
Age of First Reproduction (Days)	7	9	5
Lifetime Parthenogenetic Potential	11.9	8.9	20.1
Per Capita Sexual Reproduction	0	1	0

Expanding this pilot study to include differential temperature regimes, including lower temperatures in the range that *D. lumholtzi* is found in winter populations will characterize the relationship between temperature and other traits that affect the distribution and colonization capacity in this species.

Looking to the future, these data also form the underpinnings of a model using *Daphnia lumholtzi* to investigate the role of DNA damage in answering a long-standing question in evolutionary biology, namely: Why do organisms age? A description of this project is given in Chapter 5 of this dissertation.

CHAPTER 5: *DAPHNIA LUMHOLTZI* AS A MODEL ORGANISM FOR INVESTIGATING DNA DAMAGE DURING AGING

PROJECT SUMMARY

Senescence, or aging, is the development of increased somatic deterioration and loss of function over time. It occurs nearly universally among organisms and leads to decreased survival and reproduction with age. Because aging decreases fitness, it is a critical factor in explaining the life history evolution of organisms, as well as how they balance survival and reproduction in the context of environmental challenges. Since energy acquisition and processing are limited by environmental factors and physiological constraints, an organism's overall fitness results from trade-offs in how limited metabolic resources are allocated among the competing needs of somatic maintenance and growth, which support survival, and reproduction. Evolutionary theories of aging propose that aging results from effects of genes that exert negative effects later in life, either through accumulation of deleterious mutations or through the pleiotropic effects of genes that are beneficial during early stages but become harmful later. The disposable soma model integrates the ideas of antagonistic pleiotropy and mutation accumulation into the context of life history trade-offs, suggesting that senescence occurs because of an organism allocating more energy toward ensuring germ line integrity and reproduction at the expense of the overall health of the soma. Besides being a generalizable evolutionary theory of aging, it makes testable predictions about molecular changes

that can potentially be used as markers of senescence. The disposable soma theory predicts that somatic tissues within an organism will show deterioration with age while the germ line will be maintained in a healthy state to ensure that the genes passed on to the next generation are relatively free of damage. This suggests that DNA in germ tissues should remain relatively undamaged throughout the reproductive life of the organism while DNA in somatic tissues should accumulate more DNA damage with age. While damage to somatic tissues may take on many forms, DNA damage is a very powerful marker that can have negative effects on many other downstream physiological processes. Therefore, a comparison of DNA damage between germ and somatic tissues with age will not only allow us to test the validity of a major hypothesis in the disposable soma evolutionary theory of aging, but also provide us with a molecular marker of deterioration.

For aquatic ectotherms like the crustacean zooplankton *Daphnia*, both temperature and predation are primary drivers of life history trade-offs. Elevated temperatures can induce faster growth rates and reproductive maturation but are also associated with decreased adult survival and reproductive output. Several species of *Daphnia* respond to predator cues by shifting resources toward the growth of protective spines. In *Daphnia lumholtzi*, elevated water temperature is a secondary cue for predator presence that induces the growth of substantial head and tail spines. The development and maintenance of body armor has been shown to improve survival, but is expected to divert resources to somatic maintenance, resulting in trade-offs in reproduction and

longevity. This study aims to employ both life history and molecular studies to investigate the correlation of DNA damage with age-related changes during normal aging and in response to elevated culture temperature in *D. lumholtzi*. This will not only allow us to test molecular changes associated with age-related decline in the context of the evolutionary processes that underlie senescence but may also provide us with a specific molecular marker of somatic deterioration.

PROJECT DESCRIPTION

Aging, or senescence, is characterized by the accumulation, over time, of physical and physiological traits associated with the deterioration of the soma (non-reproductive tissues) and loss of function. These changes alter the individual phenotype in some manner that impacts fitness by decreasing reproductive capacity and survival with age. As the evolutionary biologist, George C. Williams, (1957) famously reasoned: morphogenesis is such an intricate and complex process that it seems miraculous, so why is it that an organism can achieve morphogenesis and yet be unable to merely maintain the body once it is formed? The underlying assumption of Williams' (1957) statement is that expression of traits related to senescence are unavoidable and are, in most if not all cases, opposed by natural selection.

From Williams' observation the question really becomes: if senescence is opposed by natural selection, why do organisms age? In general, natural selection drives organisms

to optimize survival and fecundity within current environmental conditions (Kirkwood 1977; Stearns, 1992). The force of natural selection is strongest during times when reproductive output is highest and diminishes as reproductive output declines during an organism's lifespan (Kirkwood & Rose, 1991). This decline is most evident in organisms with a clear separation of parent and offspring through a distinction between the germ line and the soma (Bell, 1984; Kirkwood & Rose, 1991). The somatic deterioration associated with aging in these organisms results from the inability of natural selection to eliminate genes with deleterious effects that are expressed after the reproductive stage (Rose, 1991). Evolutionary theories of aging (ETA) propose that deleterious alleles can be maintained in the population through neutral mechanisms explained by Williams' Antagonistic Pleiotropy Theory (1957) and selective mechanisms proposed by Medawar's Mutation Accumulation Theory (1955). Mutation Accumulation (MA) theory suggests that alleles with detrimental effects only in late life could accumulate in the population because they are neutral relative to fitness, not expressed at all during peak reproductive stages, or do not reach a threshold accumulation of mutation that would have an effect on the organism. Somatic mutations have been shown to accumulate in the liver of C57/BL6 mice with age (Busuttil et al., 2003), and to accumulate more slowly in the liver of the long-lived Ames dwarf mouse (Garcia et al., 2008). The Antagonistic Pleiotropy theory proposes that these alleles may be beneficial or required for reproductive performance early in life but become detrimental in some manner related to fitness later in life when they are not subject to removal by selective forces. Evidence for antagonistic pleiotropic effects has been found in studies of natural population

genetics (e.g., Charmantier et al., 2006), and more often, has been provided using laboratory models (reviewed in Leroi et al., 2005). Kirkwood (1977) recognized that these evolutionary genetic models suggest the existence of trade-offs between early-life fitness benefits and physiological costs in late life, and that these trade-offs result from the priority for reproduction imposed by natural selection (Kirkwood,1977; Kirkwood & Holliday 1979: Kirkwood & Rose, 1991).

Thus, evolutionary theories of aging are based upon age-specific differences in the contribution of reproduction to the organism's overall fitness. More specifically, senescence will evolve if an incremental increase in reproduction at an earlier age will yield a greater increase in fitness than an equal incremental increase at later ages (Bell, 1984). For example, life span has been shown to be positively correlated with age at first reproduction in female fruit flies and flour beetles (Clarke and Maynard Smith, 1961; Charlesworth, 1981; Sokal, 1970; Mertz, 1975; Partridge & Barton, 1993; Zwaan et al., 1995; Rose & Luckinbill et al., 1984; Sgro & Partridge, 1999; Wattiaux, 1968;).

Individuals that mature and become reproductively active early in life tend to die sooner than those that delay reproduction. Life span has also been shown to be negatively affected by increases in overall reproductive output in male and female fruit flies, beetles, water bugs and mammals (Creighton et al., 2009; Clutton-Brock et al. 1988; Mertz 1975; Murdoch 1966; Snell and King 1977; Law 1979; Partridge and Farquhar

1981; Nur 1984; Fowler and Partridge 1989; Rose et al. 1989; Prowse and Partridge 1997; Gilg and Kruse 2003).

The relationship between reproduction and lifespan illustrates a central idea of evolutionary theories of aging: since energy acquisition and processing is limited by environmental factors and physiological constraints, the suite of fitness characteristics an organism possesses is the result of trade-offs in how metabolic resources are allocated among the competing compartments of maintenance, growth, reproduction, and storage (Stearns 1992; Kirkwood and Rose 1991). Additionally, early in life, resources must be allocated to support maintenance and growth in order to reach sexual maturity. After sexual maturity is reached, however, resources will generally be shifted to reproduction. Since reproduction is costly, its onset leads to trade-offs in life history traits as organisms must now allocate resources in a manner that maximizes the balance between investing in current reproduction and investing in maintenance and survival in order to ensure further reproductive bouts within a given environment (Gadgil and Bossert 1970; Stearns, 1992; Gilg & Kruse, 2003: Kirkwood, 2005; Leroi et al., 2005, Rosenheim at al. 2010). One possible trade-off is that an individual that allocates more energy to reproduction will necessarily have less energy available for maintenance of somatic cells and tissues. Therefore, an increase in reproductive output generally results in a decrease in lifespan.

Snell and King (1977) provided evidence for a direct trade-off between increased reproductive output and lifespan in rotifers. Lifespan was reduced in rotifers that had higher reproductive output at earlier ages as compared to rotifers that had low reproductive rates over a longer period of time. Similar trade-offs have also been shown in female and male fruit flies (Fowler and Partridge, 1989; Service, 1989; Partridge and Farquhar, 1981; Prowse and Partridge, 1997) and waterbugs (Gilg and Kruse 2003). Likewise, fruit flies (Drosophila) that were selected for longer lifespan in laboratory experiments showed an overall decline in reproductive output (Zwaan et al., 1995).

Brood care and environmental conditions during reproductive stages can have significant impacts on the resources required for both somatic maintenance and reproduction, resulting in reduced longevity. Gilg and Kruse (2003) examined life history trade-offs between reproduction and lifespan versus parental care and lifespan using the giant waterbug, *Belostoma flumineum*. In this organism, males provide all the parental care. For both male and female waterbugs, those that were not allowed to mate outlived those that mated. Males that mated and brooded their young had significantly lower life spans than virgin males. In the beetle (*Nicrophorus orbicollus*) life history trade-offs were observed when resource availability was altered experimentally. Parents of this species procure a small vertebrate carcass that provides food resources for both them and their young, and adjust their brood size to match the available resources by estimating carcass volume. In the beetle, resource scarcity was induced by reducing the carcass size after females had estimated the carcass volume. Females in

the study that had the most food resources invested more in current reproduction at the expense of later reproductive output. All breeding females had reduced life spans compared to non-breeders. Animals in the resource scarcity group additionally experienced significant declines in fecundity, body mass and lifespan.

Other environmental factors, such as temperature fluctuations and the presence of predators have significant impacts on life history strategies (Stearns, 1992, Gadgil & Bossert 1970, Snell & King 1977, Weetman & Atkinson 2004, Crowl & Covich 1990, Tollrian 1995, Stibor 1992, Boersma et al. 1998, Weider & Pijanowska 1993, Yurista 2000, Sorenson & Sterner 2006). For aquatic ectotherms like the crustacean zooplankton *Daphnia*, both temperature and predation are primary drivers of life history shifts, but temperature has been shown to have greater effect (Weetmen & Atkinson 2004). Elevated temperatures are generally associated with faster juvenile growth rates and reproductive maturation (Schwartz 1984, Weetman & Atkinson 2004) however they are also associated with decreased adult survival and reproductive output (Schwartz 1984, Orcutt and Porter 1984). Several species of *Daphnia* are also known to respond to the presence of predators by altering their morphology to produce anti-predator defenses. For example, anti-predator defenses are quite pronounced in *D. lumholtzi*, which develops barbed head and tail spines that can be up to three times its core body length (Sorenson 1992). Experiments involving predation trials showed that these spines are quite effective at deterring predatory juvenile fish (Yurista, 2000). The development and maintenance of body armor is expected to divert resources to somatic

maintenance, resulting in trade-offs in reproduction and longevity. Thus, trade-offs play an important role in life history strategies and the evolution of senescence in organisms with distinct germ and soma lines.

The Disposable Soma (DS) theory explains the evolution of senescence in the context of how life histories are shaped by the competing demands between current reproduction and somatic maintenance to ensure later reproduction (Kirkwood 1977, Kirkwood and Holliday 1979, Kirkwood and Rose 1991). This model integrates the ideas of antagonistic pleiotropy and mutation accumulation into the context of life history trade-offs, suggesting that senescence occurs because of an organism allocating more energy toward ensuring germ line integrity and reproduction at the expense of the overall health of the soma. Besides being a generalizable evolutionary theory of aging, the Disposable Soma theory makes testable predictions about molecular changes that can potentially be used as markers of senescence. It predicts that somatic tissues within an organism will show deterioration with age while the germ line will be maintained in a healthy state. Maintenance of the germ line is important to ensure that the genes passed on to the next generation are relatively free of damage. This suggests that DNA in germ tissues should remain relatively unbroken and undamaged throughout the reproductive life of the organism while DNA in somatic tissues should accumulate more DNA damage with age. Evolutionary theories of aging also predict that DNA damage in somatic tissues should only be apparent at some time after reproductive maturity is reached, since degeneration of the soma prior to reproduction would be strongly

opposed by natural selection. While damage to somatic tissues may take on many forms, DNA damage is a very powerful marker that can have negative effects on many other downstream physiological processes. Therefore, a comparison of DNA damage between germ and somatic tissues in reproductive individuals will not only allow us to test the validity of a major hypothesis in the disposable soma evolutionary theory of aging, but also provide us with a molecular marker of deterioration.

The concept that DNA damage is important to the aging process is not new to people studying the mechanistic causes of senescent deterioration. Bernstein and Bernstein (1991) suggest that DNA damage is the underlying mechanism of senescence (see also: Avise, 1993). Structural irregularities in DNA such as single and double stranded breaks, modified bases, depurinations, and cross-links inevitably arise from both endogenous (oxidative damage) and exogenous (UV irradiation) sources. Damage to DNA has been linked to impaired DNA replication (Rupp and Howard-Flanders, 1968; Villani et al., 1978), decreased RNA transcription (Park and Buetow, 1990; Slagboom et al. 1989), decreased protein synthesis (Dwyer et al., 1980; Pluskal et al., 1984), reduced cell viability and tissue function (Doggett et al., 1981; Goldspink and Alnaqeeb, 1985; see also Holmes et al. 1992), and cell death (Cleaver, 1970; Maher et al., 1976). Several studies have shown that in certain tissues, DNA damage increases with increasing age of the organism (Bernstein and Bernstein, 1991, Vijg, 2000). Similarly, age related differences in DNA repair processes have also been observed (Bernstein and Bernstein, 1991). Most of these studies, however, have concentrated on somatic tissues in

mammals, including humans and mice. The results from these studies confirm that damaged DNA has many downstream effects known to be linked to senescence.

While there is clearly a common tie between the evolutionary viewpoints of the disposable soma theory and the more mechanistic view of DNA damage causing senescence, to date evolutionarily based theories of senescence using DNA damage as a marker have never to our knowledge been adequately tested. In fact, it is a rare occurrence when mechanistic processes correlated with aging are viewed from an evolutionary perspective, although some studies have laid important groundwork supporting this idea (Holmes et al., 2001; Austad, 2001; Tatar and Yin, 2001; Austad and Fischer, 1991; Van Voorhies and Ward, 1999; Van Voorhies, 2001). Much of the research involving DNA damage associated with aging has focused on increased DNA damage caused by irradiation or other DNA damaging processes and the corresponding decrease in life span or some indication of premature aging (Bernstein and Bernstein, 1991).

These studies have produced mixed results, potentially from the many possible side effects of such manipulations. It seems that a less intrusive method of assessing the relationship between DNA damage and life span would be to manipulate life span using a life history trade-off and measuring the accumulation of DNA damage in the various treatments. Preliminary studies in our lab using mosquito fish (Gambusia) show that DNA damage increased during normal aging in female fish that were kept individually as virgins for the duration of their lifespan. Physical and behavioral changes associated

with senescence (e.g., decreased body condition, fin deterioration, reduction in feeding rates) were also evident during later ages. Further, altering the resources available for somatic maintenance by allowing females to reproduce resulted in a decrease in longevity, confirming the predictions of the disposable soma model. These pilot studies suggest that comparing the relative levels of DNA damage between germ and somatic tissues under conditions that alter somatic maintenance requirements provides a means to integrate the evolutionary processes of senescence with specific molecular changes that result in somatic decline. Interestingly, in *D. lumholtzi*, certain environmental factors, such as elevated temperature, constitute stressors that induce the growth of large head and tail spines, which are initiated during embryonic development and persist through adult stages. Thus, growing *D. lumholtzi* at elevated temperatures provides a convenient method for increasing costs of somatic maintenance.

PURPOSE AND AIMS

The study aims to employ both life history and molecular studies to investigate the correlation of DNA damage with age-related changes during normal aging and in response to environmental challenges in *D. lumholtzi*. This will not only allow us to test molecular changes associated with age-related decline in the context of the evolutionary processes that underlie senescence but may also provide us with a specific molecular marker of somatic deterioration.

We hypothesize that DNA damage will be absent or minimal in germ tissues but increase significantly in somatic tissues during normal aging and increase faster or occur to a greater extent under environmental conditions that require increased somatic maintenance and may thus be an important biomarker of age-related somatic changes. We propose two specific aims to test this hypothesis:

1) Determine whether DNA damage increases with age in somatic and germ tissues in female *Daphnia lumholtzi***.** DNA breakage will be measured over the duration of the lifespan in female *Daphnia*. Life history responses will be monitored using life tables of longevity (days from release from brood chamber to death), growth (number of molts over duration of life span), age of first reproduction, and reproductive output (number of parthenogenetic offspring released). DNA breakage in somatic tissue will be assessed in pre-reproductive juveniles at 1 and 3 days following release from the brood chamber. DNA breakage will be measured in mature adults on 4 days after release from the brood chamber and every 10 days for the duration of the lifespan (approximately 40 days based on preliminary life tables in our lab). DNA breakage in germ tissue will be measured using parthenogenetic eggs newly (within 24 hours) deposited into the brood chamber.

2) Determine the impact of increased somatic maintenance requirements in terms of DNA repair in female *Daphnia lumholtzi*. DNA breakage will be measured over the duration of the lifespan in adult female *Daphnia* raised at preferred and elevated temperatures commonly experienced in natural environments. Life tables will be used

to assess alterations in life history characters including longevity, growth, age of first reproduction and reproductive output DNA breakage in somatic tissue will be assessed in pre-reproductive juveniles at 1- and 3-days following release from the brood chamber. DNA breakage will be measured in mature adults on 4 days after release from the brood chamber and every 10 days for the duration of the lifespan (approximately 40 days based on preliminary life tables in our lab). DNA breakage in germ tissue will be measured using parthenogenetic eggs newly (within 24 hours) deposited into the brood chamber.

Model Organism

Daphnia lumholtzi is a small crustacean zooplankton found in freshwater and estuarine habitats. The adult core body includes a non-segmented head and thoracic and abdominal regions whose combined length ranges from approximately 1.5 - 3mm (Swaffar & Obrien 1996, Ebert 2005). Locomotion is provided by two large biramous antennae positioned adjacent to the posterior margin of the head. A set of phyllopod appendages beats continuously within the ventral abdominal area, filtering algae, bacteria and other small protists from the water (Ebert 2005). Soft tissues of the body are surrounded by a chitinous carapace, which is opened to the environment along the ventral aspect. *Daphnia* have indeterminate growth, shedding the carapace every few days. Reproductively mature females develop a brood chamber within the carapace

adjacent to the dorsal body wall. Eggs develop and hatch within the brood chamber, and then are released into the environment as fully formed neonates.

D. lumholtzi is native to subtropical and tropical lakes of Africa, Asia and Australia (Green 1967, Green 1971, Havel et al. 2000, Mergeay 2005a). In Africa, it is found in lakes with temperatures ranging from 14°C to 29°C (Lewis, 1996- CIRES Tropical Lakes, Mergeay 2005a, Havel 2000, King & Greenwood 1992, Hebert 1977, Green 1967, 1971). It was likely introduced to the U.S. through the stocking of reservoirs with Nile perch from Africa in the late 1980's (Havel & Hebert 1993, Sorensen & Sterner 1992). Since then, it has spread to freshwater systems throughout the US, including reservoirs, rivers, lakes and estuaries (Benson, Maynard & Raikow 2011). *D. lumholtzi* populations in the U.S. have been studied most extensively in reservoirs, where high thermal tolerance has been hypothesized to allow this species to exploit a vacant thermal niche during warmer periods when native species undergo seasonal declines (Work & Gophen 1999, East et al. 1999, Lennon et al. 2001). For example, in Lake Okeechobee (Florida), *D. lumholtzi* population densities are low during cooler spring periods, when native species abundances reach seasonal peaks. However, during summer months, when water temperatures exceed 27 °C, native species undergo a seasonal decline, while *D. lumholtzi* populations rapidly expand to their peak densities (East et al. 1999).

In addition to its broad range of thermal tolerance, *D. lumholtzi* has several other characteristics that have facilitated its rapid expansion in the United States. Under favorable conditions, *D. lumholtzi* expands rapidly by producing large clutches of

parthenogenetic female offspring (typically 8-14) every few days, resulting in dense seasonal populations composed primarily of females. When environmental conditions decline, females begin to produce male or mixed sex clutches, followed by a switch to sexual reproduction. During this sexual phase, females produce one or two haploid eggs that are fertilized by males and then deposited into environmentally resistant cases (ephippia). These typically settle into the sediment and remain dormant until environmental cues induce development to resume (Ebert 2005), allowing the population to renew seasonally.

As a model organism, *Daphnia* may be best known for their phenotypic plasticity, which allows them to change their morphology, physiology, and reproductive activities in response to environmental factors. Warmer water temperatures and/or the presence of predators induce the development of defensive structures as outgrowths of the carapace, including neck teeth (Havel 1985), helmets (Hrbacek 1959, O'Brien et al 1979) and head and tail spines (Sorenson et al 1992, Tollrian 1994, Yurista 2000). Spine formation in *D. lumholtzi* is associated with predation defense (Kolar & Wahl 1998, Dzialowski et al. 2003, Engel & Tollrian 2009), but temperature alone has also been shown to significantly affect the length of head and tail spines in both wild (Sorenesen & Sterner 1992) and laboratory populations (Yurista 2000). Molting rate also increases with temperature (Work & Gophen 1999b), suggesting that spine formation requires significantly greater somatic maintenance at higher temperatures.

Daphnia lumholtzi is particularly well-suited to studies on life history responses and aging. They're amenable to laboratory culture, maturing within 4-10 days and producing a clutch of eggs every 3-6 days for the duration of their lifespan, which is approximately 40 days in the laboratory. Parthenogenetically reproducing females can be isolated from wild populations, and isofemale lineages (clones) can be established in the laboratory. This makes it possible to produce large numbers of genetically identical offspring for experimental manipulation. Since eggs are visible during development and carried within the brood chamber, it's possible to separate germ and somatic tissue, allowing us to test the predictions of evolutionary theories of aging. Manipulation of growth, reproduction and lifespan by varying environmental conditions allows the comparison of life history responses among groups of experimental clones exposed to different conditions.

Further, *the Daphnia* genome is composed of more genes universal among the Bilateria than other arthropods, and therefore has more genes in common with humans than do other arthropods, including *Drosophila* and *Caenorhabditis* (Colbourne et al., 2011).The sequencing of the *D. pulex* genome makes it possible to couple life history studies with underlying molecular changes that accompany trade-offs and alterations in life cycle events (e.g. Eads 2007).

EXPERIMENTAL DESIGN

Culture Conditions

Daphnia lumholtzi clones will be captured from wild populations and acclimated to culture in the laboratory for at least three months, which is at least 5 generations. Laboratory batch cultures will be maintained at 22 ±1 ^{o}C with a 16-hour photoperiod in modified lake water. *This* water is prepared by adjusting aerated, glass-fiber filtered lake water to 60-100 mg/L total hardness (as CaCO3), electrical conductivity in the range of 400-600µS/cm, and pH 7.2- 7.4. This culture water is filtered over a sterile 0.2-micron filter into sterile media bottles. To generate animals for experiments, female neonates will be isolated from batch cultures and reared individually in order to generate isofemale cultures (clones). Clones will be maintained with a 16-hour photoperiod at either 22 ±1 ^{o}C (normal temperature), which is the ambient temperature of the laboratory, or in a heated water bath set to at 30 ±1 ^{o}C (stress temperature; to alter life history responses). Individual animals used in experiments will be kept in 150mL beakers, and culture water will be changed every 2 days. All cultures will be fed a mixture of commercially available *Nannochloropsis* and *Selenastrum* in excess of what they can filter daily.

Life Table Design

For life table experiments, 10-15 neonates will be isolated from acclimated laboratory clones and reared individually in 150mL beakers to produce broods of parthenogenetic offspring. The first brood will be counted and preserved for morphological measurements. Second brood offspring will be measured for core body length (CB), head spine length (HS) and tail spine length (TS), and then transferred to individual 150mL beakers. A subset of these will be harvested for morphological measurement and DNA damage analysis at intervals that correspond to major life cycle events: Neonates (age 0-3 days, immature), Juveniles (age 4-6 days, pre-reproductive with no brood chamber development), and Adults (age 10-40 days, post-reproductive). For the Adult group, a subset of animals will be harvested every 7-10 days until all animals have died. Water in the 150mL beakers will be changed every 2 days for the duration of the study. During water changes, the number of molts, live offspring released, (dormant) sexual egg production and male production will be recorded. Life table data will be used to calculate age of first reproduction (AFR), lifetime reproductive output (total live offspring and dormant sexual eggs), mean clutch size, molting rate, and longevity in order to document life history trade-offs at the two temperatures.

EXPERIMENTS

Question 1: Determine whether DNA damage increases with age in somatic and germ tissues in female *Daphnia lumholtzi*

HW1: DNA damage will be absent or minimal in germ tissues (eggs cells) but will increase significantly in somatic tissues during over the lifespan

These experiments will use a life table design to identify age classes and major life cycle events, and to monitor life history trade-offs. For life table experiments, 10-15 neonates will be isolated from acclimated laboratory clones and reared individually in 150mL beakers to produce broods of parthenogenetic offspring. Second-brood offspring will be used as experimental animals. When eggs from the second brood appear in the brood chamber, a subset of pregnant females will be removed to collect newly deposited eggs (4 eggs from each clone), which will be used to estimate percentage DNA breakage in germ tissue. The remaining females will be maintained until brood 2 neonates are released from the brood chamber. Release from the brood chamber will be regarded as "age zero" in order to determine average longevity and age class for DNA damage analysis (comet assay). Newly released offspring (<24 hours) will be measured for CB, HS and TS lengths, and then transferred to individual 150mL beakers. A subset of these will be harvested for morphological measurement and DNA damage analysis at intervals

that correspond to major life cycle events: Neonates (age 0-3 days, immature), Juveniles (age 4-6 days, pre-reproductive with no brood chamber development), and Adults (age 10-40 days, post-reproductive). For the adult group, a subset of animals will be harvested every 10 days until all animals have died. Water in the 150mL beakers will be changed every 2 days for the duration of the study. During water changes, the number of molts, live offspring released, dormant (ephippial) egg production and male production will be recorded. The last four adult females will be maintained until death to estimate longevity. Two replicates of this experiment will be conducted, each using a distinct clone generated from separate wild-caught females. According to the disposable soma theory we expect to see an increase in DNA damage in somatic tissues with age. DNA damage in germ tissue should either occur at lower levels or increase at a much slower rate than in somatic tissue. Evolutionary theories of senescence also predict that deterioration of the body begins at some point after an individual reaches sexual maturity. Therefore, DNA damage should be minimal prior to sexual maturity but show increases afterward.

Question 2: Determine the cost of increased somatic maintenance requirements in terms of DNA maintenance

HW2: DNA damage will increase faster or occur to a greater extent under environmental conditions that require increased somatic maintenance.

Clones for these experiments will be maintained with a 16-hour photoperiod at either 22±1 ^{o}C (normal temperature) or 30±1 ^{o}C (stress temperature; to alter life history responses). The two thermal regimes will allow us to test differences in life history traits between treatments with differing somatic maintenance costs. The long head and tail spines of *Daphnia lumholtzi* can reach up to three times its core body length at temperatures greater than 29^{o}C (Sorensen & Sterner 1992, Yurista 2000). These spines are effective anti-predator defenses (Kolar & Wahl 1998, Dzialowski et al. 2003, Engel & Tollrian 2009), but they require specific environmental cues for induction, including elevated water temperature (Kolar & Wahl 1998, Dzialowski et al. 2003, Engel & Tollrian 2009, Sorensen & Sterner 1992, Yurista 2000, Work & Gophen 1999a, 1999b), and they are also accompanied by increased molting rates (Work & Gophen 1999b, Lennon Smith & Williams 2001). Therefore, the group maintained at 30°C should have greater somatic maintenance costs compared to *D. lumholtzi* kept 22°C. According to the disposable soma theory, these increases in somatic maintenance costs should cause DNA damage in this group to occur at higher levels and accumulate at a faster rate than in the low temperature group since resources for repair and maintenance will be preferentially allocated to protecting the germ line.

A subset of whole *Daphnia* from each thermal treatment will be used to determine percent DNA breakage in somatic tissues. Four neonates, four juveniles and four adult females will be collected from each clone in the 22°C treatment and the 30°C treatment,

measured for body size and spine length, and used to assay DNA breakage in somatic tissues. Additional subsamples will be collected from the adult group in each thermal treatment every 7-10 days until the end of the experiment. Each pool of four individuals will be placed in separate individually labeled microcentrifuge tubes containing high salt (100mM) STE buffer and stored at -70°C for subsequent DNA breakage analysis.

The percentage of DNA breakage in each tissue will be determined by the comet assay (Collins 2004), which is a standardized protocol for performing single-cell gel electrophoresis. One significant advantage of this technique over other protocols for assessment of DNA breakage (alkaline unwinding) is that it requires only a small amount of tissue. Other studies have demonstrated the usefulness of this technique for biomonitoring and toxicology studies in a wide variety of organisms, including yeast, plants, annelids, bivalves, insects, crustaceans, amphibians, fish, dolphins, and humans (reviewed in Mitchelmore 1998, Lee & Steinert 2003, Collins 2004, Lee, Kim & Choi 2009, Azqueta & Collins 2013, Pellegri et al. 2014).

In the comet assay, a single-cell suspension is prepared from homogenized tissues and embedded in low melting point agarose (LMA) on either a microscope slide or a commercial cassette developed specifically for processing small samples (Trevigen, Inc.). We will be using *Daphnia* hemolymph prepared using the protocol of Pellegri et al. (2014), with slight modifications. Briefly, four animals are pooled in a microcentrifuge tube and rinsed with a non-cytotoxic buffer (Buffer P, Pellegri at al., 2014). The buffer is

removed and replaced with 200µl of fresh buffer containing glass tissue-disrupter beads (50 micron). The tube is pulse- vortexed for 1 second at 4200 rpm to extract the hemolymph. After centrifuging briefly to sediment tissues, the extract is filtered over 40-micron mesh to prepare a single-cell suspension of hemocytes. This suspension is centrifuged to pellet the cells, and the supernatant is removed. The pellet is then mixed gently with 25µl of 1% low-melting point agarose, and this mixture is transferred to the comet assay cassette. The cells are lysed in place on the cassette overnight, using a high salt buffer (2.5M NaCl) to disrupt the plasma membrane and remove cellular components and histones from the DNA that is coiled around the nucleoid core (Collins 2004, Collins & Azqueta 2012, Azqueta & Collins, 2013). The cassette is incubated in an alkaline buffer for 5 minutes to induce relaxation of any alkali-labile strand breaks (such as apurinic/apyrimidinic sites) in the supercoiled DNA (Collins 2004, Collins & Azqueta 2012, Azqueta & Collins, 2013). Electrophoresis of the gels on the cassettes is performed in 0.3M NaOH buffer (pH 8>13) at low voltage (0.78 V/cm, 300mA) for 10 minutes. Intact DNA coiled around the nucleoid will have low mobility and will remain in the gel as a round “comet” of undamaged DNA. Coils that contain breaks will migrate toward the anode, forming a characteristic “tail” of damaged DNA becoming stretched away from the intact head of the comet (Collins 2004, Collins & Azqueta 2012, Azqueta & Collins, 2013). The gels are stained with a fluorescent DNA dye (SYBR Gold, Thermo Fisher Scientific), and the intensity of staining in the tail relative to the comet head is used as an index of DNA damage (Collins & Azqueta 2012). Staining intensity will be

evaluated using OpenComet, an automated, open-source software program that is freely available on the web (http://www.cometbio.org).

Status and Future Work

The *Daphnia* hemolymph cell extraction method has been tested and hemolymph cells successfully collected from male and female *D. lumholtzi* (Figure 5.1).

The comet assay has been tested comet cells from female *D. lumholtzi* have been successfully stained and imaged (Figure 5.2). This needs to be validated with controls and then a pilot study can be undertaken.

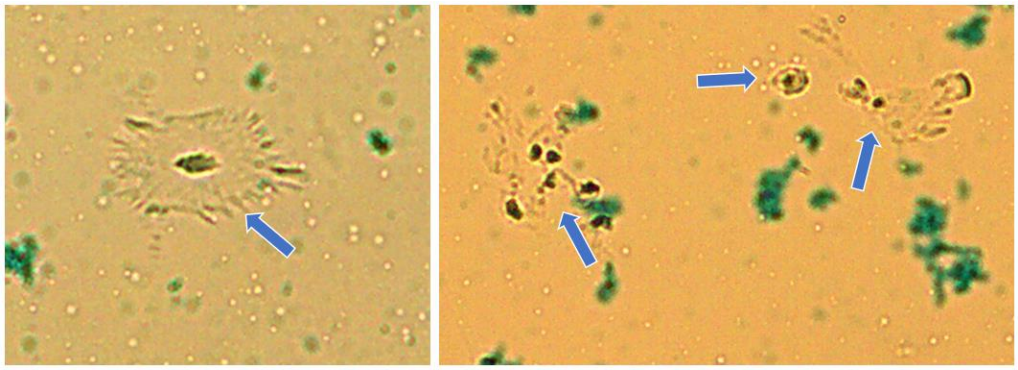

Figure 5.1. Cells extracted from the hemolymph of *D. lumholtzi* indicated by blue arrows.

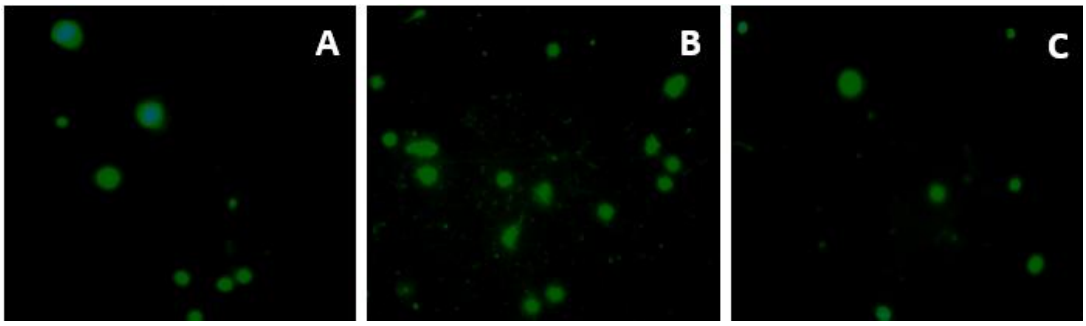

Figure 5.2. Stained cells from the Comet assay procedure using hemolymph cells extracted from early-stage embryos (A), late-stage embryos (B) and an adult female (C).

References for Chapters One, Four, and Five

Austad, S. N. (2001). An experimental paradigm for the study of slowly aging organisms. *Experimental gerontology*, *36*(4-6), 599-605.

Austad, S. N., & Fischer, K. E. (1991). Mammalian aging, metabolism, and ecology: evidence from the bats and marsupials. *Journal of gerontology*, *46*(2), B47-B53.

Avise, J. C. (1993). Perspective: The evolutionary biology of aging, sexual reproduction, and DNA repair. *Evolution*, *47*(5), 1293-1301.

Azqueta, A., & Collins, A. R. (2013). The essential comet assay: a comprehensive guide to measuring DNA damage and repair. *Archives of toxicology*, *87*, 949-968.

Beaver, J. R., Renicker, T. R., Tausz, C. E., Young, J. L., Thomason, J. C., Wolf, Z. L., ... & Koenig, *D.* T. (2018). Winter swarming behavior by the exotic cladoceran *Daphnia lumholtzi* Sars, 1885 in a Kentucky (USA) reservoir. BioInvasions Rec, 7, 43-50.

Bell, G. (1984). Measuring the cost of reproduction. II. The correlation structure of the life tables of five freshwater invertebrates. *Evolution*, 314-326.

Benson, A., E. Maynard, D. Raikow, J. Larson, T.H. Makled, and A. Fusaro, 2023, *Daphnia lumholtzi* G.O. Sars, 1885: U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, and NOAA Great Lakes Aquatic Nonindigenous Species Information System, Ann Arbor, MI, https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?Species_ID=164, Revision Date: 9/12/2019, Access date: 02/12/2023.

Bernstein, Carol, and Harris Bernstein. Aging and Sex, DNA Repair in. Wiley-VCH Verlag GmbH & Co. KGaA, 1991.

Boersma, M., Spaak, P., & De Meester, L. (1998). Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. *The American Naturalist*, *152*(2), 237-248.

Busuttil, R. A., Rubio, M., Dollé, M. E., Campisi, J., & Vijg, J. (2003). Oxygen accelerates the accumulation of mutations during the senescence and immortalization of murine cells in culture. *Aging cell*, *2*(6), 287-294.

Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Cochran, P. A., Elser, J. J., Elser, M. M., ... & von Ende, C. (1987). Regulation of lake primary productivity by food web structure. *Ecology*, *68*(6), 1863-1876.

Charmantier, A., Perrins, C., McCleery, R. H., & Sheldon, B. C. (2006). Quantitative genetics of age at reproduction in wild swans: support for antagonistic pleiotropy models of senescence. *Proceedings of the National Academy of Sciences*, *103*(17), 6587-6592.

Clarke, J. M., Smith, J. M., & Sondhi, K. C. (1961). Asymmetrical response to selection for rate of development in *Drosophila subobscura*. *Genetics Research*, *2*(1), 70-81.

Cleaver, J. E. (1970). DNA repair and radiation sensitivity in human (xeroderma pigmentosum) cells. *International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine*, *18*(6), 557-565.

Clutton-Brock, T. H. (Ed.). (1988). *Reproductive success: studies of individual variation in contrasting breeding systems*. University of Chicago Press.

Creighton, J. C., Heflin, N. D., & Belk, M. C. (2009). Cost of reproduction, resource quality, and terminal investment in a burying beetle. *The American Naturalist*, *174*(5), 673-684.

Collins, A. R. (2004). The comet assay for DNA damage and repair: principles, applications, and limitations. *Molecular biotechnology*, *26*(3), 249-261.

Crowl, T. A., & Covich, A. P. (1990). Predator-induced life-history shifts in a freshwater snail. *Science*, *247*(4945), 949-951.

DeVries, *D.* R., Wright, R. A., & DeVries, T. S. (2006). *Daphnia lumholtzi* in the Mobile River drainage, USA: Invasion of a habitat that experiences salinity.

Doggett, D. L., Chang, M. P., Makinodan, T., & Strehler, B. L. (1981). Cellular and molecular aspects of immune system aging. *Molecular and Cellular Biochemistry*, *37*, 137-156.

Dumont, H. J., & Van de Velde, I. (1977). Report on a collection of Cladocera and Copepoda from Nepal. *Hydrobiologia*, *53*(1), 55-65.

Dumont, H. J., & Verheye, H. M. (1984). The nature and origin of the crustacean zooplankton of Sahelian Africa, with a note on the *Limnomedusa*. In *Tropical Zooplankton* (pp. 313-325). Springer, Dordrecht.

Dwyer, B. E., Fando, J. L., & Wasterlain, C. G. (1980). Rat brain protein synthesis declines during postdevelopmental aging. *Journal of neurochemistry*, *35*(3), 746-749.

Dzialowski, A. R., Lennon, J. T., O'Brien, W. J., & Smith, V. H. (2003). Predator-induced phenotypic plasticity in the exotic cladoceran *Daphnia lumholtzi*. *Freshwater Biology*, *48*(9), 1593-1602.

Eads, B. D., Andrews, J., & Colbourne, J. K. (2008). Ecological genomics in *Daphnia*: stress responses and environmental sex determination. *Heredity*, *100*(2), 184-190.

East, T. L., Havens, K. E., Rodusky, A. J., & Brady, M. A. (1999). *Daphnia lumholtzi* and *Daphnia ambigua*: population comparisons of an exotica and a native cladoceran in Lake Okeechobee, Florida. *Journal of Plankton Research*, *21*(8), 1537-1551.

Ebert, D. (2005). Introduction to *Daphnia* Biology. In Ecology, Epidemiology, and Evolution of Parasitism in *Daphnia* [Internet]. National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK2042/

Elias-Gutierrez, M., Jeronimo, F. M., Ivanova, N. V., Valdez-Moreno, M., & Hebert, P. *D.* (2008). DNA barcodes for Cladocera and Copepoda from Mexico and Guatemala, highlights and new discoveries. *Zootaxa*, *1839*(1), 1-42.

Elton, C. S. (2020). The ecology of invasions by animals and plants. Springer Nature.

Engel, K., & Tollrian, R. (2009). Inducible defences as key adaptations for the successful invasion of *Daphnia lumholtzi* in North America?. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1663), 1865-1873.

Fernando, C. H. (1980). The species and size composition of tropical freshwater zooplankton with special reference to the Oriental Region (South East Asia). *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, *65*(3), 411-426.

Fowler, K., & Partridge, L. (1989). A cost of mating in female fruitflies. Nature, 338(6218), 760-761.

Frisch, D., & Weider, L. J. (2010). Seasonal shifts in genotype frequencies in the invasive cladoceran *Daphnia lumholtzi* in Lake Texoma, USA. *Freshwater Biology*, *55*(6), 1327-1336.

Gadgil, M., & Bossert, W. H. (1970). Life historical consequences of natural selection. *The American Naturalist*, *104*(935), 1-24.

Garcia, A. M., Busuttil, R. A., Calder, R. B., Dollé, M. E., Diaz, V., McMahan, C. A., ... & Vijg, J. (2008). Effect of Ames dwarfism and caloric restriction on spontaneous DNA mutation frequency in different mouse tissues. *Mechanisms of ageing and development*, *129*(9), 528-533.

Gerritsen, J. (1980). Sex and parthenogenesis in sparse populations. *The American Naturalist*, *115*(5), 718-742.

Gilg, M. R., & Kruse, K. C. (2003). Reproduction decreases life span in the giant waterbug (*Belostoma flumineum*). *The American Midland Naturalist*, *149*(2), 306-319.

Gillooly, J. F., & Dodson, S. I. (2000). Latitudinal patterns in the size distribution and seasonal dynamics of new world, freshwater cladocerans. Limnology and Oceanography, 45(1), 22-30.

Goldspink, G., & Alnaqeeb, M. A. (1985). Aging of skeletal muscle. *CRC Handbook of cell biology of aging*, 179-194.

Green, J. (1971). Associations of Cladocera in the zooplankton of the lake sources of the White Nile. *Journal of Zoology*, *165*(3), 373-414.

Green, J. (1967). The distribution and variation of *Daphnia lumholtzi* (Crustacea: Cladocera) in relation to fish predation in Lake Albert, East Africa. Journal of Zoology, 151(1), 181-197.

Haney, J. F., & Lampert, W. (2013). Spatial avoidance of *Microcystis aeruginosa* by *Daphnia*: fitness consequences and evolutionary implications. *Limnology and oceanography*, *58*(6), 2122-2132.

Hansen, G. J., Ahrenstorff, T. D., Bethke, B. J., Dumke, J. D., Hirsch, J., Kovalenko, K. E., ... & Wagner, T. (2020). Walleye growth declines following zebra mussel and *Bythotrephes* invasion. *Biological Invasions*, *22*(4), 1481-1495.

Havel, J. E. (1985). Cyclomorphosis of *Daphnia pulex* spined morphs 1. *Limnology and Oceanography*, *30*(4), 853-861.

Havel, J. E., Colbourne, J. K., & Hebert, P. *D.* (2000). Reconstructing the history of intercontinental dispersal in *Daphnia lumholtzi* by use of genetic markers. Limnology and Oceanography, 45(6), 1414-1419.

Havel, JE., & Graham, JL. (2006). Complementary population dynamics of exotic and native *Daphnia* in North American reservoir communities. Archiv Fur Hydrobiologie, 167(1–4), 245–264.

Havel, J. E., & Hebert, P. *D.* (1993). *Daphnia lumholtzi* in North America: another exotic zooplankter. *Limnology and Oceanography*, *38*(8), 1823-1827.

Havel, JE, Kovalenko, KE, Thomaz, SM, Amalfitano, S, & Kats, LB (2015). Aquatic invasive species: Challenges for the future. Hydrobiologia, 750(1), 147–170. https://doi.org/10.1007/s10750-014-2166-0

Havel, J. E., & Shurin, J. B. (2004). Mechanisms, effects, and scales of dispersal in freshwater zooplankton. Limnology and Oceanography, 49(4part2), 1229-1238.

Havens, K. E., Beaver, J. R., East, T. L., Work, K., Phlips, E. J., Cichra, M. F., ... & Rosati, T. C. (2012). The outcome of the invasion of Florida lakes by *Daphnia lumholtzi*. *Freshwater Biology*, *57*(3), 552-562.

Havens, K. E., East, T. L., Marcus, J., Essex, P., Bolan, B., Raymond, S., & Beaver, J. R. (2000). Dynamics of the exotic *Daphnia lumholtzi*i and native macro-zooplankton in a subtropical chain-of-lakes in Florida, USA. *Freshwater Biology*, *45*(1), 21-32.

Hebert, P. *D.* (1977). A revision of the taxonomy of the gennus *Daphnia* (Crustacea: Daphnidae) in south-eastern Australia. *Australian Journal of Zoology*, *25*(2), 371-398**.**

Hebert, P., & Finston, T. (1996). Genetic differentiation in *Daphnia obtusa*: a continental perspective. Freshwater Biology, 35(2), 311-321.

Hebert, P. *D.*, Schwartz, S. S., & Weider, L. J. (1989). Geographical patterns in genetic diversity and parthenogenesis within the *Daphnia pulex* group from the southern United States. American Midland Naturalist, 59-65.

Hebert, P. D., Witt, J. D., & Adamowicz, S. J. (2003). Phylogeographical patterning in *Daphnia ambigua*: Regional divergence and intercontinental cohesion. Limnology and Oceanography, 48(1), 261-268.

Holmes, G. E., Bernstein, C., & Bernstein, H. (1992). Oxidative and other DNA damages as the basis of aging: a review. *Mutation Research/DNAging*, *275*(3-6), 305-315.

Holmes, D. J., Flückiger, R., & Austad, S. N. (2001). Comparative biology of aging in birds: an update. *Experimental gerontology*, *36*(4-6), 869-883.

Hrbáček, J. (1959). Circulation of water as a main factor influencing the development of helmets in *Daphnia cucullata* Sars. *Hydrobiologia*, *13*, 170-185.

Jobling, M. (1981). Temperature tolerance and the final preferendum—rapid methods for the assessment of optimum growth temperatures. Journal of fish biology, 19(4), 439-455.

Johnson, J. L., & Havel, J. E. (2001). Competition between native and exotic *Daphnia*: in situ experiments. *Journal of plankton research*, *23*(4), 373-387.

King, C. R., & Greenwood, J. G. (1992). The productivity and carbon budget of a natural population of *Daphnia lumholtzi* Sars. Hydrobiologia, 231(3), 197-207.

Kirkwood, T. B. (1977). Evolution of ageing. *Nature*, *270*(5635), 301-304.

Kirkwood, T. B. (2005). Understanding the odd science of aging. *Cell*, *120*(4), 437-447.

Kirkwood, T. B., & Holliday, R. (1979). The evolution of ageing and longevity. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *205*(1161), 531-546.

Kirkwood, T. B., & Holliday, R. (1979). The evolution of ageing and longevity. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *205*(1161), 531-546.

Kivivuori, L. A., & Lahdes, E. O. (1996). How to measure the thermal death of *Daphnia*? A comparison of different heat tests and effects of heat injury. Journal of thermal Biology, 21(5-6), 305-311

Kolar, C. S., Boase, J. C., Clapp, *D.* F., & Wahl, *D.* H. (1997). Potential Effect of Invasion by an Exotic Zooplankter, *Daphnia Iumholtzi*. *Journal of Freshwater Ecology*, *12*(4), 521-530.

Kolar, C. S., & Wahl, D. H. (1998). Daphnid morphology deters fish predators. *Oecologia*, *116*, 556-564.

Kotov, A. A., & Taylor, *D.* J. (2014). *Daphnia lumholtzi* Sars, 1885 (Cladocera: Daphniidae) invades Argentina. *Journal of Limnology*, *73*(2).

L'Amour, L. (1983). *Ride the River: The Sacketts: A Novel* (Vol. 5). Bantam.

Lampert, W., Fleckner, W., Rai, H., & Taylor, B. E. (1986). Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase 1. Limnology and oceanography, 31(3), 478-490.

Lee, R. F., & Steinert, S. (2003). Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutation Research/Reviews in Mutation Research*, *544*(1), 43-64.

Lee, S. W., Kim, S. M., & Choi, J. (2009). Genotoxicity and ecotoxicity assays using the freshwater crustacean *Daphnia magna* and the larva of the aquatic midge *Chironomus*

riparius to screen the ecological risks of nanoparticle exposure. *Environmental toxicology and pharmacology*, *28*(1), 86-91.

Lennon, J. T., Smith, V. H., & Williams, K. (2001). Influence of temperature on exotic *Daphnia lumholtzi* and implications for invasion success. *Journal of Plankton Research*, *23*(4), 425-433.

Leroi, A. M., Bartke, A., De Benedictis, G., Franceschi, C., Gartner, A., Gonos, E., ... & Zwaan, B. (2005). What evidence is there for the existence of individual genes with antagonistic pleiotropic effects?. *Mechanisms of ageing and development*, *126*(3), 421-429.

Luckinbill, L. S., Arking, R., Clare, M. J., Cirocco, W. C., & Buck, S. A. (1984). Selection for delayed senescence in *Drosophila melanogaster*. *Evolution*, 996-1003.

MacIsaac, H. J., Hebert, P. D., & Schwartz, S. S. (1985). Inter-and intraspecific variation in acute thermal tolerance of *Daphnia*. *Physiological zoology*, *58*(3), 350-355.

Mantovano, T., Bailly, D., Ferreira, J. H. D., de Oliveira da Conceicao, E., Cassemiro, F. A., de Campos, R., ... & Lansac-Tôha, F. A. (2021). A global analysis of the susceptibility of river basins to invasion of a freshwater zooplankton (*Daphnia lumholtzi*). *Freshwater Biology*, *66*(4), 683-698.

Medawar, P. B. (1955, January). The definition and measurement of senescence. In *Ciba Foundation Symposium-General Aspects (Colloquia on Ageing)* (Vol. 1, pp. 4-15). Chichester, UK: John Wiley & Sons, Ltd..

Mergeay, J., Verschuren, D., & Meester, L. D. (2005). *Daphnia* species diversity in Kenya, and a key to the identification of their ephippia. *Hydrobiologia*, *542*, 261-274.

Mergeay, J., Verschuren, *D.*, Kerckhoven, L. V., & Meester, L. *D.* (2004). Two hundred years of a diverse *Daphnia* community in Lake Naivasha (Kenya): effects of natural and human-induced environmental changes. Freshwater biology, 49(8), 998-1013.

Mertz, D. B. (1975). Senescent decline in flour beetle strains selected for early adult fitness. *Physiological Zoology*, *48*(1), 1-23.

Mitchelmore, C. L., & Chipman, J. K. (1998). DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *399*(2), 135-147.

Montero-Pau, J., Gómez, A., & Muñoz, J. (2008). Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography: Methods*, *6*(6), 218-222.

Murdoch, W. W. (1966). Population stability and life history phenomena. *The American Naturalist*, *100*(910), 5-11.

Nur, N. (1984). The consequences of brood size for breeding blue tits I. Adult survival, weight change and the cost of reproduction. *The Journal of Animal Ecology*, 479-496.

Obreshkove, V., & Fraser, A. W. (1940). Growth and differentiation of *Daphnia magna* eggs in vitro. The biological bulletin, 78(3), 428-436.

Orcutt Jr, J. D., & Porter, K. G. (1984). The synergistic effects of temperature and food concentration of life history parameters of *Daphnia*. *Oecologia*, *63*(3), 300-306.

Park, G. H., & Buetow, D. E. (1990). RNA synthesis by hepatocytes isolated from adult and senescent Wistar rat liver. *Gerontology*, *36*(2), 76-83.

Partridge, L., & Barton, N. H. (1993). Evolution of aging: testing the theory using *Drosophila*. *Genetica*, *91*, 89-98.

Partridge, L., & Farquhar, M. (1981). Sexual activity reduces lifespan of male fruitflies. *Nature*, *294*(5841), 580-582.

Pellegri, V., Gorbi, G., & Buschini, A. (2014). Comet assay on *Daphnia magna* in eco-genotoxicity testing. *Aquatic Toxicology*, *155*, 261-268.

Pluskal, M. G., Moreyra, M., Burini, R. C., & Young, V. R. (1984). Protein synthesis studies in skeletal muscle of aging rats. I. Alterations in nitrogen composition and protein synthesis using a crude polyribosome and pH 5 enzyme system. *Journal of Gerontology*, *39*(4), 385-391.

Prosser, S., Martínez-Arce, A., & Elías-Gutiérrez, M. (2013). A new set of primers for COI amplification from freshwater microcrustaceans. *Molecular ecology resources*, *13*(6), 1151-1155.

Prowse, N., & Partridge, L. (1997). The effects of reproduction on longevity and fertility in male *Drosophila melanogaster*. *Journal of Insect Physiology*, *43*(6), 501-512.

Ricciardi, A. (2015). Ecology of invasive alien invertebrates. In *Thorp and Covich's freshwater invertebrates* (pp. 83-91). Academic Press.

Rose, M. R., & Charlesworth, B. (1981). Genetics of life history in *Drosophila melanogaster*. II. Exploratory selection experiments. *Genetics*, *97*(1), 187-196.

Rose, MR, Dorey, ML, Coyle, AM, & Service, PM (1984). The morphology of postponed senescence in *Drosophila melanogaster*. *Canadian journal of zoology* , *62* (8), 1576-1580.

Rosenheim, J. A., Alon, U., & Shinar, G. (2010). Evolutionary balancing of fitness-limiting factors. *The American Naturalist*, *175*(6), 662-674.

Rupp, W. D., & Howard-Flanders, P. (1968). Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. *Journal of molecular biology*, *31*(2), 291-304.

Rutledge, W. R., & Lyons, B. W. (1976). Texas peacock bass and nile perch: status report. In *Proceedings of the annual conference Southeastern Association of Fish and Wildlife Agencies* (Vol. 39, pp. 18-23).

Schwartz, S. S. (1984). Life history strategies in *Daphnia*: a review and predictions. Oikos, 114-122.

Sgro, C. M., & Partridge, L. (1999). A delayed wave of death from reproduction in *Drosophila*. *Science*, *286*(5449), 2521-2524.

Simões, N. R., Robertson, B. A., Lansac-Tôha, F. A., Takahashi, E. M., Bonecker, C. C., Velho, L. F. M., & Joko, C. Y. (2009). Exotic species of zooplankton in the upper Paraná river floodplain, *Daphnia lumholtzi* Sars, 1885 (Crustacea: Branchiopoda). *Brazilian Journal of Biology*, *69*, 551-558.

Slagboom, PE, & Vijg, J. (1989). Genetic instability and aging: theories, facts, and future perspectives. *Genome* , *31* (1), 373-385.

Snell, T. W., & King, C. E. (1977). Lifespan and fecundity patterns in rotifers: the cost of reproduction. *Evolution*, 882-890.

Sokal, R. R. (1970). Senescence and genetic load: evidence from *Tribolium*. *Science*, *167*(3926), 1733-1734.

Sorensen, K. H., & Sterner, R. W. (1992). Extreme cyclomorphosis in *Daphnia lumholtzi. Freshwater Biology*, *28*(2), 257-262.

Stearns, S. C. (1992). *The evolution of life histories* (Vol. 249, p. xii). Oxford: Oxford university press.

Stibor, H., & Lüning, J. (1994). Predator-induced phenotypic variation in the pattern of growth and reproduction in *Daphnia hyalina* (Crustacea: Cladocera). *Functional Ecology*, 97-101.

Stoeckel, J. A., Camlin, L., Blodgett, K. *D.*, & Sparks, R. E. (1996). Establishment of *Daphnia lumholtzi* (an exotic zooplankter) in the Illinois River. *Journal of Freshwater Ecology*, *11*(3), 377-379.

Swaffar, SM., & O'Brien, WJ. (1996). Spines of *Daphnia lumholtzi* create feeding difficulties for juvenile bluegill sunfish (Lepomis macrochirus). Journal of Plankton Research, 18(6), 1055–1061. https://doi.org/10.1093/plankt/18.6.1055

Tatar, M., & Yin, C. M. (2001). Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. *Experimental gerontology*, *36*(4-6), 723-738.

Taylor, *D.* J., Finston, T. L., & Hebert, P. *D.* (1998). Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia* laevis complex. Evolution, 52(6), 1648-1670.

Tollrian, R. (1994). Fish-kairomone induced morphological changes in *Daphnia lumholtzi* (Sars). *Archiv für Hydrobiologie*, 69-75.

Tollrian, R. (1995). Predator-induced morphological defenses: costs, life history shifts, and maternal effects in Daphnia pulex. *Ecology*, *76*(6), 1691-1705.

Villani, G., Boiteux, S., & Radman, M. (1978). Mechanism of ultraviolet-induced mutagenesis: extent and fidelity of in vitro DNA synthesis on irradiated templates. *Proceedings of the National Academy of Sciences*, *75*(7), 3037-3041.

Wattiaux, J. M. (1968). Cumulative parental age effects in *Drosophila subobscura*. *Evolution*, 406-421.

Weetman, D., & Atkinson, D. (2004). Evaluation of alternative hypotheses to explain temperature-induced life history shifts in *Daphnia*. *Journal of Plankton Research*, *26*(2), 107-116.

Weider, L. J., & Pijanowska, J. (1993). Plasticity of *Daphnia* life histories in response to chemical cues from predators. *Oikos*, 385-392.

Williams, G. C. (2001). Pleiotropy, Natural Selection, and the Evolution of Senescence: Evolution 11, 398-411 (1957). *Science of Aging Knowledge Environment*, *2001*(1), cp13-cp13.

Work, K. A., & Gophen, M. (1999). Factors which affect the abundance of an invasive cladoceran, *Daphnia lumholtzi*, in US reservoirs. *Freshwater Biology*, *42*(1), 1-10.

Young, J. (1978). Sexual swarms in *Daphnia* magna, a cyclic parthenogen. Freshwater Biology, 8(3), 279–281. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2427.1978.tb01448.x

Yurista, P. M. (2000). Cyclomorphosis in *Daphnia lumholtzi* induced by temperature. Freshwater Biology, 43(2), 207-213.

Yurista, P. M. (2004). Bioenergetics of a Semi-Tropical Cladoceran, *Daphnia lumholtzi*. Journal of Freshwater Ecology, 19(4), 681-694.

Zwaan, B., Bijlsma, R., & Hoekstra, R. F. (1995). Direct selection on life span in *Drosophila melanogaster*. *Evolution*, *49*(4), 649-659.

APPENDIX I. *DAPHNIA* MITOCHONDRIAL COI POLYMERASE CHAIN REACTION MIXTURE AND AMPLIFICATION PROFILE.

Daphnia mitochondrial COI polymerase chain reaction mixture and amplification profile:

Source: Prosser, Sean, Arely Martínez-Arce, and Manuel Elías-Gutiérrez. "A new set of primers for COI amplification from freshwater microcrustaceans." *Molecular ecology resources*13.6 (2013): 1151-1155.

Primers: ZplankF1_t1: tgtaaaacgacggccagtTCTASWAATCATAARGATATTGG
ZplankR1_t1: caggaaacagctatgacTTCAGGRTGRCCRAARAATCA

Ingredient	Stock Concentration	Reaction Concentration
D-(+)-trehalose dihydrate	10%	2.50%
Taq Buffer	10X	1X
$MgCL_2$	50mM	2.5mM
dNTP mixture	10mM	0.2mM
Primer Forward	10µM	0.1µM
Primer Reverse	10µM	0.1µM
Taq	5U/µL	0.35U
PCR-grade water	to 12.5µL final volume (V_f)	

Add 2 µL DNA template to each reaction V_f = 12.5µL

PCR reaction profile

2min @ 94°C
5 x [94°C x 1min ; 45°C x 40s ; 72°C x 1min]
35 x [94°C x 1min ; 51°C x 40s ; 72°C x 1min]
1 x [72°C x 5min]
Hold at room temperature