CHARACTERIZING ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) CONDITION, POPULATIONS, AND COMMUNITY EFFECTS IN OKLAHOMA HABITATS

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TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

CHAPTER I

PROLOGUE

One of the most widely recognized aquatic invasive species in North America is the zebra mussel (*Dreissena polymorpha*). Native to the Black and Caspian Sea region in Eastern Europe and Western Asia (Karnaukhov and Karnaukhov 1993, Ludyanskiy 1993), this organism invaded the North American Great Lakes region in the mid-1980's probably as a result of at least one trans-Atlantic ship releasing ballast water into Lake St. Clair (Hebert et al. 1989). By 1991 zebra mussels were established in the Lower Mississippi River, and by 1994 they could be found throughout the Mississippi River and most of its major southern tributaries (Allen et al. 1999). Zebra mussels were first discovered in Oklahoma in 1993 in the Arkansas River along the McClellan-Kerr Navigation Channel, their introduction into Oklahoma probably the result of barge traffic along this route (Laney 2005). Since that time, zebra mussels have continued to spread to reservoirs largely in the northeastern part of the state. These infestations have occurred through human-mediated overland transport as well as passive downstream transport through connected reservoirs (Laney 2005).

Effects from zebra mussels occur on species that occupy several trophic levels (Strayer et al. 1999, Beekey et al. 2004, Strayer and Malcom 2006) through direct interactions (Schloesser et al. 1997, Ricciardi et al. 1998, Martel et al. 2001), as well as through habitat alteration such as increased water clarity as a result of their filtering abilities (Ludyanskiy et al. 1993, Ram and McMahon 1996, Raikow 2004). The goal of this study is to understand zebra mussel condition, reproduction, and effects on aquatic communities in Oklahoma. As Oklahoma is positioned on the southern edge of the projected zebra mussel distribution in North America, an understanding of how well this organism is able to live and grow under local conditions can have important management implications. By understanding when zebra mussels are growing, when they are reproducing, and when they may be stressed, stake holders can determine when eradication procedures are most effective or human mediated dispersal events are most probable. In addition, characterizing how well zebra mussels are capable of coping with Oklahoma's climate, and understanding the effects of zebra mussels on other organisms under that climate, may help determine the long term survival of species that compete with zebra mussels for resources. If zebra mussels routinely experience a reduction in densities during the warm summer months, then effects on other organisms may remain fairly minor. On the other hand, if zebra mussels in the state are slightly more tolerant of warmer temperatures than those in the Great Lakes, their densities could remain high during the summer and may lead to chronic effects on other organisms.

LITERATURE REVIEW

Dreissena polymorpha life cycle

Zebra mussels are dioecious, (an individual is either male or female), and utilize external fertilization by releasing gametes into the surrounding water (Ackerman et al. 1994). The releasing of gametes generally occurs, either synchronously or asynchronously, between water temperatures of 12 and 27°C (Ludyanskiy et al. 1993, Neumann et al. 1993, Sprung 1993, Laney 2005). These gametes range in size from as small as 40 μm to 96 μm (Ackerman et al. 1994). After fertilization, the larvae grow and the organism develops a velum, a ciliated organ that allows for feeding and limited locomotion in the pelagic zone of a body of water. Depending on water temperature and available nutrients, the veliger will remain in the water column for a few days to several weeks (Ludyanskiy et al. 1993, Ackerman et al. 1994), until it begins to form an umbone (protuberance near the hinge of the bivalve). After developing an umbone, the veliger will settle out of the water column and find appropriate habitat to attach to, which is generally any firm substrate such as rocks, woody debris, native mussels or other zebra mussels, or macrophytes. Once firm substrate is established, the juvenile zebra mussel attaches with byssal threads that are secreted by a gland at the base of the foot. These structures are probably conserved from the marine ancestors of bivalves and are unique among the freshwater bivalves to *Dreissena* (Ackerman et al. 1994). After settlement, the juvenile mussel continues to metamorphose from the planktonic veliger to the settled adult form by increasing in length, losing the velum and developing the mantle tissue, gills, and mouth (Mackie 1991, Ackerman et al. 1994). Individuals usually become reproductively mature in the second year, however under optimum conditions early season spawned individuals may mature by the end of that season (Mackie and Schloesser 1996, Juhel et al. 2003). Once mature, they usually reach maximum lengths of 25-35mm (Chase and Bailey 1999), with female zebra mussels capable of

releasing up to 1,000,000 eggs per reproductive season (Neumann et al. 1993). They generally live for 3-5 years, with occasional reports of greater longevity (Karatayev et al. 2006).

Given the high reproductive rates of zebra mussels, once established they can reach very high densities. Larval densities have been reported at nearly 500/L in the Great Lakes in just a few years after their discovery (Fraleigh et al. 1993), and adult densities have been estimated as high as 700,000/m^2 (Schloesser et al. 1996). Growth rates can also be quite high approaching 0.07mm/day in both European and North American systems (Dorgelo 1993, Allen et al. 1999).

Zebra mussel ecosystem effects

Nutrients

Karatayev et al. (1997) provides a review of pertinent literature on zebra mussel effects on abiotic components in European systems. Consistent trends were: increase in water clarity, decrease in organic matter, and a decrease in biological oxygen demand**.** Given their filtering efficiencies and high densities, zebra mussels are strong benthic-pelagic couplers, in that they remove nutrients and particulates from the pelagic zone and deposit them in the benthos. As a result, water clarity increases after zebra mussel invasion (Holland 1993, MacIsaac 1996, Idrisi et al. 2001, Boeckman and Bidwell unpublished data). Also pervasive in the literature, chlorophyll levels decline within lake systems after zebra mussel introduction (Leech 1993,

Idrisi et al. 2001, Miller and Watzin 2007, Qualls et al. 2007). Arnott and Vanni (1996) and Bykova et al. (2006) report zebra mussels serve as a significant sink for nitrogen and phosphorus in general and furthermore excrete nutrients at low N:P ratios, which promote cyannobacterial proliferation (further discussed below).

Biotic components

MacIsaac (1996) provides an excellent schematic model to represent some of these relationships (Figure 1.1).

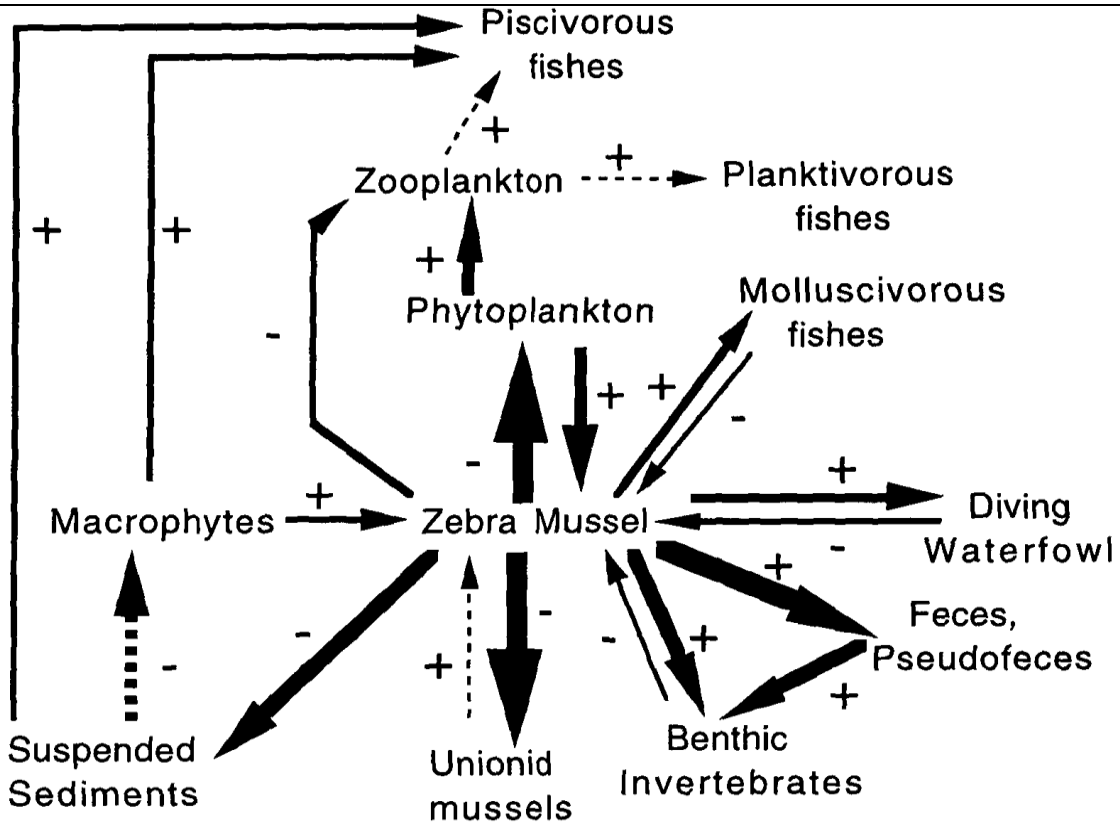

Figure 1.1. Taken from MacIsaac (1996) where solid lines are observed effects with potential effects shown in dashed lines. Thicker arrows indicate more strong associations with taxa benefiting from zebra mussels labeled with (+) and taxa adversely affected by zebra mussels shown as (-).

As previously discussed zebra mussels have strong negative effects on suspended solids by removing them from the pelagic zone via their high filtration rates. This in turn may have positive effects on macrophytes in the system which can proliferate with the increased water clarity. Interestingly, these macrophytes can then benefit zebra mussels by providing new substrate for them to colonize (Burlakova et al. 2006).

Reduction in phytoplankton is another often observed phenomenon in lakes after zebra mussel introduction. Dionisio Pires et al., (2004) found zebra mussels preferentially ingest phytoplankton over detrital material, implicating direct reduction of phytoplankton rather than changes in abiotic components causing the decline. Karatayev et al. (1997) summarizes zebra mussel effects on phytoplankton in European systems with the majority of studies noting decreases between 1 and 4x the pre-invasion levels of phytoplankton. Fahnenstiel et al. (1995) reported a 59% decrease in chlorophyll 3 years after zebra mussel introduction in Saginaw Bay, Lake Huron. Similarly, Idrisi et al. (2001) documented a 46% decline in chlorophyll in Oneida Lake, New York in years after colonization.

Also of interest is the potential change in phytoplankton community structure associated with zebra mussel colonization. Zebra mussels have the ability to selectively ingest food resources (Juhel et al. 2006 a, b, Naddafi et al. 2007). Selective feeding combined with excretion of material with low N:P ratios, suggests zebra mussels may allow for proliferation of cyanobacteria, which can fix nitrogen and out-compete other green algae under low

nitrogen conditions. In fact, this scenario has been documented in Lakes Huron and Erie (Vanderploeg et al. 2001), where *Microcystis* has shifted from low levels pre-invasion, to one of the most common phytoplankton taxa in years following zebra mussel colonization.

Zebra mussel effects on zooplankton have also been documented. MacIsaac et al. (1995) found rotifers (and other larger zooplankton) declined in Lake St. Clair after zebra mussel invasion. They hypothesize rotifers experience direct predation by zebra mussels, with larger zooplankton reduction due to indirect effects of reduced food resources. Similarly, Pace et al. (1998) demonstrated a 70% decline in zooplankton of the Hudson River following zebra mussel invasion. They also suggested direct predation of smaller zooplankton (rotifers) with larger taxa inhibited by low chlorophyll levels.

Not all aquatic organisms are negatively affected by zebra mussels. As Figure 1.1 shows, benthic macroinvertebrates generally benefit from the pelagic derived pseudofeces (rejected food particulates) and feces deposited by zebra mussels. Also important, zebra mussel shells and the matrices they form when aggregated together, also provide habitat for benthic invertebrates (Griffiths 1993, Stewart et al. 1998). The increased complexity of the benthic habitat caused by the aggregation of zebra mussel shells has been shown to reduce fish predatory success (Gonzalez and Downing 1999, Mayer et al. 2001, Beekey et al. 2004). Other benthic macroinvertebrates (native mollusks) however, do not fare so well. Perhaps one of the best known detrimental effects of zebra mussel introductions in some freshwater habitats is the dramatic decrease in native mussels (Burlakova et al. 2000, Martel et al. 2001,

Strayer et al. 2004, Schloesser et al. 2006). Zebra mussels colonize the shell of native mussels and can reach densities high enough to limit movement, burying, and siphoning ability (Ricciardi et al. 1996, 1998, Baker and Hornbach 1997, 2000). Additional effects stem from direct competition for food resources (Baker and Levinton 2003, Strayer and Malcom 2007). While early estimates of near complete unionid extinction were common, more recent studies have shown native mussels to co-exist with zebra mussels but at much reduced densities (Schloesser et al. 1997, Nichols and Amberg 1999, Bowers and De Szalay 2004, Strayer and Malcom 2007). However, associated with reduced densities of unionids are decreased genetic diversity and perhaps less "population level" resistance to additional forms of stress.

Finally, zebra mussels have hypothesized effects on fish. This is shown in Figure 1.1 as the lack of arrows connecting zebra mussels and fish directly, indicating associations are generally through indirect effects. They can benefit molluscivorous fish such as Drum (*Aplodinotus grunniens*), Redear (*Lepomis microlophus*), Blue Catfish (*Ictalurus furcatus*), Pumpkinseed Sunfish (*Lepomis gibbosus*), and Redhorse (*Moxostoma spp*.), by providing an abundant food source (French 1993, Magoulick and Lewis 2002). However, French and Bur (1996) found Drum in Lake Erie to be significantly longer before the zebra mussel invasion, indicating a growth consequence of utilizing zebra mussels as a main component of the diet, perhaps due to zebra mussels being a low energy resource when compared with other forage items (Magoulick and Lewis 2002). Furthermore, Bartsch et al. (2003) and Raikow (2004) have shown reduced growth rates of larval (2-6 week old) planktivorous fish such as fathead

minnows and bluegill when grown in the presence of zebra mussels. They hypothesize that through competition for food, zebra mussels can have negative effects on planktivorous fish.

Feeding and trophic level

The zebra mussel diet has been the subject of a litany of research with different methods applied in almost each study; however, a few basic principles are beginning to emerge. Adult zebra mussels are efficient filter feeders capable of siphoning over 200mL/hr in some laboratory experiments (Diggins 2001). They are able to utilize resources from 1 to 1000µm diameter (Wong and Levinton 2005) as well as capable of discerning between palatable and non-palatable food sources (Dionisio Pires et al. 2004), (although see Nicholls and Hopkins 1993, Horgan and Mills 1997). They have been shown to selectively reject more toxic strains of *Microcystis aeruginosa*, as "pseudodirrhoea" through the pedal gap, rather than through the usual rejection of pseudofeces out of the incurrent siphon (Juhel et al. 2006a, b). Naddafi et al. (2007) suggests zebra mussel diet changes seasonally, to focus on the highest quality phytoplankton at any given time, and Baines et al. (2007) demonstrated their ability, under low food conditions, to utilize dissolved organic matter, (via uptake across the gill surfaces, Baines et al. 2005), and lengthen time-to-death over zebra mussels held without organic matter.

Zebra mussel larvae (veligers) on the other hand represent an understudied life stage of the organism with respect to feeding. Far fewer studies have been conducted on veligers, as they are not as easy to work with and because of the relatively short time spent in that life stage.

However, Barnard et al. (2006) used ^{13}C and ^{15}N and radio-labeled isotopes to determine if veligers in an estuarine transition zone were utilizing bacteria, dissolved organic carbon and small freshwater algae. Dissolved organic carbon, originating from radio-labeled algal lysates, were quickly assimilated into veliger soft tissues, indicating the ability to use dissolved carbon as a resource. MacIsaac et al. (1992) found Lake Erie-derived veligers, capable of ingesting inert beads of 2-3µm in size with clearance rates increasing with length of the veliger, however those rates were orders of magnitude lower than that reported for settled individuals.

Given the wide array of potential food resources that zebra mussels can consume, and the fluctuating nature of the plankton that comprise part of their diet (Hutchinson 1961, Matthews and Mazumder 2005) it should be no surprise that their trophic position is also quite variable. One way to elucidate trophic position is through the use of naturally occurring stable isotopes such as ^{15}N and ^{13}C. As these isotopes are heavier than ^{14}N and ^{12}C, they are not as readily used in metabolic processes within the body and therefore accumulate at known rates (Gannes et al. 1997). Typically, predators have ^{15}N values that are 3.4‰ enriched over their prey, and 1‰ enriched in ^{13}C (Post 2002). Therefore, organisms with similar ^{15}N or ^{13}C tissue concentrations may be utilizing similar food resources (Limen et al. 2005). Garton et al. (2005) demonstrated zebra mussels from Lake Erie and other smaller lakes had similar stable isotopic compositions to those of zooplankton, indicating direct competition for seston. Mitchell et al. (1996) also found zebra mussel ^{15}N signatures to be similar to *Daphnia* spp. in Oneida Lake, NY, however ^{13}C signatures

between the two groups were different, implying *Daphnia* were selectively assimilating carbon whereas zebra mussels were reflecting a more general carbon source.

One potentially confounding principle in stable isotopic studies investigating trophic position is the regional specificity of such studies. For instance, Fry and Allen (2003) demonstrate that zebra mussels, collected from the entire span of the Mississippi River, could be assigned to their respective watershed based upon their stable isotopic composition alone. Zebra mussels collected from the upper Mississippi River had different isotopic compositions than those collected in the lower Mississippi River. In other words, habitat specific inputs of carbon and nitrogen cause variation in stable isotopic composition of the organisms in that system. Given the habitat specificity of stable isotope studies, direct comparisons of organism isotope composition between studies needs to be done with caution therefore site specific studies investigating trophic positioning need to be conducted when possible (Gannes et al. 1997, Vander Zanden and Rasmussen 1999, 2001, Post 2002).

Tolerance/condition of zebra mussels in Oklahoma

After the discovery of zebra mussels in Lake St. Claire in the 1980's, initial projections of zebra mussel spread throughout North America, (based upon European derived thermal tolerance values, and lake size, depth, water hardness, and water transparency) indicated zebra mussels may not be able to tolerate the warmer summertime conditions in the more southern latitudes (Strayer 1991). Since that time, upper thermal tolerance experiments conducted with North American zebra mussels have indicated zebra mussels in the U.S. are

more tolerant of warmer temperatures (Hernandez et al. 1995, McMahon and Ussery 1995, McMahon et al. 1995). These studies have shown zebra mussels in the U.S. have increased tolerance times to water temperatures above 30˚C as compared to European zebra mussels.

While temperature tolerance differences between European and North American zebra mussels have been fairly well characterized, McMahon (1996) reports general agreement in most other physiological aspects of zebra mussel biology between European and North American zebra mussels. Matthews and McMahon (1999) report zebra mussels are able to tolerate low dissolved oxygen (< 3% saturation) for 3-48 days, with the variability due to differences in acclimation temperature. Mussels acclimated to 5˚C were significantly more tolerant of low oxygen than those acclimated to 25˚C, and larger mussels were also more tolerant than smaller mussels. When compared with other freshwater bivalves, zebra mussels are among the most sensitive to low dissolved oxygen levels, with *Corbicula* twice as tolerant and Sphaeriid and Unionid bivalves better adapted to cope with hypoxic conditions (McMahon 1996).

With zebra mussels being fairly sensitive to hypoxic conditions, and upper thermal tolerances near 30˚C, zebra mussels in Oklahoma may experience declines in density during the summer months after spawning due to rising water temperatures. Aldridge et al. (1995) demonstrated zebra mussel metabolic expenditure increased by 265% when temperature was raised from 20 to 32˚C and above 28˚C, energy expenditure exceeded energy gained through food intake. Furthermore Sprung (1991) found post-spawn females lost 30% of their original

body weight. The indications may be that post-reproduction, zebra mussels in Oklahoma may experience environmental conditions such as warm temperatures, low flow, and low dissolved oxygen concentrations which may result in physiological demands that exceed the energy available from food resources. These factors may contribute to population die-offs already observed in Oologah Lake (Boeckman and Bidwell, unpublished data).

These die-off events are not unique to Oklahoma. Early studies conducted in some European lakes also documented declines in zebra mussel densities (Stanczykowska and Lewandowski 1993). Most die-off events occurred in eutrophic lakes with high phosphorus concentrations, in years following extremely high densities, similar to what occurred in Oologah Lake. Some populations re-established and subsequently crashed again, while others disappeared from the lakes all together.

One factor that may help in co-existence of unionids and zebra mussels in Oklahoma, is unionids have been shown to be slightly more tolerant of low dissolved oxygen and high temperatures than zebra mussels (Polhill et al. 1996). McMahon (2002) describes zebra mussels as having an r-selected (density independent) life history strategy that allows for rapid recolonization of areas, in contrast to native unionids being k-selected (density dependent) life histories that have evolved greater tolerance to environmental extremes but requiring relatively longer colonization times. The ability of unionids to tolerate these more extreme environmental conditions, that may occur during Oklahoma's warm summer months, may allow unionids to survive as zebra mussels are eliminated.

Since their introduction into North America, zebra mussels have significantly altered aquatic habitats ecologically and forced industry to spend billions of dollars to deal with their effects on industrial equipment (MacIsaac 1996, Pimental et al. 2000, 2005). Oklahoma has not been immune to these effects with major cities, electricity generating facilities, golf courses, and others, all having to significantly modify operating procedures after zebra mussel introduction into various lakes and rivers in northeastern Oklahoma (Laney 2005, personal observation). Effects on aquatic communities in Oklahoma have also occurred, although the severity of these effects is still largely unexplored. In the 1920's, Isely considered rivers in northeastern Oklahoma to harbor one of the greatest diversity of unionids in the state (Isely 1924). Surveys of these rivers during the 1990's indicated some decline in richness and abundance of unionids in the last 80 years (Vaughn 1998), however there is more recent evidence of increases of unionids in the Kansas portion of the Verdigris River (Miller and Lynott 2006), yet little data is available for Oklahoma.

As discussed above, zebra mussels can have negative effects on aquatic systems and biota inhabiting them, particularly native mussels. Oklahoma represents an area where *D. polymorpha* may experience stress during the summer months due to warm water temperatures. If these periods of stress result in decreased zebra mussel densities, this may mitigate negative effects on other components of aquatic ecosystems, specifically native mussels. This study sought to characterize *D. polymorpha* density and reproduction in several Oklahoma reservoirs, assess their physiological condition on a seasonal scale, and

determine if they have had a negative effect on native mussel communities in the Verdigris River.

The following chapters are organized and formatted for publication in various scientific journals and books. Chapter two characterizes an 8 year study of zebra mussel growth and reproduction in Oologah Lake, OK, and a 4 year study on Sooner Lake, OK. It is currently accepted for publication as a chapter in the book entitled "*Quagga and Zebra Mussels: Biology, Impacts and Control*" with T.F Nalepa and D.W. Schloesser as editors, with an anticipated publication date of late 2011. Chapter three outlines a study assessing the potential effects of *D. polymorpha* on native mussel communities in the Verdigris River, OK. Chapter three was published in American Malacological Bulletin in 2008 (vol. 25: pgs. 1-8). Chapters four and five, discuss various zebra mussel body condition metrics and glycogen concentrations, upper thermal tolerance assays and oxygen consumption/ammonia excretion in several different populations in the region. Chapters four and five will be submitted for publication following defense of this dissertation. Each chapter is meant to "stand alone" and therefore, each is followed by a literature cited and tables and figures sections.

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CHAPTER II

CHARACTERIZING ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) POPULATION DYNAMICS IN TWO OKLAHOMA RESERVOIRS

ABSTRACT

Zebra mussels (*Dreissena polymorpha*) were first introduced into Oklahoma in 1993 and have spread to 10 different reservoirs in the state. This study characterizes zebra mussel population dynamics in two of these reservoirs; one had a 'natural' temperature regime and the other had a warm-water discharge that significantly altered the thermal profile. Zebra mussels were discovered in Oologah Lake in 2003 and monitoring began that year and continued through 2010. In Sooner Lake zebra mussels were discovered in 2006 and monitoring began in 2007 and also continued through 2010. Veliger densities peaked between 500 and 600/L in 2006 in Oologah Lake and 2010 in Sooner Lake, respectively. Peak veliger densities usually occurred in the month of June, with adult zebra mussel densities achieving 150,000/m^2 in the first year of study. Maximum growth ranged between 0.1 and 0.14 mm/day, however in 2009-10 growth rates moderated slightly. In most years, summer dieoffs of older, reproductively mature *D. polymorpha* were observed. It is hypothesized dieoffs resulted from a combination of poor physiological condition after reproduction, and stress induced by water temperatures of 30˚C, usually

achieved in July and August in Oklahoma reservoirs. Substantial floods in 2007, 2008, and 2009, following one such dieoff, also contributed to a prolonged recovery of the zebra mussel population in Oologah Lake. At present, it appears temperature may serve as an important control on *D. polymorpha* in Oklahoma reservoirs and, possibly, throughout southern North America.

INTRODUCTION

Zebra mussels, (*Dreissena polymorpha*), were first reported in the McClelland-Kerr Navigation System, Arkansas River, located in the eastern part of Oklahoma in 1993 (Laney 2010). Mussels were confined to the navigation system for nearly 10 years until 2003 when they were discovered in Oologah Lake (Rogers and Nowata Counties) northeast of Tulsa, Oklahoma (Figure 2.1). *D. polymorpha* currently infests at least 10 different reservoirs in Oklahoma, primarily in northeastern portions of the state. However, one reservoir (Lake Texoma) on the Oklahoma-Texas border has also been colonized. While Oklahoma has a few natural oxbow lakes, most lakes are impounded reservoirs linked a river. These rivers facilitated the spread of mussels in the state (Havel et al. 2005). For example, mussels clearly spread downstream along the Arkansas River from Kansas into Oklahoma (Bidwell 2010).

Initial predictions of the potential range of zebra mussels in the United States delineated a southern limit near the border of Oklahoma and Texas due to summer water temperatures that were thought to exceed the thermal tolerance of dreissenid mussels (e.g. Strayer,

1991). Reservoirs in Oklahoma are warm-monomictic during an average year, with no period of ice cover and stratification only during the warmest months (July – August). Systems in the northern part of the state have a mean annual temperature range of approximately 5 to 25˚C while those in the southern part of the state have a mean range of 7 to 29˚C (OWRB 2007). Reservoirs across the state may have late summertime water temperatures that exceed 30˚C for several weeks in July and August, which may also be coupled with low flow conditions. While thermal tolerance experiments conducted with North American populations indicate zebra mussels are more tolerant of warm temperatures than European populations (e.g. Hernandez et al. 1995, McMahon and Ussery 1995, McMahon et al. 1995), they may still exhibit a negative scope for growth when sustained water temperatures exceed 28˚C (Aldridge et al. 1995). As such, there is significant potential for zebra mussels in Oklahoma reservoirs to experience stressful thermal conditions for part of the year. This would be particularly true for systems in southern Oklahoma and into Texas.

Nichols (1996) highlights the scarcity of data regarding zebra mussel population dynamics in southern North America. Much of what is known about population dynamics in southern latitudes is derived from accounts in the Mississippi River (Allen et al. 1999) where dispersal of zebra mussel veligers and adults from upstream sources is a confounding variable. The present study was initiated as a basic program to monitor the density, reproduction, and growth of zebra mussels in two Oklahoma reservoirs. It represents one of the few long-term studies of zebra mussel population dynamics in southern reservoirs in what has been considered the limit of the southern range for this

organism. Data generated from one of these reservoirs stimulated a retrospective consideration of the role temperature may play in zebra mussel populations in this region.

METHODS

Oologah Lake

Oologah Lake is an impoundment on the Verdigris River, completed in 1974 for the purposes of flood control, water supply, and navigation (OWRB 1990). The lake has a storage capacity of 0.7 km^3 at normal pool and drains 11,000 km^2 in northeastern Oklahoma and southeastern Kansas with a maximum depth of approximately 20m (USACE 2002).

Zebra mussels were first reported in Oologah Lake in 2003. Mussels were most likely brought into the reservoir by recreational boaters or sport fisherman since no source populations upstream of the system were known to occur and because it is a popular site for sport fishing tournaments. Monitoring of Oologah Lake began in June 2003 at 4 sites (Spencer Creek, Blue Creek, Redbud Marina, and Hawthorn Bluff) in the south/southeastern part of the lake (Figure 2.2).

Samples were collected weekly from June 2003 to 2007 and monthly from 2008 to 2010. During each sampling event, temperature (˚C), conductivity (mS/cm), dissolved oxygen (mg/L), and pH (standard units) were collected using a Hydrolab Quanta multi-parameter

probe (Hach Hydromet Corporation, Loveland, CO) at the surface, middle and bottom of the water column. Secchi disk depth was also recorded at each site. In addition, four vertical plankton tows were taken from boat docks at each site with a 64µm mesh Wisconsin style zooplankton net with a 20 cm aperture. Horizontal tows were obtained at Spencer Creek because this location did not have a boat dock, however similar volumes were sampled between all sites. Redbud Marina was the deepest site averaging 4 m depth, followed by Hawthorn Bluff and Blue Creek at approximately 3 m and Spencer Creek with a depth of 1.5 m. Each sample was rinsed into individually labeled 125 mL polyethylene bottles and preserved with 70% ethanol. The volume of water sampled was estimated from the equation of the volume of a cylinder ($V= \pi r^2 h$), where r is equal to the radius of the net aperture (0.2 m), and h is equal to the length of the tow.

At Blue Creek, Redbud Marina, and Hawthorn Bluff, densities of settled veligers were enumerated using modified glass microscope slide boxes suspended approximately 1 m below the surface. The top and bottom of each box were removed to allow water exchange and four glass microscope slides were inserted and held in place using plastic zip ties. During each sampling event, slides were removed, placed in 100 mL glass bottles filled with lake water, and transported back to the laboratory for settled-veliger enumeration. Veligers collected in plankton tows and on glass slides were enumerated under 12.5X magnification with an Olympus SZX-ILLD100 dissecting microscope (Olympus America Inc., Center Valley PA) fitted with cross-polarization filters as described by Johnson (1995).

Densities of adult mussels were estimated on 10 x 20 cm concrete panels suspended at three sampling sites. Five panels were attached to a 1.25 m section of PVC pipe and suspended approximately 1m under boat docks at Hawthorn Bluff, Redbud Marina, and Blue Creek. Each panel was sampled by laying a wood-framed wire grid (50 total- 2 x 2 cm squares) on its surface and counting the number of zebra mussels in 10 randomly selected grids on each panel. Five grids were counted on the front and five on the back of each panel if mussel densities were high enough to warrant sub-sampling.

Growth estimates of mussels were obtained from mussels placed in modified polyethylene tackle boxes (10x20 cm) with 12 chambers per box. The top and bottom of each box was removed and replaced with a rigid plastic mesh (2x2 mm grids) to allow for water exchange. Zebra mussels were collected from boat hulls or the dock itself at Redbud Marina. Individuals of approximately 8-10 mm were isolated by cutting the byssal threads with a scalpel and measured with digital calipers to the nearest 0.01 mm total length. One zebra mussel was placed in each of the 12 chambers per box and boxes were suspended at several sites and depths for 5-9 weeks. After the growth trial was complete, mussels were re-measured and growth rates were expressed as mm/day.

Sooner Lake

Sooner Lake is an impoundment of Greasy Creek, a tributary of the Arkansas River, constructed in 1976 to provide cooling water for a coal-fired electric generating facility (OWRB 1990). The lake has a capacity of 0.2 km^3 at normal pool with an average depth of 8.5 m and a maximum depth of 27 m (Angyal et al. 1987). The power plant on the lake releases heated effluent which is directed from the discharge area to the main body of the lake by a series of dikes that facilitate cooling prior to the water being taken into the plant again via an intake channel. This creates a series of discrete temperature zones in the lake that range from 10-15˚C above ambient at the discharge, to ambient temperature in the main lake.

Zebra mussels were first reported in Sooner Lake in 2006. While introduction due to boating/fishing activities is a possibility, the presence of established mussel populations in the Arkansas River above and below Sooner Lake (Bidwell 2010) suggest make-up water taken from the Arkansas River was the most likely source of mussels in the system. Beginning in January 2007, samples were collected from six sites within Sooner Lake (Figure 2.3). These sites were selected in part to investigate the potential influence of the thermal effluent on zebra mussel reproduction dynamics. Sites at the discharge buoy, end discharge channel, dam, and intake buoy were accessed by boat, while those at the discharge bridge and plant intake were accessed from the shore. Water chemistry, veliger collection and enumeration, and adult densities were assessed as described for Oologah Lake with the exception that at the boat-accessed sites a buoy was deployed with the concrete panel apparatus suspended approximately 1 m below the lake surface, rather than being hung from boat docks. Beginning in July 2008, temperature loggers (HOBO

pro v2, Onset Computer Corporation, Pocasset MA) were suspended below each buoy location (discharge buoy, end discharge, dam, and intake buoy) as well as at the east boat ramp, in association with growth experiments. Loggers were programmed to record temperature every hour.

Growth experiments at Sooner Lake were conducted to investigate the potential effect of the warm water discharge on zebra mussel growth rates. Experiments were conducted in the same polyethylene tackle boxes as described for Oologah Lake. Zebra mussels used to measure growth were collected from a boat dock outside the zone of the warm-water discharge. Each box was then suspended under the buoys supporting the adult settling panels at approximately 1 m below the surface and remained in the lake for 5-9 weeks with longer deployments occurring in winter months. At the conclusion of the growth trial, each box was collected and the total length of individual mussels was again determined using digital calipers. Growth rates were expressed as the increase in shell length mm/day.

Statistical evaluation of growth rates was accomplished with one-way analysis of variance (ANOVA, $\alpha = 0.05$) followed by Holm-Sidak post-hoc tests with appropriate alpha level corrections to compare growth rates between sites. Data that did not meet assumptions of normality or equal variance were natural log-transformed prior to ANOVA. If transformations did not alleviate non-normality or equal variances, an ANOVA on ranks was performed with Dunn's post-hoc procedure (Zar 1999).

RESULTS

Oologah Lake

In Oologah Lake, maximum seasonal temperatures reached just over 30°C in late August of most years, and consistently fell below 5°C in January-February (Figure 2.4). Because of their shallow depths, no depth-related temperature stratification was observed at the monitoring sites. As with temperature, dissolved oxygen exhibited significant seasonal variation, with peaks greater than 10 mg/L observed in late winter/early spring and lows (as low as 2 mg/L) occurring in mid-late summer. As observed for temperature, no significant depth-related oxygen stratification was noted. Water pH was slightly alkaline over the study period with periodic extreme values ranging from 6 to over 9 standard units. Conductivity at Oologah Lake ranged from 0.200 to 0.650 mS/cm. Alkalinity and hardness were indicative of moderately hard water with alkalinity values ranging from 40 to 160 mg/L, and hardness from 80 to 240 mg/L as $CaCO_3$.

From 2003 to 2006 mean veliger densities in Oologah Lake increased each year (Figure 2.4). In 2003, veliger densities peaked at 30/L in September (Figure 2.5). The peak in veliger densities increased in each successive year, from 170/L in 2004 to 480/L in 2006 (Figure 2.5). In 2007, a decrease in veliger production resulted in annual densities of less than 1/L until 2010. In order to examine variability in the timing of veliger production in this reservoir, veliger densities from 2003 to 2006 were presented for each year (Figure 2.5). Years 2005 and 2006 represented typical zebra mussel reproduction timing, with

veligers first observed in the water column in May when water temperatures usually ranged between 15 and 24˚C. Peak densities usually occurred in late May or early June, associated with water temperatures between 24 and 28˚C. As water temperatures increased during July and August, veliger densities decreased, and then as temperatures fell to between 16 and 26˚C in September and October, a secondary peak in veliger production often occurred. This secondary peak in veliger densities was usually much reduced as compared to the spring density peak. Veliger densities in 2004 did not fit this typical seasonal pattern. Veligers were first observed in early May with peak densities occurring in July and August (Figure 2.5). Mean water temperature in July and August at mid column depth was significantly lower in 2004 than in 2003, 2005 and 2006 (26. 8 vs. 28.5, 28.9 and 28.2˚C, respectively with $P < 0.005$ for all years). Peak veliger settling increased from 200,000/m^2 in 2003 to near 1.2 million/m^2 in early 2006 (Figure 2.6). Greatest settling rates were observed 2-3 weeks after maximum pelagic veliger densities. No settling was detected between January 2007 and July 2010, associated with low pelagic veliger concentrations in this period.

Zebra mussel densities sampled from concrete panels also exhibited seasonal trends with peak densities generally occurring in July shortly after planktonic veligers matured and settled (Figure 2.7). In the summer of 2004, concrete panels suspended at Redbud Marina were excessively colonized, which made the grid enumeration system (outlined in methods section) inadequate to determine density. Those panels were harvested and replaced with clean panels, which were recolonized six weeks later. Harvested panels were brought into the laboratory where a more accurate estimate of zebra mussel density

could be conducted. More thorough examination of the harvested panels revealed a density of 155,000/m^2. In 2005 and 2006, declines in abundance reflected dieoffs in late July and August (Figure 2.7). Between 2007 and 2009, no adult mussels were found on concrete panels at any site until July 2010. In July 2010 mussels reappeared at Blue Creek and Redbud Marina. Panels suspended at Hawthorn Bluff were repeatedly colonized during the course of the study, however, these panels were located at the end of a long fetch and subjected to wave action greater than at the other sites that displaced mussels and lead to artificially low density estimates for this site.

From 2000 to 2006, water transparency significantly increased in Oologah Lake ($P < 0.001$, Table 2.1). Pre-invasion/ low zebra mussel density mean Secchi disk data from 2000-2002 were provided by the United States Army Corps of Engineers, Tulsa District, (personal communication, Tony Clyde) with data from 2003-2010 derived from the current study. Interestingly, a marked decline in water transparency occurred in 2007. While mean Secchi disk values were not significantly different between 2003-06 and 2007-10 ($P = 0.065$) the decline from 0.99 m to 0.84 m followed a lake-wide dieoff of zebra mussels in Oologah Lake. However, Oologah Lake also experienced substantial flooding in 2007, 2008 and 2009 (Figure 2.8), therefore the decline in water transparency cannot be strictly tied to the dieoff alone.

Initial growth experiments were conducted in Oologah Lake in 2005-2006 with caged mussels. Mussel growth rates in Oologah Lake approached 0.14 mm/day at Redbud

Marina, Blue Creek, and Hawthorn Bluff in June/July 2006 (Figure 2.9). Mean water temperatures during the trial ranged from 26.9˚C at Redbud Marina to 27.5˚C at Blue Creek. To investigate the influence of depth on growth rates, cages were suspended at 1, 2 and 3 m at Redbud Marina in June 2005 (Figure 2.9). Growth rates approached 0.1 mm/day at the 1 and 2 m and were significantly different from the 0.05 mm/day observed at the 3 m depth ($P<0.001$). Mean temperatures ranged from 28.9˚C at 1 m to 28.2˚C at 3 m. A second depth vs. growth trial was initiated at Redbud Marina in September 2005 with cages suspended at 1, 2, and 3 m (Figure 2.9). Growth of zebra mussels was significantly greater at 2 m than 3 m ($P=0.015$) but was not different between 1 and 3 m ($P=0.097$). Mean temperatures ranged from 22.2˚C at 1 m to 22.1˚C at 3 m. Finally, to determine winter growth rates, zebra mussels were suspended at 1 m at Redbud Marina in January 2006 (Figure 2.9). Maximum winter growth rates (mean temperature of 6.9˚C) approached 0.01 mm/day.

Sooner Lake

Based on data from the temperature loggers, sites closest to the warm-water discharge had higher temperatures throughout the year compared to sites farther away. Peak summer temperatures were near 40˚C and winter temperatures varied between 5 and 10˚C at the discharge buoy. Sites located farthest from the warm-water discharge (east boat ramp, dam, and intake buoy) reflected typical Oklahoma reservoir water temperatures of near 30˚C in July-August and below 5˚C in January-February.

Other water quality parameters were more consistent among sites within Sooner Lake. Dissolved oxygen ranged between 1 and 14 mg/L with highest concentrations recorded during the winter and early spring and low values associated with warmer temperatures in July and August. Sooner Lake stratified during July and August in 2007, 2008, and 2010 although due to the small size of the lake, length of fetch across the lake, and prevailing winds, two of the three stratification events were quite short in duration. Stratification in 2007 and 2008 lasted about 3 weeks, while the 2010 stratification persisted for nearly 7 weeks. These stratifications were most evident at the dam location with a maximum depth of 27 m. During each stratification event, surface dissolved oxygen concentrations of 7 mg/L were depleted to 1.5 mg/L at 15 m. Water pH ranged from 6.8 to 8.9 standard units during the course of the study, with conductivity ranging between 1.3 and 2.1 mS/cm. Alkalinity and hardness in Sooner Lake were indicative of hard to very hard water with alkalinity ranging between 100 and 200 mg/L as $CaCO_3$ and hardness between 100 and 240 mg/L as $CaCO_3$.

Zebra mussels were first reported in the lake in 2006, and peak veliger densities generally increased thereafter: 150/L in 2007, 580/L in 2008, 350/L in 2009, and 600/L in 2010 (Figure 2.10). The seasonal timing of zebra mussel reproduction at sites away from the heated discharge in Sooner Lake (dam, intake buoy, and plant intake) was generally similar to the seasonal timing in Oologah Lake, with veligers first observed in the pelagic zone in May and peak densities observed in June. Reproduction occurred earlier at the sites nearest the discharge (discharge bridge, discharge buoy, and end discharge sites), with veligers being observed in April and peak densities occurring in May (Figure 2.10).

In contrast to Oologah Lake, densities of settled veligers on glass slides in Sooner Lake decreased each year after first introduction, from 1.7 million/m^2 in 2007 to less than 100,000/m^2 in 2010 (Figure 2.11). Peak settling was observed 2-3 weeks after peak pelagic veliger densities, usually in late June or July. Sites within the discharge channel generally had lower settling when compared with sites located further from the warm-water discharge.

Adult zebra mussel densities peaked at 150,000/m^2 in 2007, 50,000/m^2 in 2008, 60,000/m^2 in 2009, and 30,000/m^2 in 2010 (Figure 2.12). At the discharge buoy location, zebra mussels were extirpated by July each year and never exceeded 10,000/m^2, however at the end of the discharge channel they reached 60,000/m^2 in 2009 (Figure 2.12). Away from the discharge channel, peak densities were recorded in July and August associated with settling and growth of young of the year mussels. In July and August 2010 however, adult zebra mussel densities were reduced to 2500/m^2.

Growth experiments conducted in Sooner Lake indicate water temperatures > 32˚C were lethal to transplanted mussels. Maximum growth rates exceeded 0.1 mm/day in June 2008 and October 2009 at the warmest site (discharge buoy, Figure 2.13). Mean temperatures during these periods were 27.3˚C in June 2008 and 25.6˚C in October 2009. Trials conducted June-September 2007, July-September 2008, and September 2009 were not successful as mussels died within one week of deployment. Mean temperatures

during these unsuccessful mussel growth periods ranged from 32.1 to 35.3˚C. Maximum winter growth rates were 0.05 mm/day in January 2009 when the mean temperature was 16.1˚C during the growth period.

Maximum growth rates observed at the intake buoy (site farthest from the warm-water discharge) exceeded 0.11 mm/day in June and September 2007, with the lowest growth occurring in January and May 2009 at less than 0.04 mm/day (Figure 2.13). Mean water temperatures in June and September 2007 were 19.8 and 28.0˚C, respectively. The lowest growth rates were associated with mean temperatures of 10.0˚C in January 2009 and 10.4˚C in May 2009 growth period. Mussels at the intake buoy also did not survive in fall of 2009 when associated mean water temperatures were 27.3˚C and 21.6˚C for September and October, respectively.

DISCUSSION

Since Oklahoma is positioned near the predicted southern boundary of zebra mussels in North America (Strayer 1991), the present study provides an opportunity to evaluate population dynamics of the organism under environmental conditions that may cause stress during certain times of the year, especially in relation to maximum lethal temperatures. Characterizing zebra mussel population dynamics in Oklahoma reservoirs may allow for a re-evaluation of the southern limit for mussel dispersal. The present study compares zebra mussel dynamics in two reservoirs, one exhibited a natural

Oklahoma temperature regime and the second received a warm-water discharge that altered water temperature in part of the system.

Oologah Lake

From 2003 to 2006, the zebra mussel population in Oologah Lake expanded nearly exponentially. Peak veliger densities generally occurred in late May or June, which contrasts to the Great Lakes where veliger peaks occurred in July and August during the initial populations expansion (Garton and Haag 1993; Fraleigh et al. 1993; Smit et al. 1993). However, in 2004, peak veliger densities occurred in August when the mean water temperature was 2˚C cooler in July and August than in any other year of study. This supports conclusions drawn by McMahon (1996) indicating that water temperatures play an important role in zebra mussel reproduction and population dynamics. Apparently, the relatively mild year in 2004, may have provided low enough temperatures to allow mussels to reproduce well into the summer months. Further support for this was provided in 2006 when water temperatures remained below 30˚C until August. Veliger densities in 2006 peaked at 480 veligers/L, similar to maximum densities observed in the Great Lakes (Garton and Haag 1993; Fraleigh et al. 1993). Zebra mussel densities declined precipitously in late-August 2006, in association with water temperatures of 30˚C in combination with a drought which resulted in low inflow into the lake.

While the specific sequence of events that initiated the 2006 die-off of zebra mussels in Oologah Lake are not explicitly clear, a combination of factors may have played a role. Water temperature in 2006 was not greater than maximum temperatures in 2003 and 2005, therefore it is unlikely that high temperatures alone caused the dieoff of mussels. Low water levels and high zebra mussel densities however, were unique compared with previous years. Under low flow conditions, Burks et al. (2002) showed nitrate concentrations to be greater at the base of aggregations of zebra mussels (druses) than at the surface of the druse. In addition, they found dissolved oxygen declines at the base of druses when compared with overlying water. Similarly, Tuchman et al. (2004) demonstrated zebra mussels on the interior of these druses experience reduced food availability under low-flow conditions. As a result, mussels in the interior of these druses may have experienced mortality due to low dissolved oxygen, low food resources, and high nitrate concentrations as well as lethal temperatures near 30˚C. Aldridge et al. (1995) and McMahon et al. (1995) have shown zebra mussels are sensitive to temperatures above 28˚C with death occurring in a matter of hours when exposed to 30˚C. As zebra mussels on the interior of these druses begin to expire, decomposition byproducts such as ammonia and nitrite may have increased, particularly under low flow conditions, which would have resulted in a further decline in water quality inside druses. In experiments conducted with *Corbicula sp*., Cherry et al. (2005) and Cooper et al. (2005) showed these ammonia spikes may result in a cascade of changes that increased mortality of bivalves in the surrounding environment.

Dieoff events of mussels are not unique to reservoirs in Oklahoma or even to populations located in more temperate climates. Stanczykowska and Lewandowski (1993) noted large scale and rapid reductions in zebra mussel densities in Mazurian Lakes in northeastern Poland. They noted these events occurred in years after relatively high zebra mussel densities, and particularly in eutrophic systems. They also noted some populations later rebounded to previously recorded densities, while in other systems populations were eventually extirpated. Allen et al. (1999) showed that summer dieoffs of zebra mussels in the lower Mississippi River were associated with high water temperatures. While the population in Oologah Lake was not extirpated, it remained at very low levels from 2007 through 2010. After the near drought conditions in late-2006, record floods occurred in 2007, 2008 and 2009. These high-water events occurred in June, when peak reproduction occurred in three of the four previous years. While there appears to be little published information available on effects of floods on zebra mussels, these high water events may have flushed viable veligers out of the reservoir, or could have resulted in veligers that settled onto structures high in the riparian areas which later were dry as the water receded. Between 2007 and 2009, adults were undetectable and veliger densities were below 1/L until 2010 when adult zebra mussels were once again found at Redbud Marina and Blue Creek and veligers peaked at 1/L. This three year recovery period appears to have resulted from the dieoff initiated in 2006 combined with high water levels in peak-reproductive months in 2007, 2008, and 2009. The reduction in water transparency after the dieoff in 2006 may have resulted from either flooding events in 2007, 2008, and 2009 or the lack of zebra mussel filtration as the population declined.

Maximum growth rates of mussels between 5 and 11 mm initial length generated in 2005 approached 0.14 mm/day at three of the four sites. These estimates are somewhat greater than previously published growth rates ranging between 0.05 and 0.1 mm/day (Allen et al. 1999; Dorgelo 1993; Karatayev et al. 2006). In addition, zebra mussel growth rates in our study were determined for individual caged mussels which have been shown to decrease growth (Karatayev et al. 2006). Zebra mussels deployed at 3 meters depth also exhibited lower growth rates when compared with mussels suspended at 1 and 2 meters, which is consistent with data reviewed by Karatayev et al. (2006) who suggest reduced temperature and food availability may explain decreased growth in deeper waters.

While long-term population trends (i.e. survival, growth, and reproduction) of zebra mussels in Oologah Lake are still uncertain, Strayer and Malcom (2006) suggested disturbance may impact population trends of zebra mussels. They state that while regular disturbance tends to stabilize mussel populations, severe and irregular disturbances, (such as flooding) may contribute to more variable population trends. Given the inherent variability of reservoir water levels, particularly reservoirs used for flood control, zebra mussel population dynamics may continue on a boom-bust cycle not only in Oologah Lake but also in other reservoirs in the region.

Sooner Lake

Peak veliger densities rapidly increased from 150/L in 2007 to over 500/L in 2008 and 2010. However, these peaks were very short lived lasting only 1 to 2 weeks, but similar

to that observed in Oologah Lake. Peak densities were similar to those observed by Garton and Haag (1993) and Fraleigh et al. (1993) in the Great Lakes. Veligers generally first appeared at sites located within the warm-water discharge zone (discharge bridge and discharge buoy). Peak veliger densities usually occurred at the cool-water sites (dam and intake buoy) several weeks after peak densities at warm-water sites. Veliger densities in the discharge zone never exceeded 100/L because adult zebra mussel densities in this area remained low (0 to 10,000/m^2).

Water temperatures at discharge locations exceeded 30˚C in July of all years and adult mussels were routinely extirpated from sites in this location from July through September. After declines in water temperatures to below 30˚C, young zebra mussels settled on concrete panels and firm substrates within the discharge zone. Winter water temperatures at the discharge buoy generally ranged between 3 and 10˚C which allowed young newly-settled mussels to grow through much of the winter and reach reproductive maturity by spring. This cycle of repeated extirpations and reintroductions in the discharge zone, maintained low zebra mussel densities (compared to the cool water sites) throughout the study period. Cyclical extirpation and reintroduction phenomenon at warm-water sites was also described by Sinicyna and Zdanowski (2007 a, b) at the Konin heated lakes complex in central Poland.

At sites with ambient temperatures, densities of adult zebra mussels attached to concrete panels peaked near 150,000/m^2 in 2007 and subsequently, the population stabilized near

40,000/m^2 between 2008 and 2010. Isolated dieoffs of older zebra mussels were noted at the intake buoy, dam, and end discharge during July and August, associated with water temperatures near 30˚C at these locations. These dieoffs were restricted to mussels greater than 15 mm in length, while the young-of-the-year-mussels were largely able to withstand these high water temperatures. Again, adult densities and juvenile domination during summer are similar to patterns observed in the Konin heated-lakes complex in Poland (Sinicyna and Zdanowski 2007 a, b).

Growth rates of zebra mussels varied both seasonally and among sampling sites. At the discharge buoy, maximum growth rates of 0.1 mm/day were achieved in periods that ended in June 2008 and October 2009 with associated mean temperatures of 27.3 and 25.6˚C, respectively. Growth in winter of 2008-09 was 0.04 mm/day with a mean water temperature of 16˚C during the period. During each of the failed attempts at measuring growth rates, mean water temperature ranged between 32 and 35˚C. At the intake buoy site, maximum growth rates were slightly greater than in the discharge zone, reaching 0.12 mm/day with a mean temperature of 28˚C. However, experiments in 2008 and 2009 resulted in maximum growth rates of only 0.09 mm/day. Karatayev et al. (2006, and references therein) found that zebra mussel growth rates in lakes varied significantly between years, perhaps brought about by different environmental conditions such as temperature, food availability, suspended solids, etc.

While isolated dieoffs of zebra mussels greater than 15 mm were noted at all locations within Sooner Lake, there was no large-scale dieoff similar to what occurred in Oologah Lake in 2006. Small scale dieoffs in Sooner Lake occurred in association with the warmest temperatures in July and August of each year. The largest of these dieoffs occurred in 2010, associated with water temperatures that exceeded 30˚C at sites farthest from the heated discharge. Also, during the 2010 dieoff, the lake stratified, brought about by an uncharacteristic period of relatively low winds and little wind-driven mixing of the lake. Hypolimnetic dissolved oxygen values ranged between 1 and 2 mg/L during the 7-week period in July-August 2010 which may have contributed to the dieoff.

As Aldridge et al. (1995) described, given the higher metabolic rates of smaller organisms compared with larger ones, it seems counterintuitive that larger zebra mussels would be more negatively affected by high temperatures than small mussels. However, McMahon (1996) found that small zebra mussels were more tolerant of high temperatures than larger mussels. This was supported by our observations in Sooner Lake, with specific reasons for large mussels being more susceptible to high temperatures compared to small mussels, perhaps related to differences in metabolic demands.

Sprung (1991, 1993) showed zebra mussels can lose as much as 30% of their body weight after reproduction. Furthermore, Aldridge et al. (1995) demonstrated zebra mussels, 13-17 mm in length, enter negative growth at water temperatures above 28˚C where metabolic demands exceed energy gained through food resources. Zebra mussels

in Oklahoma reproduce in May or June under lower, optimal water temperatures, but are then exposed to temperatures above their 28˚C threshold in July and August. As mature mussels allocate energy toward reproduction rather than somatic growth, stored energy reserves may be inadequate to deal with high water temperatures that generally occurred in July and August in Oklahoma. This hypothesis is further supported by Stoeckmann and Garton (1997, 2001) who found large mussels did not allocate energy to growth and reproduction equally. When exposed to near lethal temperatures mussels sacrificed somatic growth for reproduction which caused a net negative growth during the summer months. Small zebra mussels, on the other hand, allocate no energy for reproduction, but devote most energy on somatic growth and metabolic demands. This may allow them extra energy reserves needed to cope with warmer water temperatures. Therefore, temperature may serve as an important limiting variable for zebra mussel populations in southern North America by affecting adult survival, and skewing populations toward juvenile dominance, especially during summer months. An extended recovery phase of the zebra mussel population in Oologah Lake appears to have been brought about by one such dieoff, in combination with flood events in three subsequent years. With the 2009 discovery of zebra mussels in Lake Texoma on the Oklahoma-Texas border, it is clear zebra mussels will continue to spread to yet warmer habitats, and continued examination of population dynamics under natural conditions will provide further insights into temperature limitations of this species.

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TABLES AND FIGURES

Table 2.1. Oologah Lake mean (± standard deviation) Secchi disk depth for periods associated with pre-zebra mussel invasion/low densities (2000-2002), high densities (2003-2006), and post-dieoff/flooding (2007-2010).

Period	Event	Mean (± S.D.) Secchi depth m	Comparison	P value
2000-2002	Pre-invasion/low densities	0.44 (0.17)	2000-2002 < 2003-2006	< 0.001
2003-2006	High densities	0.99 (0.38)	2003-2006 = 2007-2010	0.065
2007-2010	Post-dieoff/flooding	0.84 (0.33)	2000-2002 < 2007-2010	< 0.001

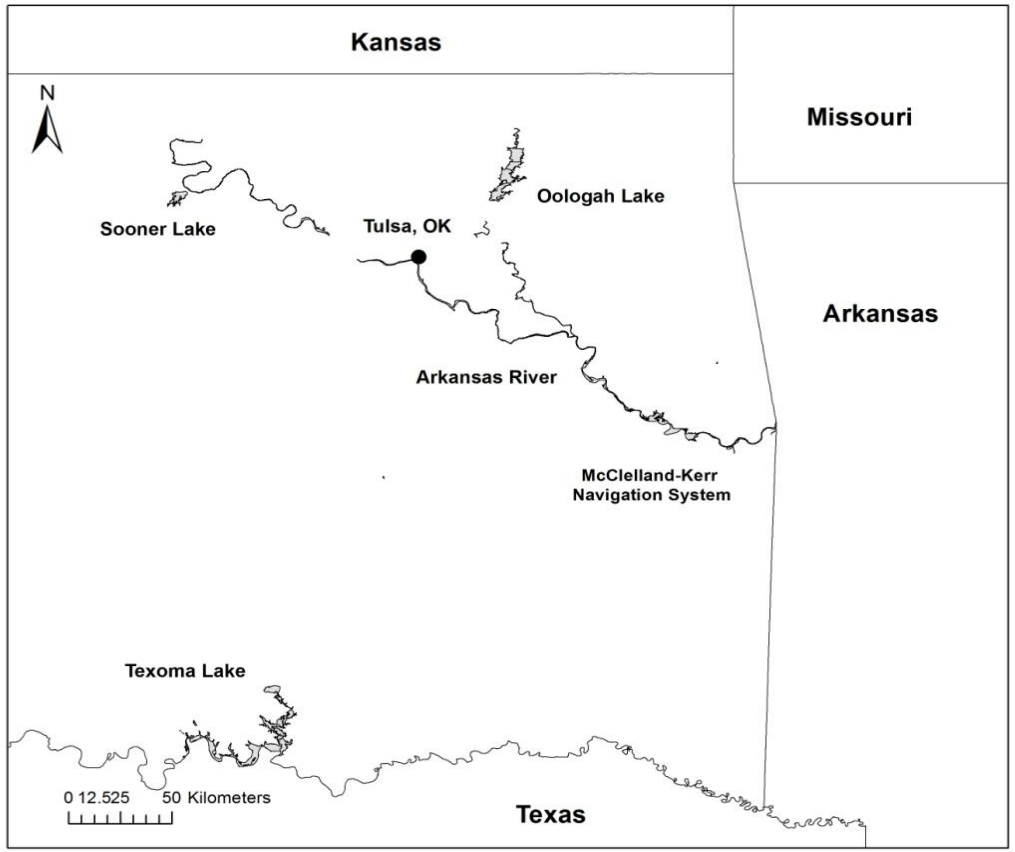

Figure 2.1. Map of eastern Oklahoma and surrounding states showing relative location of discussed features.

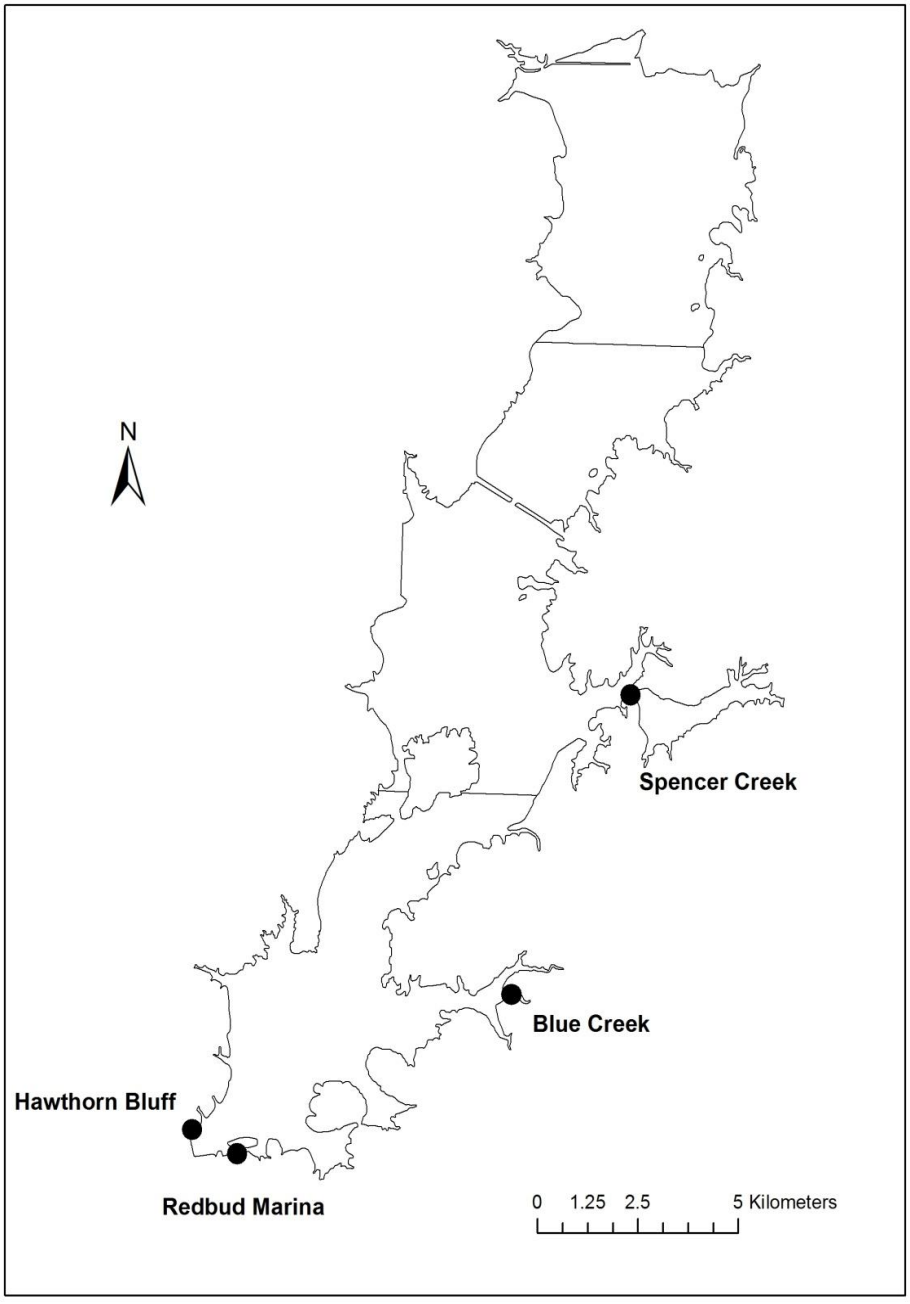

Figure 2.2. Map of Oologah Lake, Oklahoma with selected sampling sites.

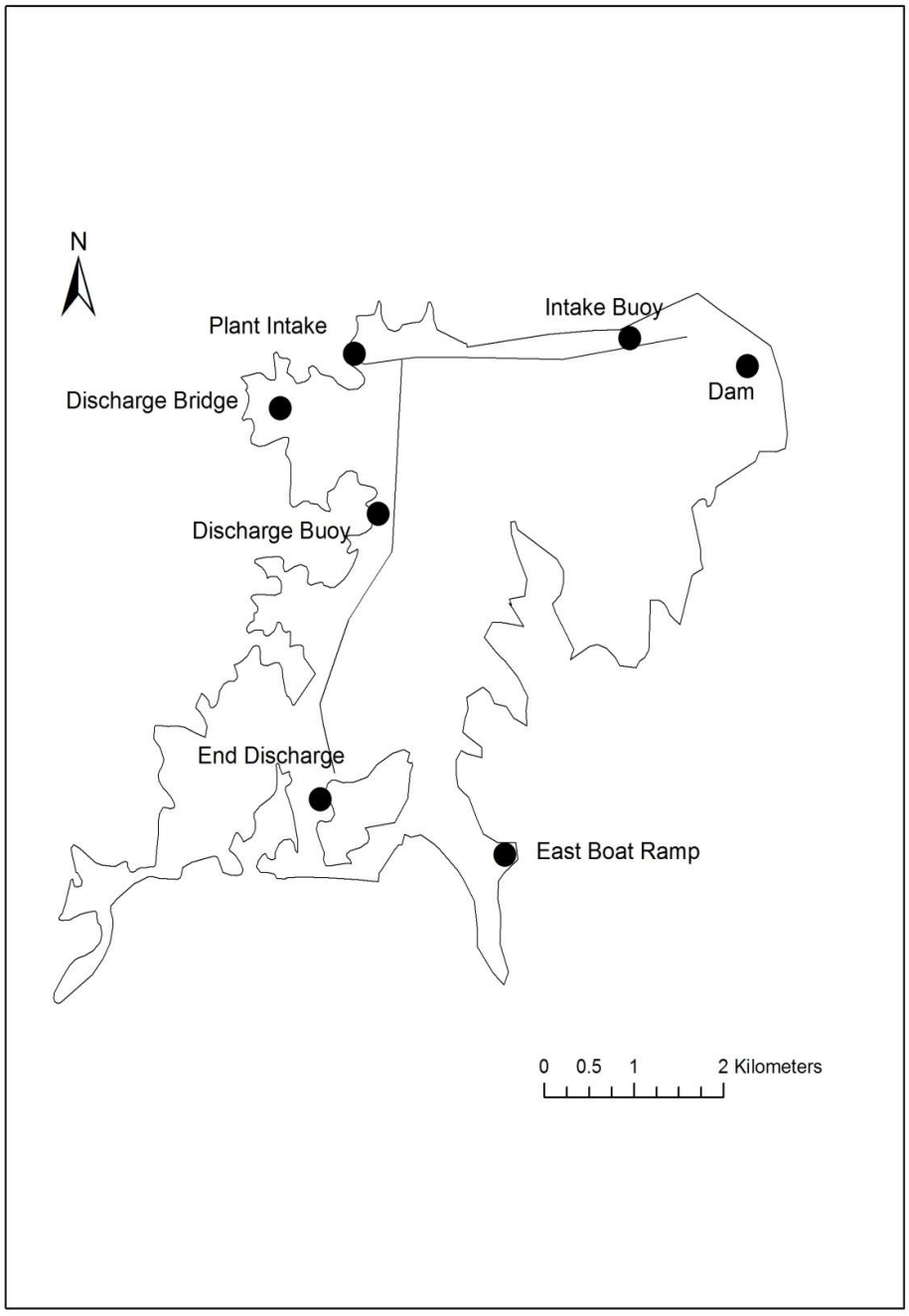

Figure 2.3. Map of Sooner Lake, Oklahoma with selected sampling sites.

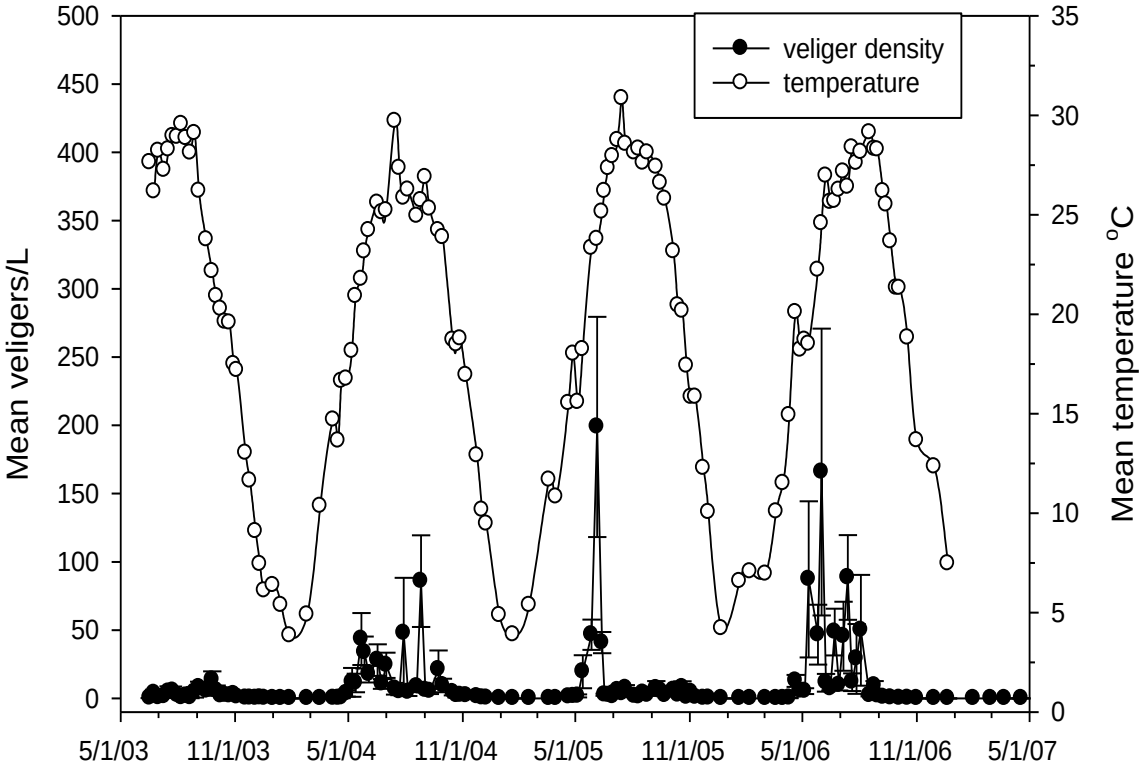

Figure 2.4. Mean (± S.E.) veliger densities at four sampling sites and mean water temperature at mid-column depth in Oologah Lake, Oklahoma, from June 2003 to April 2007. Veliger densities remained below 1/L from 2007 to 2010 and were removed from figure.

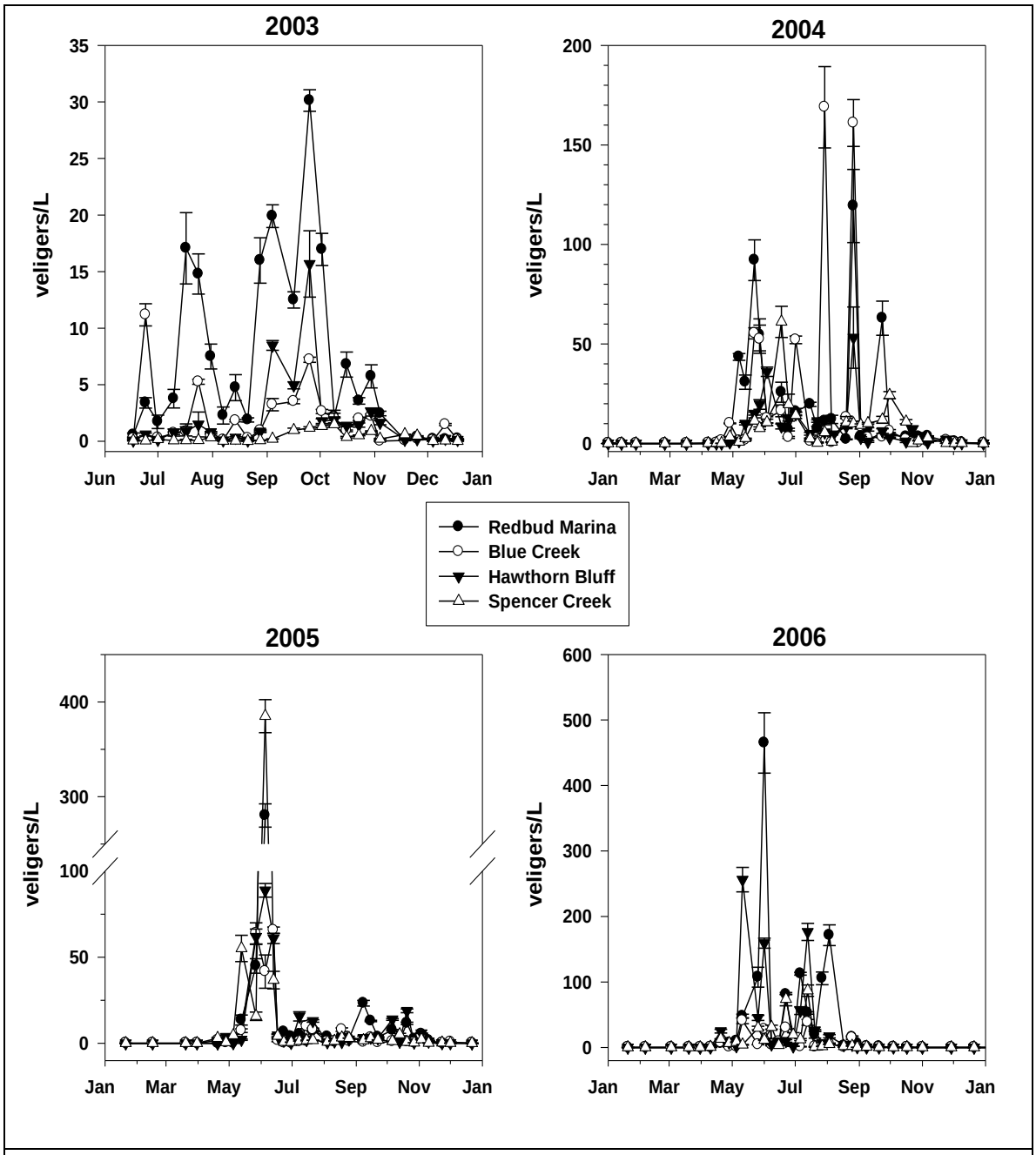

Figure 2.5. Mean (± S.E.) veliger densities at four sites in Oologah Lake, Oklahoma from 2003 to 2006.

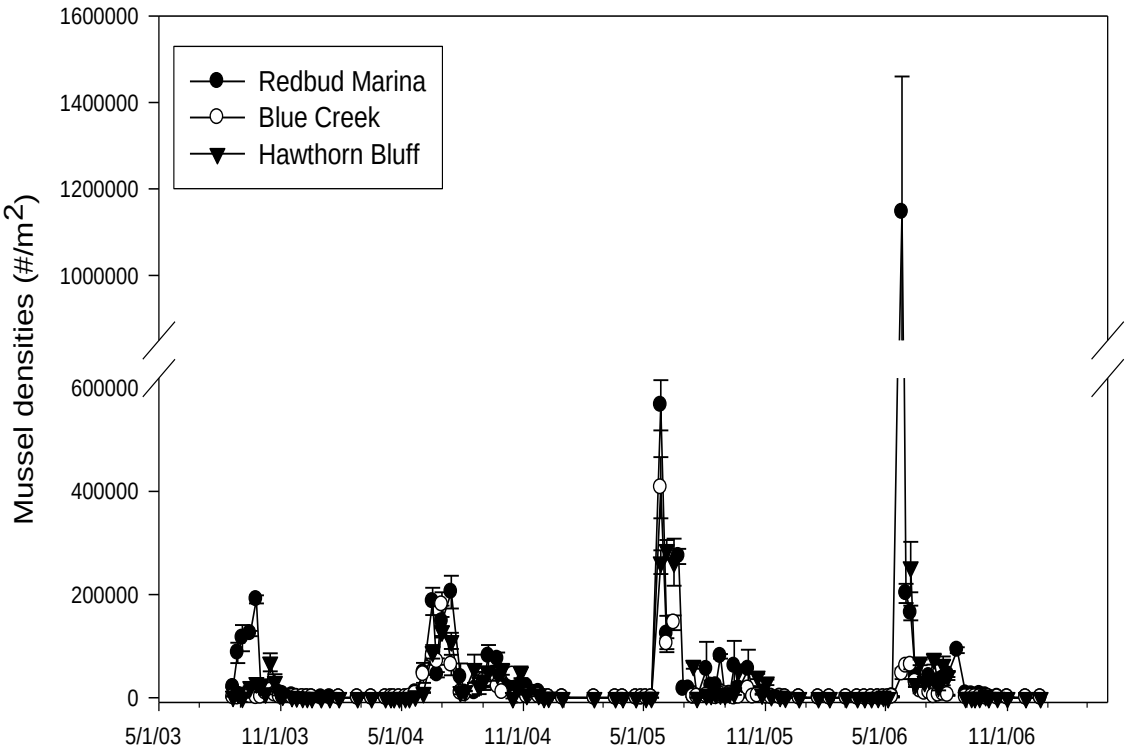

Figure 2.6. Mean (± S.E.) densities of settled zebra mussel veligers on glass slides suspended at three sites in Oologah Lake, Oklahoma, from 2003 to 2006.

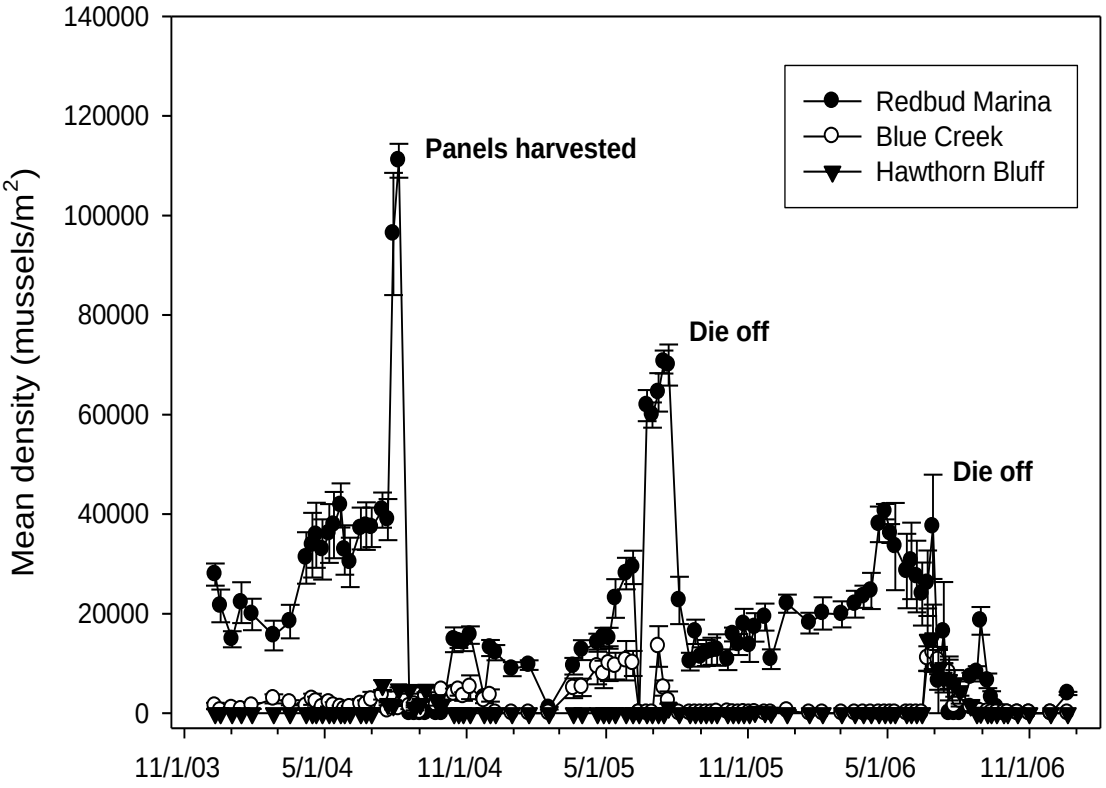

Figure 2.7. Mean (± S.E.) zebra mussel densities sampled from concrete panels suspended at three sites in Oologah Lake, Oklahoma, from 2003 to 2006.

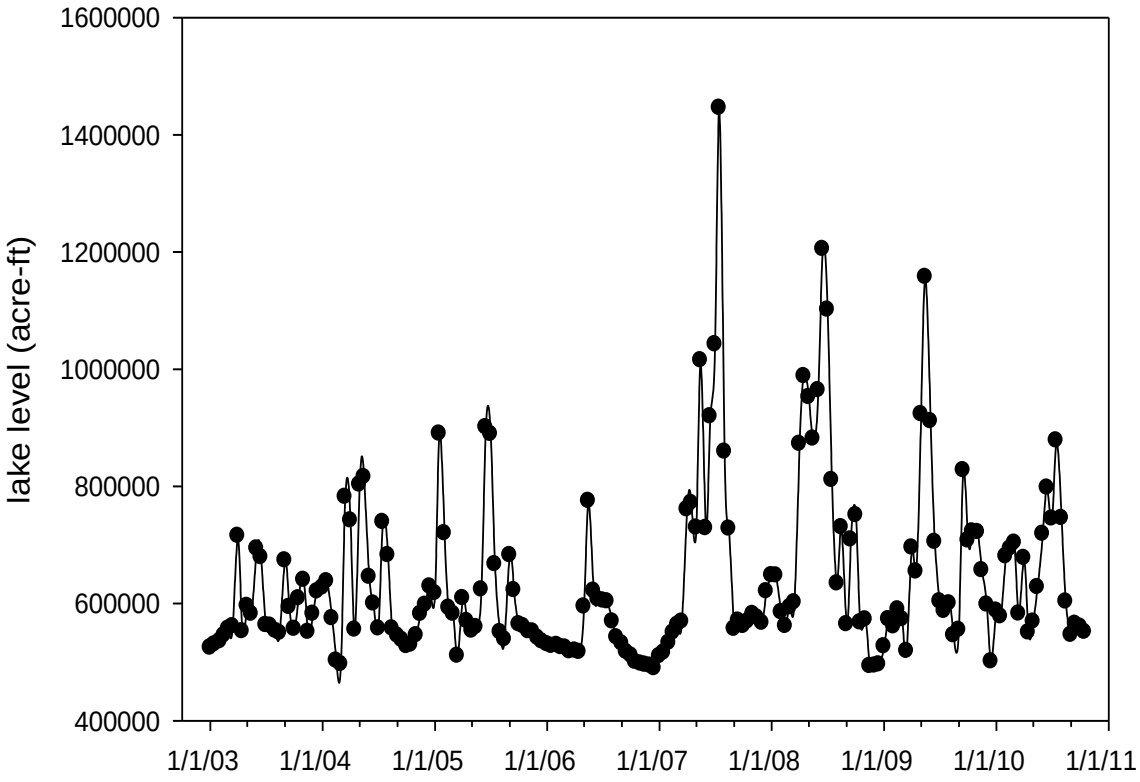

Figure 2.8. Water level on the 1st and 15th of each month for Oologah Lake, Oklahoma, from January 2003 to October 2010.

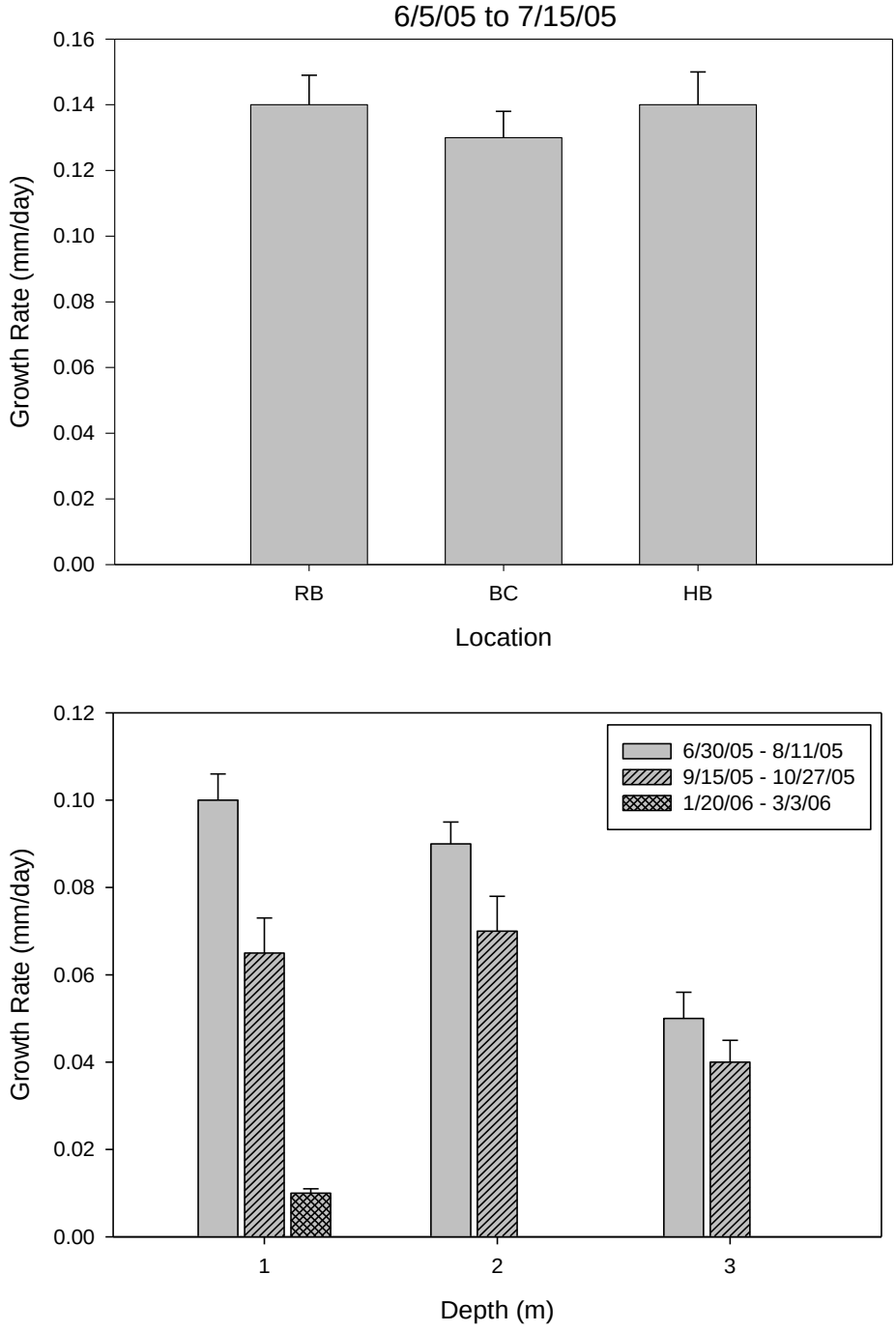

Figure 2.9. Mean (± S.E.) growth rates of zebra mussels measured from individuals chambered at various locations and depths in Oologah Lake, Oklahoma. RB = Redbud Marina, BC = Blue Creek, HB = Hawthorn Bluff. Growth rates at 1, 2, and 3 m depths were measured at Redbud Marina.

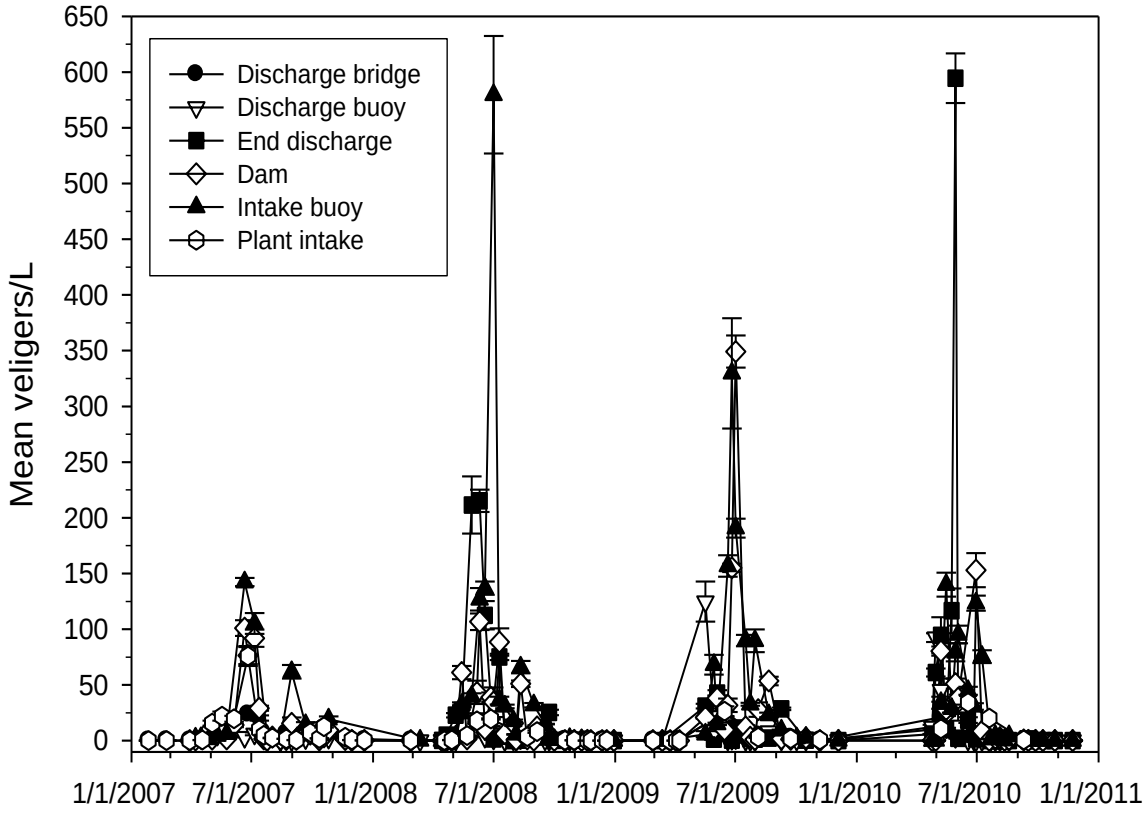

Figure 2.10. Mean (± S.E.) veliger densities from six sampling sites in Sooner Lake, Oklahoma from January 2007 to October 2010.

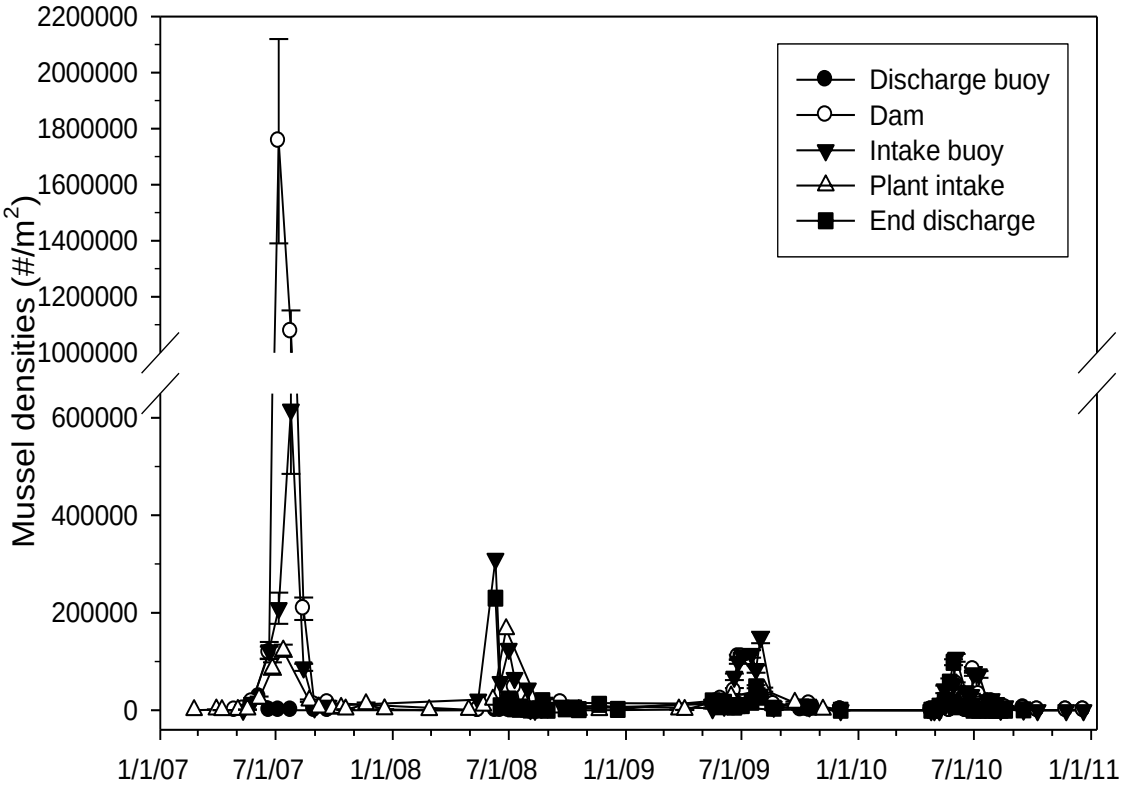

Figure 2.11. Mean (± S.E.) veliger densities settled on glass slides at five sites in Sooner Lake, Oklahoma from February 2007 to September 2010.

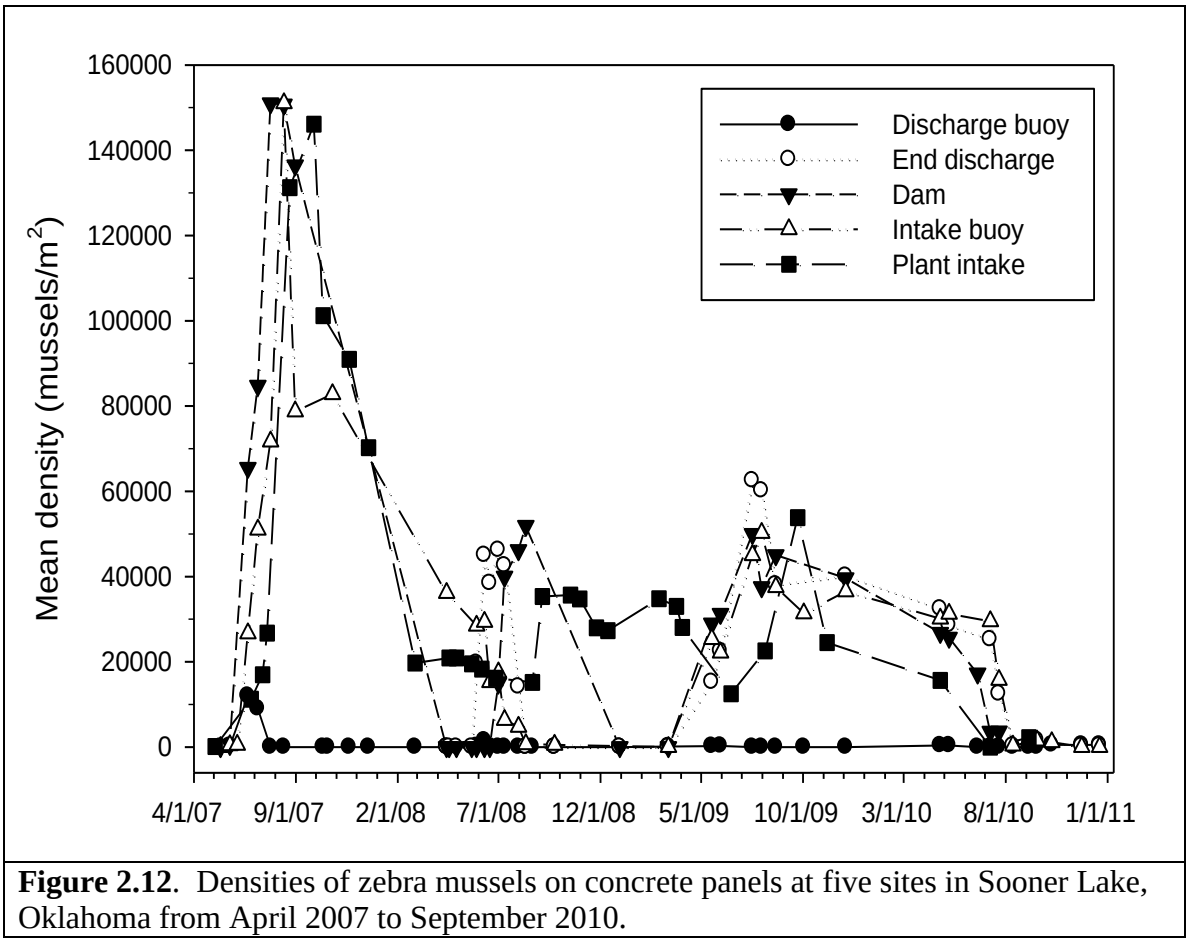

Figure 2.12. Densities of zebra mussels on concrete panels at five sites in Sooner Lake, Oklahoma from April 2007 to September 2010.

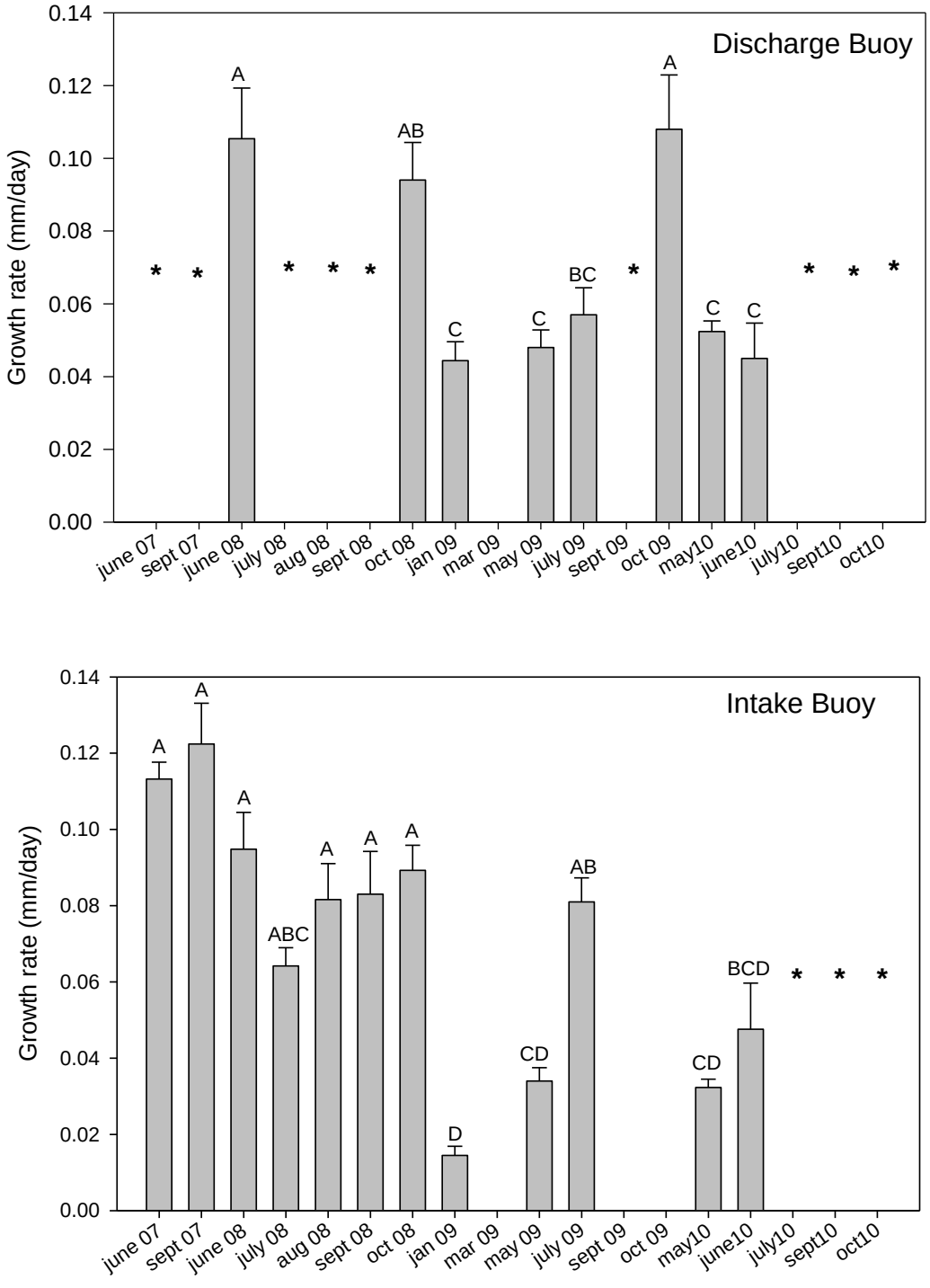

Figure 2.13. Mean (± S.E.) zebra mussel growth rates at two sites in Sooner Lake, Oklahoma. * indicates death of mussels prior to the end of the growth trial. Bars sharing a common letter are not significantly ($P < 0.05$) different.

CHAPTER III

STATUS OF FRESHWATER NATIVE MUSSELS (UNIONIDAE) IN THE OKLAHOMA SECTION OF THE VERDIGRIS RIVER AFTER INTRODUCTION OF THE ZEBRA MUSSEL (*DREISSENA POLYMORPHA*, PALLAS 1771)

ABSTRACT

The Verdigris River in Kansas and Oklahoma once held a diverse native unionid mussel fauna, although a number of these populations have undergone declines in richness and abundance. There is recent evidence that populations of some species of unionid mussels are increasing in parts of the Verdigris River in Kansas, but no current data existed for Oklahoma. In addition, zebra mussels (*Dreissena polymorpha*, Pallas 1771) have been introduced into a major impoundment on the Verdigris River, Oologah Lake, and these may further threaten mussel populations downstream from this reservoir. This study updates the distribution and abundance of native mussels in the Oklahoma portion of the Verdigris River upstream and downstream of Oologah Lake, and documents the current distribution of zebra mussels in this region. Thirty-one sites were sampled and a significant increase in species richness and abundance of native mussels was observed as compared to a 1997 study. Two species of special interest, *Cyprogenia aberti* (Conrad 1850) and *Quadrula cylindrica* (Say 1817) were found. Zebra mussels were not found at

any sample site upstream of Oologah Lake, but they were present at every downstream site. During sampling conducted in September 2006, zebra mussel byssal threads were observed on shells of a number of native mussels downstream from Oologah Lake, but unionid richness and abundance were not significantly different between sites above and below the reservoir.

INTRODUCTION

Freshwater mussels are considered one of the most imperiled groups of organisms in the world, with 70% of taxa within the family Unionidae considered species of special concern or endangered (Bogan 1993; Strayer et al. 2004; Warren and Haag 2005; Jones et al. 2006). As with many other groups of organisms that are experiencing population declines, loss of native mussels has been has been attributed to habitat alteration (Garner and McGregor 2001; Vaughn and Taylor 1999), point and non-point source pollution (Richter et al. 1997), and invasive species (Burlakova et al. 2000; Strayer et al. 2004).

The Verdigris River originates in the Flint Hills of southeastern Kansas (Schuster 1979) and flows southward into Oklahoma where it joins the Arkansas River near Muskogee, OK. Historically, the Oklahoma portion of the Verdigris contained a diverse assemblage of native unionid mussels, with Isely (1924) describing this region as having among the richest mussel fauna in the state. Since that time, significant portions of the river have been altered by impoundments and the lowest reaches have been dredged to create a navigation channel to the Arkansas River (USACE 2007). Agricultural activity and

urban and industrial development have led to pollutant and sediment input degrading water quality. Surveys conducted in both Kansas and Oklahoma in the 1990's indicated an overall decline in Verdigris River mussel populations as compared to earlier studies (Obermeyer et al. 1997a; Vaughn 1998).

Interestingly, more recent surveys in Kansas have reported increases in the densities of some unionid taxa. For example Miller and Lynott (2006) report increases in the abundance of 10 mussel species in the Kansas portion of the Verdigris between 1991 and 2003, a change they attributed to improved habitat quality. The last mussel survey conducted in the Oklahoma portion of the Verdigris was that by Vaughn (1998) in the late 1990s. In addition, zebra mussels (*Dreissena polymorpha*, Pallas 1771) were discovered in Oologah Lake, an impoundment of the Verdigris in Oklahoma, in the spring of 2003 (Laney 2005). While the distribution of *D. polymorpha* in the Verdigris River was largely unknown, the potential impact of these invasives on unionid mussels is well established (Ricciardi et al. 1996; Baker and Hornbach 1997; Schloesser et al. 2006). The objectives of this study were to survey the mussel communities in the Oklahoma portion of the Verdigris River to determine if increases similar to those described for Kansas were occurring, to characterize the extent to which zebra mussels occur along this section of the Verdigris, and to gather preliminary data regarding their potential interaction with native mussels.

METHODS

Sampling locations in the Verdigris River were selected to correspond with sites previously sampled by Vaughn (1998). Sample sites were mostly riffle areas and mussel surveys were conducted in runs immediately downstream of each riffle. Substrate at each sample location ranged from loose gravel to cobble. During each site visit, physical-chemical characteristics (temperature, dissolved oxygen, pH and conductivity) were recorded using a Hydrolab Quanta multi-parameter probe (Hydrolab Corporation, Austin, TX). Additionally, river water was collected in acid-washed polyethylene bottles and transported back to the laboratory on ice for determination of titratable alkalinity and hardness (APHA 1998).

Mussel surveys were conducted through timed snorkel searches by two individuals. Surveys were conducted for at least 15 minutes (2 person x 15 minutes = 30 minutes total search time), with longer time intervals devoted to locations that contained greater area of stable gravel substrate with good flow and low sediment. Once located, mussels were carefully removed from the substrate and placed in mesh bags. After each survey, mussels were sorted, measured to the nearest 0.01 mm maximum length using digital calipers (Fisher Scientific, Pittsburgh PA.), and identified to species using keys by Cummings and Mayer (1992), Oesch (1995), and Couch (1997). Mussels were then carefully returned to the river throughout the search area. Live voucher specimens were not collected due to limited representatives of some species; however, digital photographs and relic shells from each sample site were taken and are currently held at the Ecotoxicology and Water Quality Research Laboratory at Oklahoma State University, Stillwater, OK.

Site comparisons of mussel richness and abundance were made using 2-sample paired t-test for means ($\alpha = 0.05$) performed with SigmaStat version 3.1 (Systat Software Inc. San Jose, CA.). Regression coefficients and elevation of richness versus log-transformed abundance curves were generated for the present study and that conducted by Vaughn (1998) and were compared using t-tests at $\alpha = 0.05$ (Zar 1999).

RESULTS

A total of 31 sites (20 above Oologah Lake and 11 below) were surveyed for mussels between July and October 2006. Sites above Oologah Lake started approximately 0.5 km after the Verdigris River crossed the Oklahoma-Kansas border and ended approximately 28 km above Oologah Lake. The section of river between the last upstream site and the reservoir was not surveyed because of a general lack of riverine mussel habitat as the river widens and becomes more lake-like. Sites below Oologah Lake were located 1-25 km below the Oologah Lake dam. Habitat beyond this point also was considered unsuitable due to dredging in the McClellan-Kerr Navigation Channel.

Temperature across all sites ranged between 20-35°C, dissolved oxygen between 6.0-11.9 mg/L, pH between 6-8 standard units, alkalinity between 120 to 170 mg/L $CaCO_3$ and hardness between 112 to 156 mg/L as $CaCO_3$. Further comparisons of physical-chemical

data were not made due to potential sample collection date interferences over the 4-month sampling period.

Seventeen species of mussels were identified from the Verdigris River as a whole (Figure 3.1), with *Quadrula metanevra*, (Rafinesque 1820) being most abundant and *Quadrula nodulata*, (Rafinesque 1820) the least. *Cyprogenia aberti*, (Conrad 1850), *Lampsilis teres*, (Rafinesque 1820), and *Lasmigona complanata*, (Barnes 1823) were only found in sites above Oologah Lake, while *Megalonaias nervosa*, (Rafinesque 1820), *Quadrula cylindrica*, (Say 1817), and *Q. nodulata* were only found at sites below the lake. Generally, abundant species were also the most widely distributed except for *Q. cylindrica* which was only found at 3 locations but was the sixth most abundant species (Figure 3.2). Length distributions were developed for the 4 most abundant species, *Q. metanevra*, *Tritogonia verrucosa*, (Rafinesque 1820), *Obiquaria reflexa*, (Rafinesque 1820), and *Amblema plicata*, (Say 1817) (Figure 3.3). The distributions for all 4 species were negatively skewed, indicating more smaller individuals than might be predicted given a normal distribution, with kurtosis values generally positive except for *O. reflexa* with a -0.74 indicating a slightly more platykurtic (uniform) distribution.

Live mussels were found at all but 2 sites. Timed abundance estimates for sites with live mussels ranged from 3 to 156 mussels per hour (MPH) with a mean of 38 for all 31 sites (Table 3.1). Taxa richness ranged from 2 to 10 species per site with a mean of 5.2 species for all 31 sites combined.

No evidence of zebra mussels was found at any site above Oologah Lake, however zebra mussels and byssal threads (structures produced by zebra mussels that allow attachment to firm structure) were found on substrate and unionid shells at every site below the lake. While many native mussels collected below the lake had byssal threads on the valves, few had live zebra mussels attached. In order to assess the potential impact of zebra mussels on native mussel community composition, species richness and abundance were estimated separately for sites above and below the lake. Sample locations above the lake had a mean richness of 5.6 species per site, and an abundance of 39.3 MPH (Table 3.1). Sites below Oologah Lake had a mean richness of 4.4 species per site with an abundance of 35.7 MPH (Table 3.1). There was no significant difference in overall mussel species richness or abundance between upstream and downstream sites.

While no differences in mussel richness and abundance were apparent when sites were combined within upstream and downstream sections, a downstream longitudinal gradient in these parameters was apparent. Both mussel abundance ($r^2 = 0.697$, $P = 0.0014$) and species richness ($r^2 = 0.539$, $P = 0.0138$) were significantly positively associated with distance from the dam (Figure 3.4). However, these analyses may be influenced by two downstream sites that had the greatest abundance of any of the sample locations. When these two sites are removed from the analyses, the longitudinal relationship is no longer significant (MPH $r^2 = 0.31$, $P = 0.120$; richness $r^2 = 0.38$, $P = 0.077$).

DISCUSSION

Sampling locations in the Verdigris River were selected to correspond to a previous study (Vaughn 1998) to reassess the status of the native mussel community since the discovery of zebra mussels in Oologah Lake in June 2003. Vaughn (1998) identified 16 species of mussels, compared with 17 in this study. While species composition was largely similar between the two surveys, *Potamilus ohiensis*, (Rafinesque 1820) and *Quadrula quadrula*, (Rafinesque 1820) were not found in 2006. In contrast, live *C. aberti*, *Q. cylindrica*, and *Q. nodulata* were found in the 2006 survey while only relic shells of these species were found in the previous survey. Of these, *C. aberti* and *Q. cylindrica* are of particular interest because *C. aberti* was once thought extirpated from the state (Mather 1990; Serb 2006), and *Q. cylindrica* is a federal candidate for listing as an endangered species (personal communication, David Martinez, United States Fish and Wildlife Service). Only 2 *C. aberti* were found, and both individuals were from sites near the Oklahoma-Kansas border. As abundance of this species has increased in the Kansas portion of the Verdigris (Miller and Lynott 2006), these upstream populations may be driving the re-introduction into Oklahoma through mechanisms such as fish host dispersal or downstream transport of juveniles after detaching from the fish host (Morales et al. 2006).

While *Q. cylindrica* was the sixth most abundant species, they were only found in a short river section downstream from Oologah Lake, an area also infested with zebra mussels. Lee et al. (1998) and Berg et al. (2007) suggest host-fish vagility may explain unionid

distribution patterns, with mussels that utilize fish hosts with greater home ranges typically showing greater abundance and distribution. The known fish hosts for *Q. cylindrica* include several species of *Notropis* (Yeager and Neves 1986), which have relatively small home ranges (Goforth and Foltz 1998). This characteristic of its fish host may explain the limited distribution of *Q. cylindrica* in the Verdigris.

Length distributions of the 4 most commonly encountered mussel species did not include individuals less than 30 mm in length (70 mm for A. *plicata*), although this may have been a sampling artifact as timed snorkel searches may bias against encountering very small or particularly cryptic individuals (Hornbach and Deneka 1996; Obermeyer 1998; Metcalfe-Smith et al. 2000). Furthermore, juveniles frequently bury completely in the substrate, making detection of these cohorts difficult when using snorkel searches (Neves and Widlak 1987; Amyot and Downing 1991; Yeager et al. 1994; Sparks and Strayer 1998). Timed snorkel search techniques are however more commonly used for determining mussel richness and locating rare species (Metcalfe-Smith et al. 2000; Vaughn and Spooner 2004) and are more cost effective when surveys of large areas are needed as compared with quadrat methods.

Vaughn (1998) reported a mean abundance for all 31 sites of 14.4 MPH and a mean richness of 3.3 species per site. Current abundance and richness across all 31 sites was 38.0 MPH and 5.2 species per site, which are significantly greater than the values reported by Vaughn ($P = 0.002$, $P = 0.001$, respectively). Mean sampling time between

the two surveys was not significantly different (P = 0.65) with 46.8 min in this survey versus 49.8 min in Vaughn (1998). In order to evaluate the extent to which the increase in richness resulted from locating more mussels overall, abundance vs. richness curves were prepared from both studies (Figure 3.5). These curves were linearized by log-transforming abundance to facilitate statistical analysis. There was no significant difference in the regression coefficient (36.7 for the present study versus 65.7 for the Vaughn study, P > 0.05) or elevation (P > 0.05) derived from the best fit lines from these data, indicating that mussel abundance explained a similar degree of variation in taxa richness in both studies. Thus the greater taxa richness observed in the present study appears to have been driven by our finding a greater number of mussels during the timed searches.

Given the semi-quantitative nature of the sampling technique employed in both surveys, care should be taken to not over-interpret these data. Strayer (1999) found low statistical power for detecting population declines when using presence/absence techniques; therefore these results should at a minimum support the need for more quantitative techniques throughout the study area. Miller and Obermeyer (1997) and Miller and Lynott (2006) reported an increase in 10 different species in the Kansas portion of the Verdigris River as compared to 1991 levels using quadrat methods. They attribute this increase to improvements in habitat quality, namely reduction in pollution, increase in fish hosts, and lack of severe drought. These same factors may be working to improve native mussel populations in the Verdigris River of Oklahoma.

The detrimental effects of zebra mussels on native mussel communities are well documented (Burlakova et al. 2000; Martel et al. 2001; and Schloesser et al. 2006), although we found no difference in mussel abundances between sites upstream of Oologah Lake, in which no zebra mussels occur, and sites downstream of the dam which are infested with the mussels. However, two relatively "good" sites greater than 20 km from the dam may have influenced these analyses. A number of native mussels were found to have byssal threads on their valves indicating some degree of zebra mussel settling. It is possible that zebra mussel colonization of native mussels occurs during the spring, but the attached *D. polymorpha* may be eliminated if water temperatures increase through the summer. The impoundment at Oologah Lake was designed for hypolimnetic releases, meaning if water releases occur during the summer months, downstream habitats would receive cooler water. However, in the months from August to November of 2006, there were no releases from the reservoir. Downstream habitats would therefore not have been influenced by the hypolimnetic release of water from Oologah Lake during the summer and fall the study was conducted. Without this influence, it is reasonable to assume that water temperature in these downstream reaches of the Verdigris River is similar to that observed in other streams in the area, and approaches 30ºC in mid summer. This temperature may be lethal to zebra mussels (Karatayev et al. 1998; Matthews and McMahon 1999; Elderkin and Klerks 2005) while unionids may be slightly more tolerant of these conditions (Polhill and Dimock 1996). Additionally, monitoring of zebra mussel densities in Oologah Lake indicated a significant die-off beginning in late June 2006. Adult zebra mussel densities in the lake declined from nearly 150,000 /m^2 to less than

4,500 /m^2 from late June to September 2006 (authors unpublished data). Temperature-induced seasonal die-offs of zebra mussels in the Verdigris River may mean that zebra mussel fouling of native mussels does not reach high enough numbers to cause a significant effect. Other studies have reported mortality of unionids due to zebra mussel infestation within 2 to 8 years after initial zebra mussel colonization (Schloesser et al. 1996; Ricciardi et al. 1998; Schloesser and Masteller 1999; and Schloesser et al. 2006). Zebra mussels were first reported in Oologah Lake in June 2003 (Laney 2005), with numbers increasing steadily since that time. It may be that negative effects on unionids due to zebra mussel colonization in the Verdigris are still to come, particularly if a cooler summer that reduces the late season die-off of zebra mussels were to occur.

Lastly, several studies have shown that zebra mussels and native mussels can co-exist in habitats that have fluctuating water depth, potential wave action, and substrate soft enough to allow native mussels to bury (Schloeser et al. 1997; Nichols and Amberg 1999; Bowers and De Szalay 2004; and Strayer and Malcom 2007). The habitat below Oologah Lake does have some of these characteristics, so co-existence of zebra mussels and native unionids cannot be ruled out.

A potentially confounding factor in determining the effect of zebra mussels on unionid mussels in the Verdigris River is the influence of the Oologah Lake dam. The effects of impoundments on native mussel communities have been well established (Bogan 1993;Vaughn and Taylor 1999; Bednarek 2001; and Sethi et al. 2004). For example,

Vaughn and Taylor (1999) described a reduction in native mussel populations below an impoundment and found a distance of 20 km was needed for these populations to recover to pre-impoundment levels. In the current survey, mussel abundance at sites downstream from the reservoir was positively correlated with distance from the dam. However, this relationship may have resulted from two sites (greater than 20 km from the dam) that harbored the greatest abundance of all 31 sites surveyed. Downstream sites less than 20 km from the dam generally had poor or less than average abundance and two sites immediately downstream from the dam had no live mussels at all. Taxa richness for downstream sites was also positively correlated with distance from the dam, although less distance from the dam was needed to "recover" as compared with abundance. Again the presence of two very good sites greater than 20 km from the impoundment may be influencing these data. When these two sites were removed from the analyses, the relationship was no longer significant.

In summary, the native mussel fauna in the Oklahoma portion of the Verdigris River that was surveyed here appears to have experienced a general increase in abundance and an associated increase in richness over the last ten years. The semi-quantitative nature of the techniques employed in this study mean that the data do need to be interpreted with some care and validation of the results with a more quantitative sampling technique is warranted. *C. aberti* and *Q. cylindrica*, both species of special interest were also found in the present survey. No zebra mussels were found at sites above Oologah Lake, although they were present at every site below the lake. While it appears that zebra mussels are currently having little effect on native mussel richness and abundance below Oologah

Lake, the consequences of long-term zebra mussel colonization on native mussels in this reach of the river are still unknown. For this reason, conservation efforts such as periodic defouling or propagation and reintroduction (Hallac and Marsden 2001; Strayer et al. 2004) to sites above Oologah Lake should be directed at species of special interest, (and native mussels in general) that are currently located below Oologah Lake. *Q. cylindrica,* given its rather limited distribution in the Verdigris River (Obermeyer et al. 1997a, b), the impoundment upstream and dredging activity downstream of the current population, and the relatively limited dispersal potential of its known fish hosts, should be a high priority for such efforts.

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TABLES AND FIGURES

Table 3.1. Mean richness and abundance (standardized as mussels found per hour sampling effort, MPH) for sites within the Verdigris River, OK. Upper indicates 20 sites above Oologah Lake, with Lower representing 11 sites below the lake. All refers to all 31 sites combined.

Sites	Mean Richness	Mean MPH
Upper	5.60	39.31
Lower	4.36	35.66
All	5.16	38.01

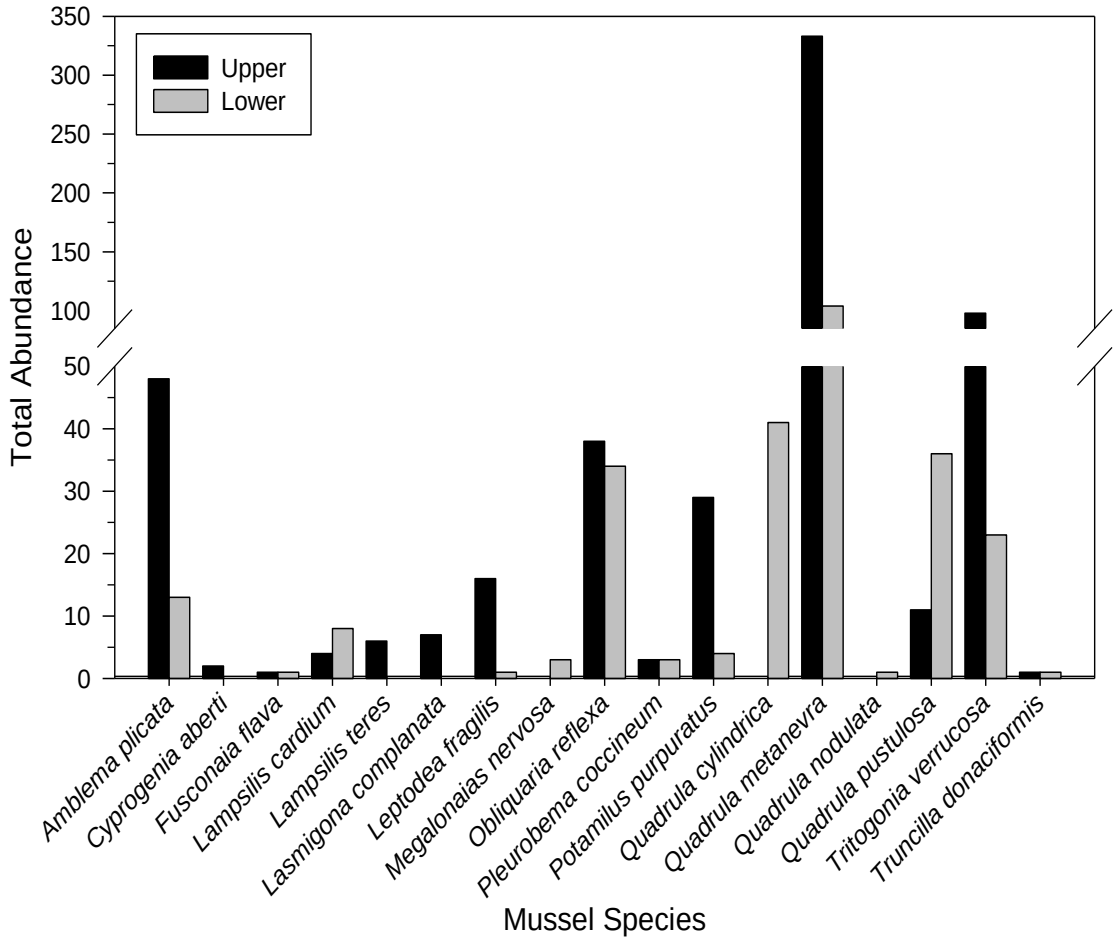

Figure 3.1. Abundance of native mussels in the Verdigris River, Oklahoma, above (upper) and below (lower) Oologah Lake

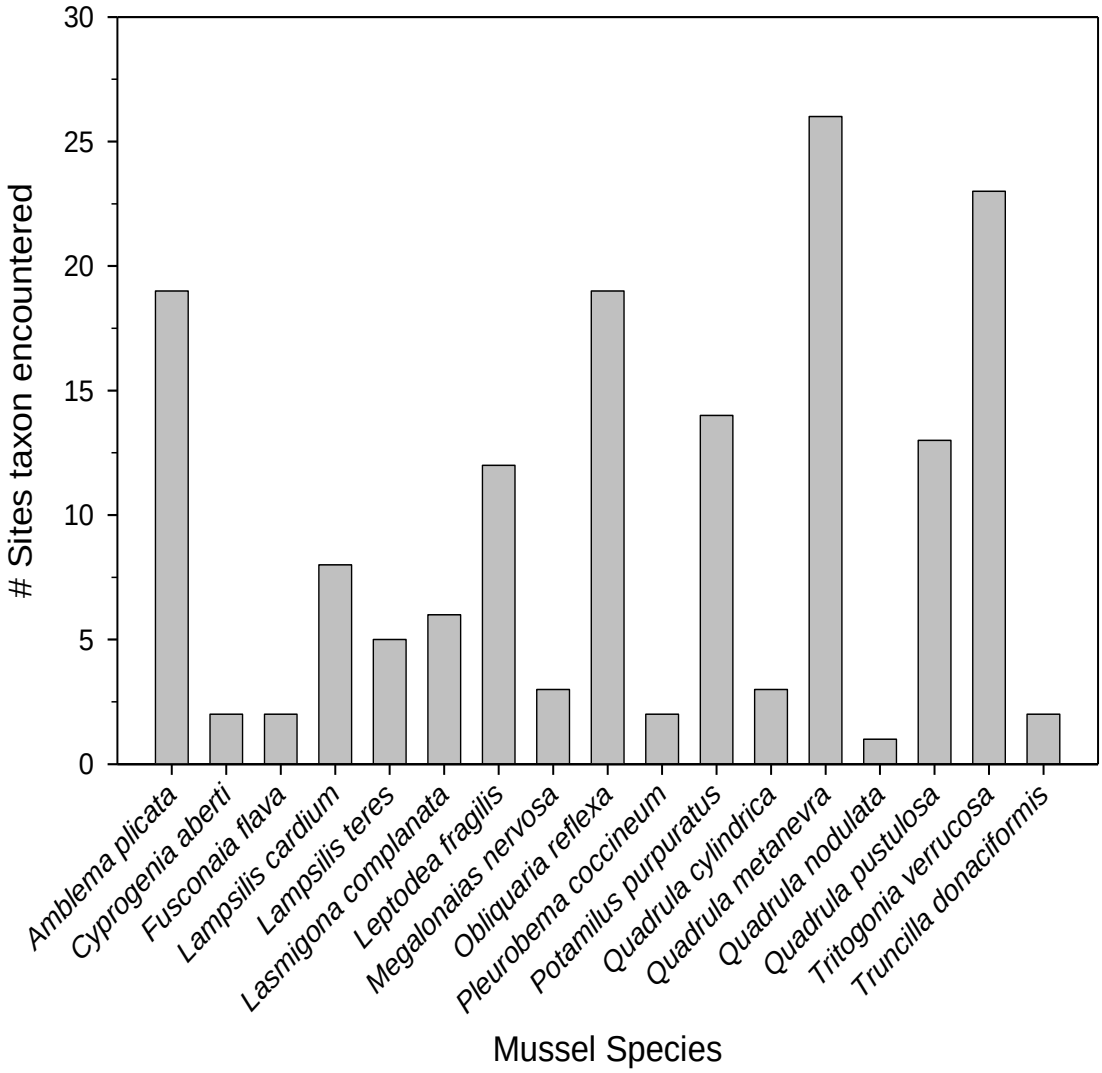

Figure 3.2. Incidence (# of sites at which a species occurred) of native mussels for the Verdigris River, Oklahoma

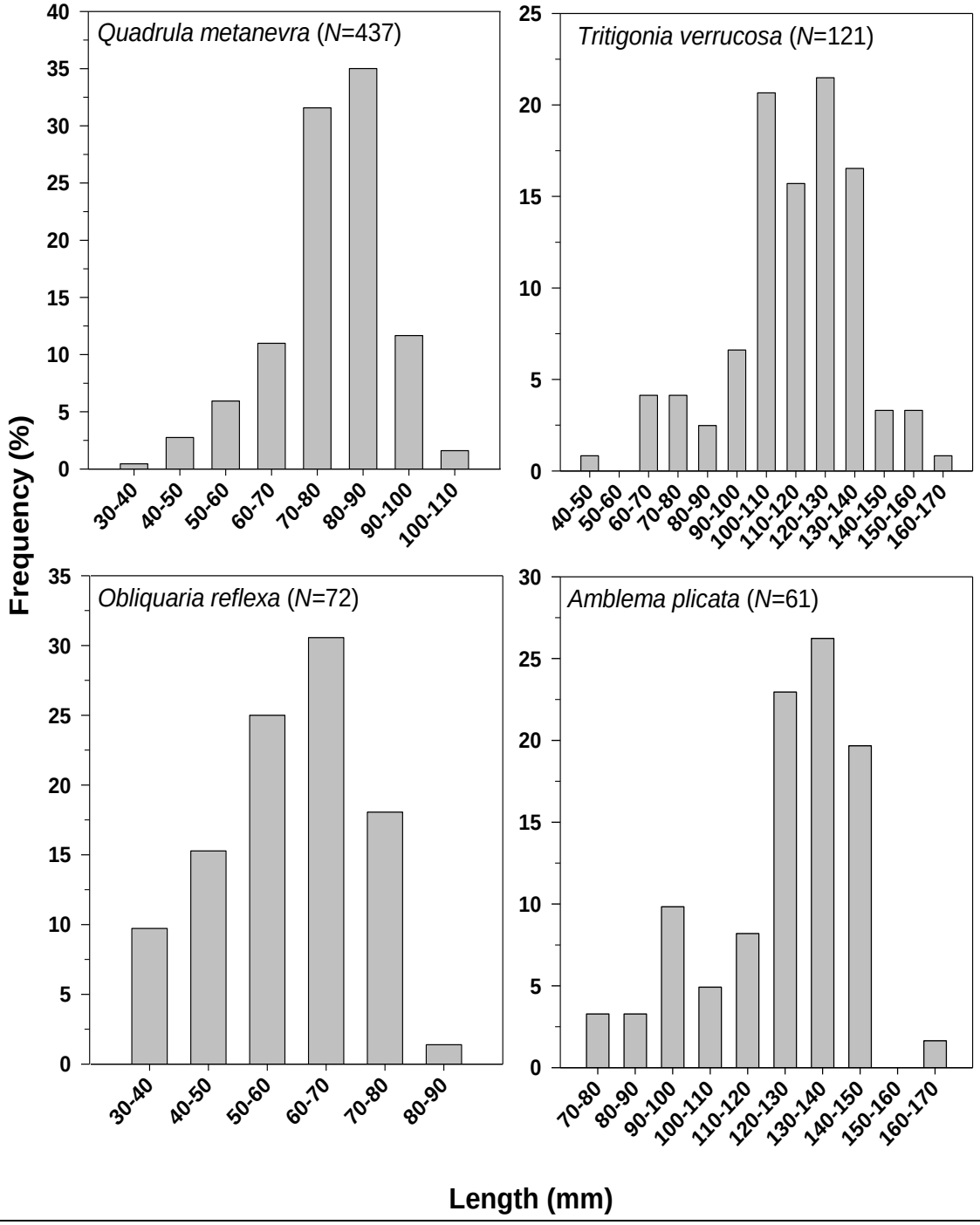

Figure 3.3. Length distributions for the 4 most abundant species in the Verdigris River, OK. N= number of observations for that species.

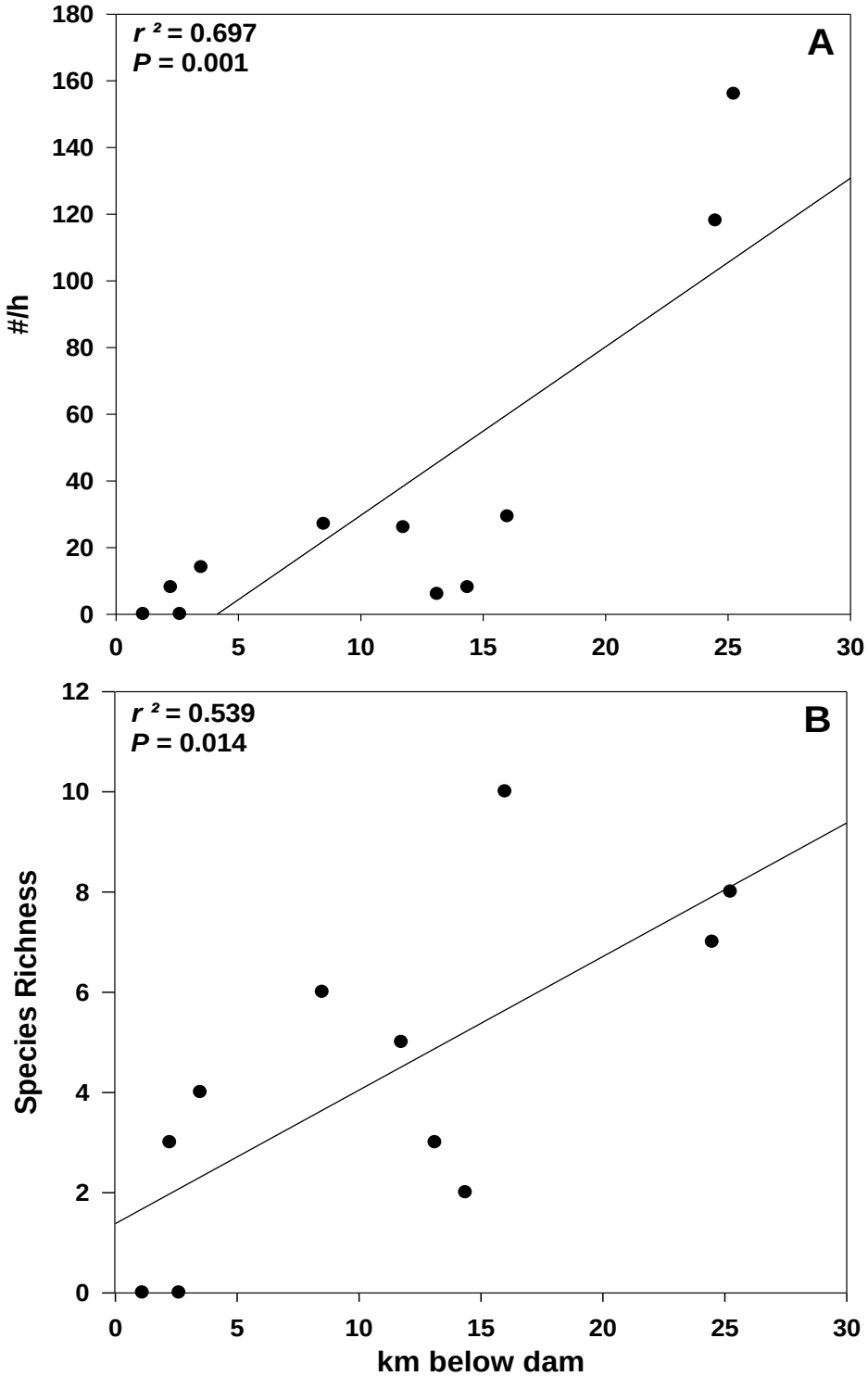

Figure 3.4. Distance from the dam vs. mussel abundance (MPH) (A) and richness (B) in Verdigris River, OK. for sites below Lake Oologah dam.

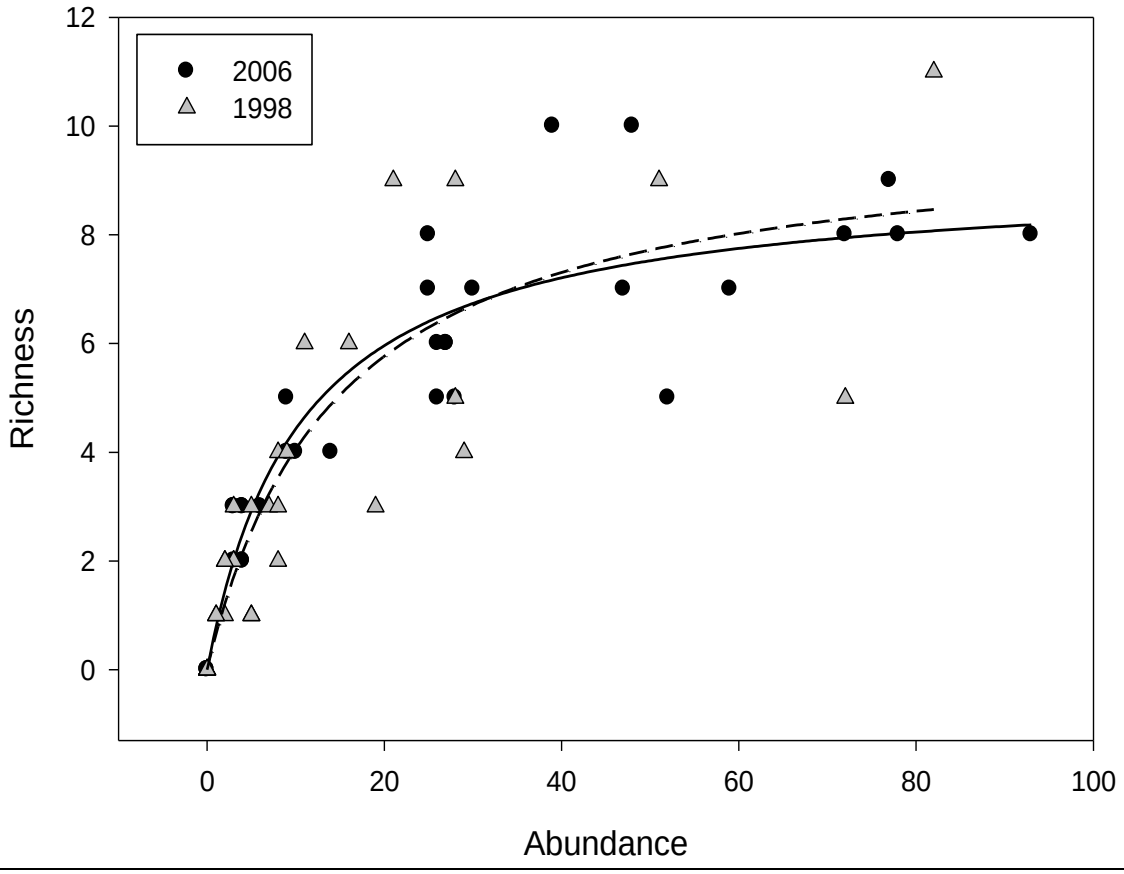

Figure 3.5. Abundance vs. richness curves for the Vaughn (1998) survey (dashed line) and the current survey (solid line). For statistical analysis, these curves were linearized by log-transforming abundance. The resulting regression equations were $y = 4.332x + 0.1135$ for the present study and $y = 4.284x + 0.0257$ for the study by Vaughn.

CHAPTER IV

ASSESSMENT OF ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) PHYSIOLOGICAL CONDITION IN OKLAHOMA RESERVOIRS I. CONDITION INDICES AND WHOLE-BODY GLYCOGEN LEVELS

ABSTRACT

It has been hypothesized that expansion of the range of zebra mussels (*Dreissena polymorpha*) may be limited by warm water temperatures in the southern United States, however few studies have addressed the physiological condition of the species in their natural environment. This study describes zebra mussel tissue water metrics and glycogen concentrations in a population located in north-central Oklahoma in a reservoir with elevated temperatures in part of the lake. A suite of tissue water metrics indicated mussels located at warm-water sites were in poorer physiological condition than those located at ambient temperature sites during the month of May, however few seasonal differences were noted. Glycogen concentrations varied significantly among seasons with the lowest concentrations observed during the summer months of July and August, however few site effects were noted. It was hypothesized tissue water metrics may be more appropriate for short term assessments (one month) of zebra mussel physiological

condition while direct evaluation of energy reserves may be better suited for seasonal characterization of body condition. While summer water temperatures in Oklahoma may serve as a natural environmental control of zebra mussel populations, it appears mussels may continue to expand further south.

INTRODUCTION

Zebra mussels (*Dreissena polymorpha*) have become one the most widespread and successful aquatic invasive species within North America (Ram and McMahon 1996). Since introduction, zebra mussels have extended their range in the United States southward with the first reported sighting in Oklahoma occurring in 1993 (Laney 2010). Within Oklahoma, their dispersal has been aided significantly by downstream transport of individuals through connected reservoirs (Bidwell 2010), however overland dispersal to isolated systems also has occurred. Currently, at least 10 different reservoirs in the state harbor populations of *D. polymorpha*. While most occur in the northeastern part of the state, one reservoir (Texoma Lake) on the Oklahoma-Texas border is also infested.

Reservoirs in Oklahoma are warm-monomictic during an average year, with no period of ice cover and a clinograde stratification during the warmest months. Systems in the northern part of the state have a mean annual temperature range of approximately 5 to 25˚C, while those in the southern part of the state have a mean range of 7 to 29˚C (OWRB 2007). Reservoirs across the state may have late summertime water temperatures that exceed 30˚C for several weeks and that may also be coupled with low

flow conditions. With reported upper thermal tolerances for zebra mussels ranging from 28 to 30˚C, (Aldridge et. al 1995, McMahon and Ussery 1995, McMahon et al. 1995), *D. polymorpha* populations in Oklahoma provide a unique opportunity to evaluate the physiological condition of organisms that may occur in systems where their thermal tolerances are regularly tested (Strayer 1991).

Monitoring of zebra mussel populations in Oklahoma has characterized summer dieoffs, particularly of older, reproductively mature mussels (Boeckman and Bidwell in review). Similar long term monitoring of zebra mussel populations in northeastern Poland noted large scale and rapid zebra mussel die-offs (Stanczykowska and Lewandowski 1993) that occurred in years following relatively high population densities, particularly in eutrophic systems. Additionally, Allen et al. (1999) demonstrated summer dieoffs of zebra mussels in the lower Mississippi River associated with high water temperatures. Sprung (1991, 1993) has shown zebra mussels can lose as much as 30% of their body weight after spawning. As such, these die-offs may be initiated by a combination of low energy reserves combined with high water temperatures following peak spawning events.

A limited number of studies have examined the biochemical effects of high temperature on zebra mussels and indicate that chronic exposure to water temperatures above 28˚C may increase metabolic demands above the energy gained through food resources (Aldridge et al. 1995, Stoeckmann and Garton 2001) and result in a decrease in overall body condition. A number of indices have been used to indicate body condition in

bivalves, including zebra mussels. For example, Smolders et al. (2002, 2004) utilized wet: dry weight ratio, tissue condition index, and tissue glycogen concentrations to characterize the negative effects of a wastewater effluent on zebra mussel body condition. Maintaining the correct ion composition and tissue water balance is an energetically-demanding requirement for freshwater organisms and measurements related to these variables may also be expected to respond during periods of physiological stress. Voets et al. (2006) demonstrated decreased energy reserves, and high tissue water content in zebra mussels from metal contaminated sites relative to reference locations. These same indices may be applied to better understand the changes in zebra mussel body condition as a result of summertime spawning events and increased water temperature and may be useful to indicate the onset of a die-off event.

We used multiple physiological condition indices and direct energy reserve determinations to assess zebra mussel physiological condition on a seasonal scale in an Oklahoma reservoir that is influenced by the thermal discharge of a coal-fired power plant. As water temperatures rise above 28˚C, it was hypothesized that energy stores (tissue glycogen levels) in the organisms would decrease, while tissue water content would increase due to a reduced capacity for osmoregulation. In addition to seasonal changes, spatial differences in these variables were also characterized in different zones of the reservoir that were influenced to varying degrees by the thermal discharge.

METHODOLOGY

Study area

Sooner Lake (Figure 4.1) is an impoundment of Greasy Creek, a tributary of the Arkansas River, and was constructed in 1976 to provide cooling water for a coal-fired electric generating facility stationed on the lake (OWRB 1990). Sooner Lake has a capacity of 149,000 acre-feet at normal pool with an average depth of 8.5 m and a maximum depth of 27 m (Angyal et al. 1987). Additionally, make-up water can be withdrawn from the Arkansas River with overflow returning to the river. The power plant on the lake releases a heated effluent which is directed from the discharge area to the main body of the lake by a series of dikes that facilitate cooling prior to the water being taken into the plant again via an intake channel. This creates a series of discrete temperature zones in the lake that range from 10-15˚C above ambient at the discharge to ambient in the main lake (Figure 4.2). Zebra mussels were first reported in Sooner Lake in 2006. While introduction due to boating/fishing activities is a possibility, the presence of established mussel populations in the Arkansas River above and below Sooner Lake (Bidwell 2010) suggest make-up water taken from the Arkansas River was the most likely source of mussels in the system.

Experimental design

Zebra mussels used in condition trials were collected from a boat dock on the southeast side of the lake, outside of the zone of warm-water influence. Individuals measuring approximately 8-10 mm were isolated by cutting the byssal threads with a scalpel and measured with digital calipers (Fisher Scientific Pittsburg, PA) to the nearest 0.01 mm

total length. Condition trials were conducted in modified polyethylene tackle boxes (10x20 cm) with 12 chambers per box. The top and bottom of each box was removed and replaced with a rigid plastic mesh (2x2 mm grids) to allow for water exchange. One zebra mussel was placed in each of the 12 chambers per box. Each box was then suspended approximately 1 m under buoys located both within and outside the warm-water effluent and remained in the lake for 5-9 weeks depending on season and temperature. At the conclusion of the condition trial, each box was collected and returned to the laboratory in coolers. The total length of individual mussels was again determined using digital calipers and 6 mussels were used for glycogen determination with the remaining 6 mussels used for soft tissue wet: dry weight ratios, tissue condition index (TCI), and percent water content.

Condition indices

All soft tissue was removed from each mussel by prying open the valves and using a small spatula to scrape tissue onto paper towels. Byssal threads were cut away from the tissue if needed. Soft tissue was then blotted dry using lint free Kimwipes® and placed in pre-dried and pre-weighed aluminum weigh pans. Wet weight of both soft tissue and shell material was recorded separately to the nearest 0.00001g using a Mettler Toledo AT261 analytical balance (Mettler Toledo, Columbia, MD) and weigh pans containing the tissues were placed in drying ovens at 60˚C for 24-48 hr. Tissue and shell dry weights were recorded using the same balance. Wet:dry weight ratios were calculated by dividing soft tissue wet weight by soft tissue dry weight. TCI was calculated by dividing

soft tissue dry weight by shell dry weight and percent water content was calculated by subtracting soft tissue wet weight from soft tissue dry weight, divided by soft tissue wet weight (Soto et al. 2000, Smolders et al. 2002).

Glycogen

D. polymorpha used in glycogen assays were treated similarly as those used for the condition indices, with the entire soft tissue mass removed as above, blotted dry, and wet weight recorded. Each tissue mass was then transferred to 2-mL screw-top cryogenic vials (Fisher Scientific, Pittsburg PA), and flash frozen in liquid nitrogen. Tissue samples were then transferred to an ultra cold freezer and stored at -80˚C until the glycogen assay was conducted. The procedure for determining tissue glycogen is described by Naimo et al. (1998) and Herod et al. (2001). In summary, tissue samples were thawed and 500 µL of 30% KOH (potassium hydroxide) was added to each vial. Vials were then heated in a 100˚C water bath for 20 min. Each vial was then vortexed for 30 seconds and placed on crushed ice for 5 min. Next, 750 µL of 95% ethyl alcohol (ETOH) was added and the vial was again vortexed for 5 s. Each vial was then placed back into the 100˚C water bath for another 15 min. The glycogen extraction, now complete, was analyzed immediately or frozen for no more than 1 month. Glycogen determinations were conducted using a Molecular Devices Spectra max 190 plate reader (Molecular Devices, Sunnyvale, CA), and analyzed in triplicate for absorbance at 490 nm, with appropriate standard curves, and matrix blanks (reagent grade water + KOH + ETOH). Standard curves were generated from serial dilution of a 2000 mg/L glycogen stock solution using

glycogen type VII (*Mytilus edulis*) (Sigma-Aldrich, Saint Louis MO). Each standard was subjected to the digestion procedure outlined above and final standard concentrations ranged from 0.5 to 8.3 µg glycogen in the well plate. Unknown samples exceeding these concentrations were diluted with reagent grade water until glycogen concentrations were brought to within range of the standard curve. Final glycogen concentrations were expressed as mg glycogen/g wet tissue weight.

Statistics

Condition indices and glycogen concentrations were analyzed for normality and heterogeneity of variance using the Shipro-Wilk and Levene procedures (Zar 1999) within SigmaPlot 11.0 (Systat Software Inc. San Jose, CA). Data not meeting parametric assumptions were natural log transformed and reanalyzed. If transformation did not alleviate violation of assumptions, original data were analyzed using a one-way ANOVA on ranks. All data meeting assumptions were analyzed using a one-way ANOVA for significant differences ($\alpha = 0.05$) both across time within a site, as well as within a time across sites. If significant differences were noted, a Holm-Sidak multiple comparison procedure was initiated with appropriate Bonferroni style α-level corrections. All statistical comparisons were conducted at $\alpha = 0.05$.

RESULTS

As shown in Figure 4.2, water temperature varied across sites with the warmest temperatures recorded at the discharge buoy of between 5 and 38˚C. Temperature at the end discharge ranged from 4 to 35˚C. Water temperatures at the east boat ramp, dam and intake buoy was not affected by the warm-water discharge and subsequently ranged from 0 to 32˚C.

For all four physiological metrics, data were analyzed for possible effects of location and associated temperature within a time period, as well as for the effect of season within one site. Differences among sites were limited, with significant differences only noted in May 2009 (Figure 4.3 P= 0.014) and May 2010 (Figure 4.3, P= 0.002). In May 2009, mussels suspended at the discharge buoy had significantly elevated wet: dry weight ratios over mussels at the end discharge, dam and intake buoy, while in May 2010 mussels deployed at the warm sites, (discharge bridge and end discharge) had significantly elevated wet: dry weight ratios compared with those at the east boat ramp and intake buoy locations. Seasonal differences within sites were also quite limited and did not necessarily follow a clear pattern. Wet: dry weight differed significantly among season within the discharge buoy (Figure 4.4, P= 0.013), end discharge (Figure 4.4, P= 0.04) and dam locations. At the dam location, *D. polymorpha* deployed during August 2008 had significantly greater wet: dry weight ratios than those in May 2009 (Figure 4.4, P< 0.001). Generally, the greatest wet: dry weight ratios were observed during the summer months, associated with the greatest water temperatures. Mussels deployed during summer and fall months, particularly at the discharge buoy, did not survive the trial period. These deaths were associated with water temperatures between 30 and 38˚C.

Deployment location had a significant effect on zebra mussel TCI in May 2009 (Figure 4.5), with mussels at the dam and intake buoy having greater TCI than those suspended at the discharge buoy and east boat ramp. Similarly, in May 2010 *D. polymorpha* deployed at the discharge buoy had significantly lower TCI than those at the end discharge, east boat ramp and intake buoy locations (Figure 4.5, P= 0.002). Zebra mussel tissue condition index did not differ among sites at any other time period. Tissue condition index differed significantly within the discharge buoy site across season with mussels in June 2008 having greater TCI than those in May 2010 (Figure 4.6, P= 0.005). Similarly mussels at the east boat ramp in July 2008 had greater TCI than mussels in September 2008 and May 2009 (Figure 4.6, P= 0.004). From 2008 to 2010, TCI decreased during summer months, potentially indicating a reduction in environmental resources, such as habitat or food quality or quantity.

Percent water content exhibited the least variability of all the physiological condition metrics evaluated. Only two site effects were noted, in May 2009, mussels at the discharge buoy had significantly greater water content than mussels exposed at the end discharge, dam and intake buoy (Figure 4.7, P= 0.005). Finally, in May 2010 mussels at the warm-water locations (discharge bridge and end discharge) had significantly greater water content than mussels at the east boat ramp and intake buoy locations (Figure 4.7, P< 0.001). The only seasonal effect occurred with mussels suspended at the dam in August 2008 having significantly greater water content than mussels in May 2009 (Figure

4.8, P= 0.025) suggesting *D. polymorpha* were in poorer physiological condition in August than in May of the following year.

Location had little influence on glycogen concentrations with the only significant difference noted in June 2008, when mussel tissue glycogen at the end discharge approached 5 mg/g wet weight, which was significantly lower than at any other site (Figure 4.9, P= 0.001). On a seasonal scale, glycogen concentration was the most sensitive metric used in this study with significant differences within all 5 sites. At the discharge buoy, glycogen levels peaked in June 2008 near 14 mg/g wet weight, which was significantly greater than the 3 mg/g wet weight observed in October 2008 (Figure 4.10, P= 0.007). At the end discharge, glycogen concentrations near 2 mg/g in September and October 2008 were significantly lower than during any other time period (Figure 4.10, P< 0.001). At the east boat ramp, glycogen was lowest during September and October 2008 and July 2010 (Figure 4.10). The greatest glycogen concentrations were observed in the summer of 2008 and spring of 2010. At the dam, glycogen stores were greater in 2008 than in July of 2009 (Figure 4.11, P= 0.001). Similarly, at the intake buoy, in September and October 2008 glycogen levels were near 2 mg/g wet weight, which was significantly lower than in June and July 2008, and May 2009 and 2010 (Figure 4.11, P< 0.001).

DISCUSSION

Established zebra mussel populations in Oklahoma provide a unique opportunity to characterize the physiological condition of this invasive species under conditions that may be stressful during certain times of the year. In particular, summertime water temperatures can reach above 30˚C which has traditionally been considered the upper thermal tolerance of *D. polymorpha*. In support of this hypothesis, Boeckman and Bidwell (in review), have noted summer dieoffs of zebra mussels in several Oklahoma reservoirs. Additionally, in native mussel surveys conducted during summer months below an infested reservoir, Boeckman and Bidwell (2008) described numerous unionid mussels with *D. polymorpha* byssal threads attached, however few live zebra mussel were present. The warm water temperatures experienced in Oklahoma may serve as a natural environmental population control on zebra mussels during some years and may mitigate their effects on species of special concern, particularly native mussels. As native mussels begin to experience sub-lethal stress at 35˚C (Spooner and Vaughn 2005), they have greater thermal tolerance than zebra mussels and can withstand these periods of high temperature. Given the altered thermal regime of Sooner Lake, OK it provided an opportunity to assess the seasonal physiological condition of zebra mussels across a broad spectrum of temperatures.

Physiological condition indices have been in use for some time, particularly in marine bivalves, as an ecophysiological tool to assess overall mussel health (Lucas and Beninger 1985). The metrics employed in this study were designed to assess the amount of water within soft tissues. As energy reserves are depleted within freshwater organisms, water content often increases within tissues (Wilkins 1967). As a result, high values for wet:

dry weight and percent water content are indicative of poor physiological condition, with low TCI numbers representative of poor condition. Patterson et al. (1999) demonstrated reduced glycogen concentrations in starved freshwater mussels as compared with fed mussels. While glycogen is the primary storage form of energy in most bivalves (Giese 1959), zebra mussels appear to utilize lipids in this regard (Sprung 1995), although glycogen is still used as a readily available form of energy in this species (Sprung 1995). For instance, Lauer and Spacie (2000) described significant declines in glycogen concentrations of sponge-covered *D. polymorpha* as compared with unfouled individuals. Similarly, Bidwell et al. (1995) showed significant reductions in whole-body glycogen concentrations of zebra mussels exposed to a surfactant based molluscicide. Therefore, glycogen concentrations can be reduced in physiologically stressed *D. polymorpha*.

Wet: dry weight ratios generally ranged between 6 and 9 throughout the study period. Similar values have been observed by Smolders et al. (2002, 2004) in their assessment of the effects of a wastewater effluent on zebra mussel condition. Fisher et al. (1993) reported a wet: dry weight value of 7.6 for non-exposed mussels which was not significantly different from Smolders et al. (2002) evaluations for mussels prior to deployment. While there appears to be some variability, wet: dry weight ratios in the 6-7 range may represent good condition for *D. polymorpha*, with elevated ratios of 10 representing stressed mussels (Smolders et al. 2002). Seasonal differences were noted at most sampling locations, although deployment location only appeared to influence wet: dry weight ratios in May 2009 and 2010 with significantly higher ratios at the warmer locations. In May 2009, temperature at the discharge buoy increased from 23 to 28˚C

while temperature at the intake buoy increased from 17 to 24˚C. In 2010, temperature increased more rapidly from 24 to 32˚C at the discharge buoy and 17 to 25˚C at the intake buoy. Aldridge et al. (1995) has shown mussels exposed to temperatures of 28˚C and above are unable to increase food consumption enough to meet the elevated physiological demands of dealing with those temperatures. Additionally, temperatures observed during May at the cool-water sites correspond to periods of maximum recorded growth in *D. polymorpha* (Karatayev et al. 2006). As such, the month of May represents a time when mussels housed at the warm-water sites appear physiologically stressed while those at the cool-water sites are subjected to more optimal growing conditions. These elevated temperatures at the warm-water sites relative to the cool water sites, may explain the poorer physiological condition observed in *D. polymorpha* suspended at warm-water locations.

Additionally, as peak reproduction is generally noted in May at these warm-water sites (Boeckman and Bidwell in review), the combination of reproductive activities and warm water temperatures may negatively affect zebra mussels at these locations. As Sprung (1991, 1993) has shown, female *D. polymorpha* can lose as much as 30% of their body weight post-reproduction which can significantly affect physiological condition (Stoeckmann and Garton 1997, 2001). Increased temperature also elevates respiration rate of zebra mussels, (Sprung 1991) causing an even larger demand for energy resources.

Zebra mussels often did not survive the deployment period at the warmest site, (discharge buoy) indicating mussels at this site were exposed to environmental conditions that exceeded their physiological limits. A more intensive sampling regime may have been necessary to detect changes in the condition indices prior to the mortality of these mussels. Generally, wet: dry weight values were greatest during the summer months of July and August and lowest during the winter and spring, again indicating zebra mussels appear to experience some reduction in physiological condition during the summer months for those same reasons as mentioned above.

Significant differences observed in zebra mussel TCI were nearly equal to differences observed with wet: dry weight, which was expected since the two metrics are influenced by tissue water content and mass. Significant seasonal differences occurred at the discharge buoy and east boat ramp, with deployment location effects noted in May 2009 and 2010. As with wet weight: dry weight the month of May appears to represent a time when mussels at the warm-water locations are in relatively poorer physiological condition than those at the cool-water sites. Again, the increased temperatures and physiological demands associated with that temperature increase may be driving the differences between zebra mussel TCI among sites.

TCI generally ranged from 0.04 to 0.12 which again is similar to those values reported by Smolders et al. (2002, 2004). *D. polymorpha* tissue condition index in 2010 tended to be lower than in 2008 and high temperatures at the warm-water sites caused decreases in

TCI. Smolders et al. (2002), citing data from their study and one other (Mersch et al. 1996), considered TCI may not be an appropriate metric for assessing zebra mussel condition due to gradual declines in TCI during both studies, which they attributed to food shortages or overall decreases in physiological condition due to winter environmental conditions. While no such gradual decline was noted in this study, the previously mentioned two studies assessed TCI over a 28 day to 4 month period and consisted of mussels from the same cohort. This 2.5 year study used multiple cohorts of *D. polymorpha* as zebra mussel life span in Sooner Lake was generally only 1 to 1.5 years (Boeckman and Bidwell in review) therefore, a similar decline in TCI may not have been detected.

Percent water content was the least variable condition metric employed, with significant effects of location only in May 2009 and 2010 and seasonal effects observed only at the dam. As with wet: dry weight ratio, greater water content occurred during summer months and at the warm-water sites, indicating warm temperatures observed in Oklahoma may have a negative effect on zebra mussel body condition for those same reasons outlined above. Increased physiological demands, and elevated energy requirements needed to supply those demands, appear to drive zebra mussels into a metabolic rate that cannot be supported for more than a few days. Some of these demands may be driven by the necessity for relatively stable ionic concentrations between freshwater organisms and their environment. Significant energy resources are devoted to maintaining this balance (Willmer et al. 2005), as slight deviations from this balance can have significant

physiological consequences. This may partially explain why little variance was observed in this metric.

Interestingly, wet: dry weight, TCI and % water content all showed significant differences among deployment locations during May 2009 and May 2010. In all cases, mussels deployed at the warm-water sites were in significantly poorer condition than those at the ambient temperature sites. For the reasons outlined above, the month of May appears to represent a unique dynamic in terms of zebra mussel physiological condition within Sooner Lake. The elevated temperatures within the discharge zone appear force zebra mussel metabolism into an unsustainable level, while at the cool water sites, zebra mussels appear to experience more optimal growing conditions. Field monitoring of adult *D. polymorpha* populations in the discharge channel also support this hypothesis, with die-offs occurring in this area shortly after the month of May (Boeckman and Bidwell in review).

Glycogen concentration exhibited the most variation on a seasonal scale, however effects of deployment location were only noted during June 2008. Glycogen concentrations ranged between 2 and 15 mg/g wet weight which is similar to those reported by Sprung (1995) and Lauer and Spacie (2000). Generally, glycogen reserves were lowest in the fall of 2008, and summer of 2009 and 2010, however mussels in the summer of 2008 tended to have greater glycogen concentrations then those assessed in all of 2009 and 2010. These annual variations may be due to differences in food quantity or quality among

these years or perhaps other environmental explanations such as water temperature or density of the zebra mussel population.

Karatayev et al. (2006) suggests annual differences in *D. polymorpha* growth rate may occur due to changes in water temperature or food resources. For instance, algal resources often peak in spring, and decline during the warmest water temperatures. Additionally, cyanobacterial blooms may also occur during the summer months, which may be impalatable to zebra mussels (Juhel 2006). These factors affecting growth rate would also negatively affect zebra mussel glycogen concentrations and physiological condition.

Interestingly, there was no effect of location/temperature during May 2009 and 2010 as was noted in the three other tissue water condition metrics that were evaluated. Considering glycogen is used as a form of stored energy available for physiological needs and the necessity for tissue water content to remain fairly constant within freshwater organisms, this result may be interpreted as counterintuitive. However, as mentioned above, while most bivalves utilize glycogen as their primary storage form of energy, zebra mussels appear to use lipids in this regard (Sprung 1995). Physiologically stressed *D. polymorpha* may preferentially deplete lipids and then utilize glycogen and protein sources for energy as lipids become depleted (Sprung 1995). Consequently, for short-term assessments, lipid analysis may be a more appropriate biochemical metric for future studies involving zebra mussels, while glycogen may be sufficient for questions involving more long-term studies.

Based on initial range projections for the zebra mussel (e.g. Strayer, 1991) Oklahoma represented the southern limit for colonization due to elevated temperatures that are characteristic of aquatic systems here. This would only be enhanced within Sooner Lake's altered thermal regime. Multiple metrics were employed in this study to characterize the physiological condition of zebra mussels both seasonally and among sites within these different thermal environments. Tissue-water based metrics indicated *D. polymorpha* deployed at warm water locations during the month of May were in poorer condition than those suspended at relatively cooler water sites. During the summer months, mussels suspended at these warm water locations did not survive the deployment period and no differences were detected among the other locations. Death of mussels at the cool water sites also occurred during July and August.

Population-based monitoring in Oklahoma systems has documented summertime die-offs of reproductively mature *D. polymorpha*, while young of the year mussels survive (Boeckman and Bidwell in review). The physiological condition metrics used in this study, appear to support the hypothesis that the combination of energy allocation for reproduction combined with warm water temperatures appear to act as a natural environmental control on adult zebra mussel populations in the state. However, selection for more thermally tolerant individuals within these populations is one important consideration for future evaluation.

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FIGURES

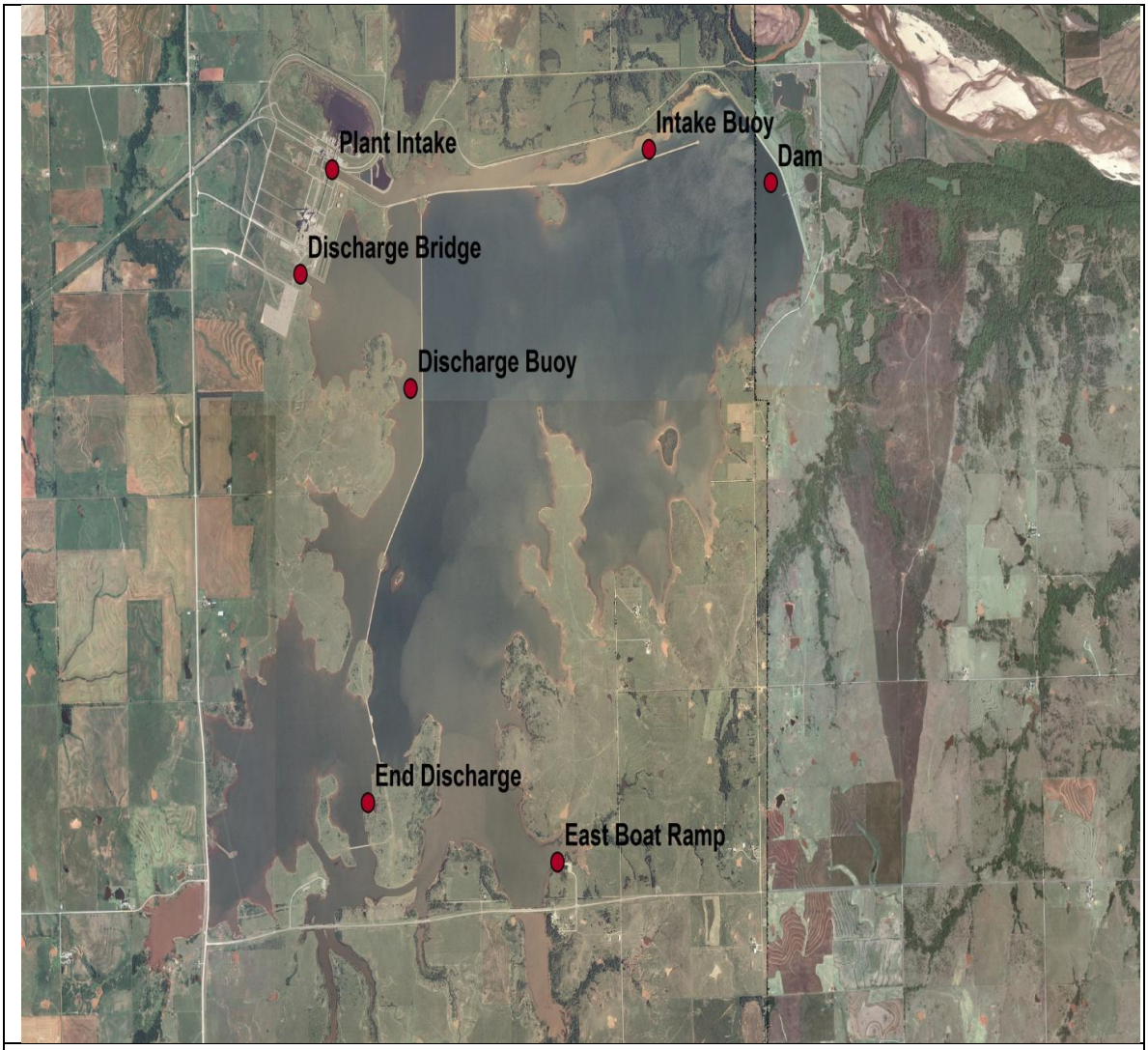

Figure 4.1. Image of Sooner Lake, OK, with selected sampling locations (National Agricultural Imagery Project, NAIP).

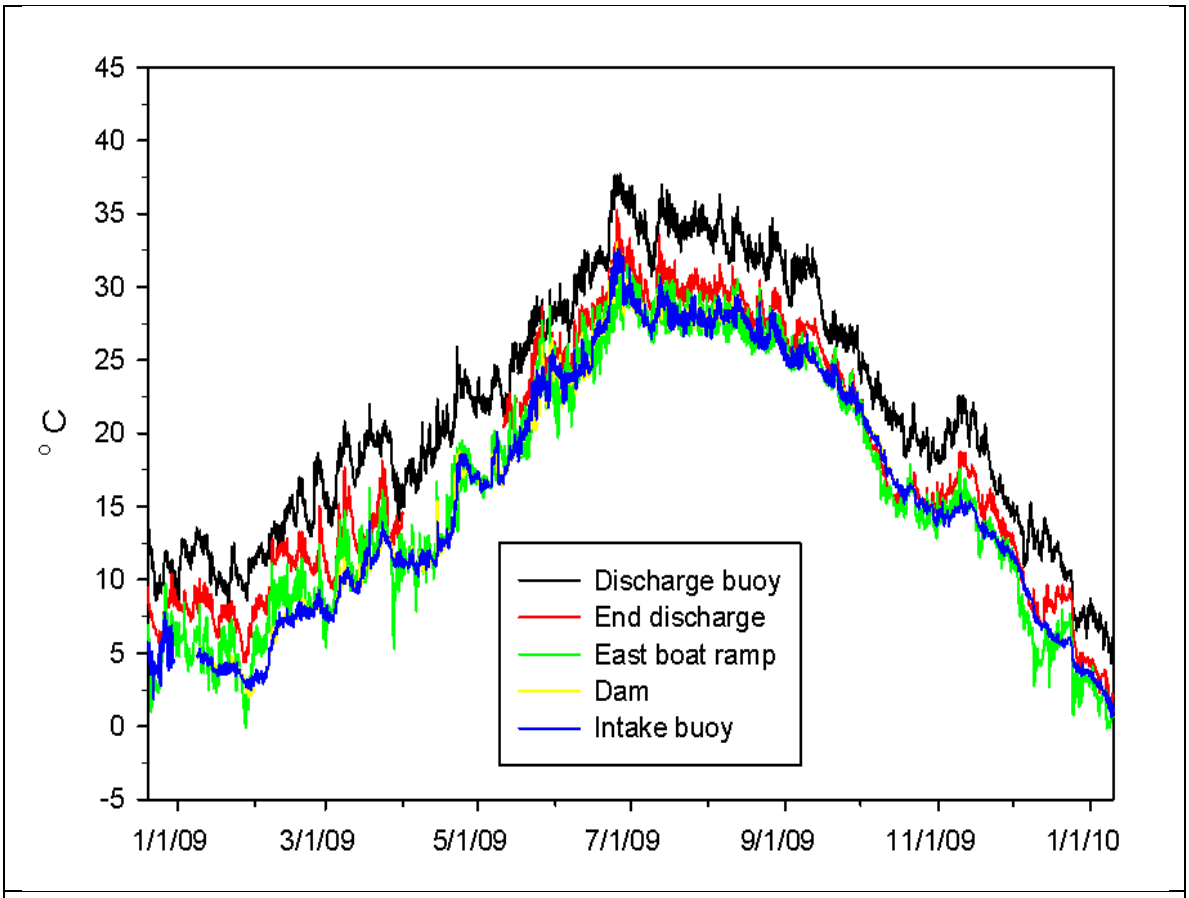

Figure 4.2. Hourly temperature recorded at 5 sites in Sooner Lake, OK, from January 2009 to January 2010.

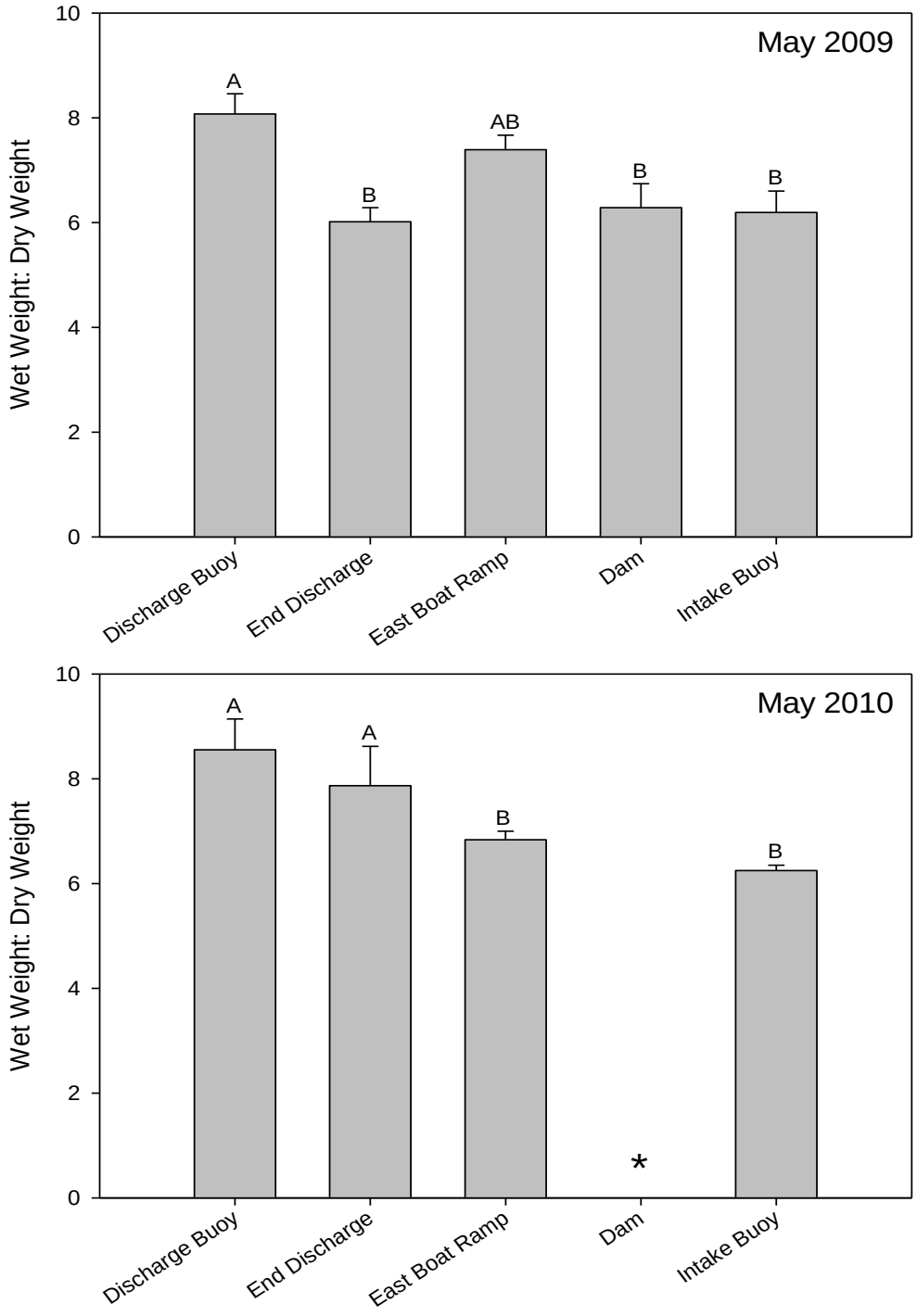

Figure 4.3. Mean wet: dry weight ratios for zebra mussels suspended at the various locations during May 2009 and 2010. * indicates death of mussels before end of trial. Error bars represent standard error of the mean. Bars sharing a common letter are not significantly different.

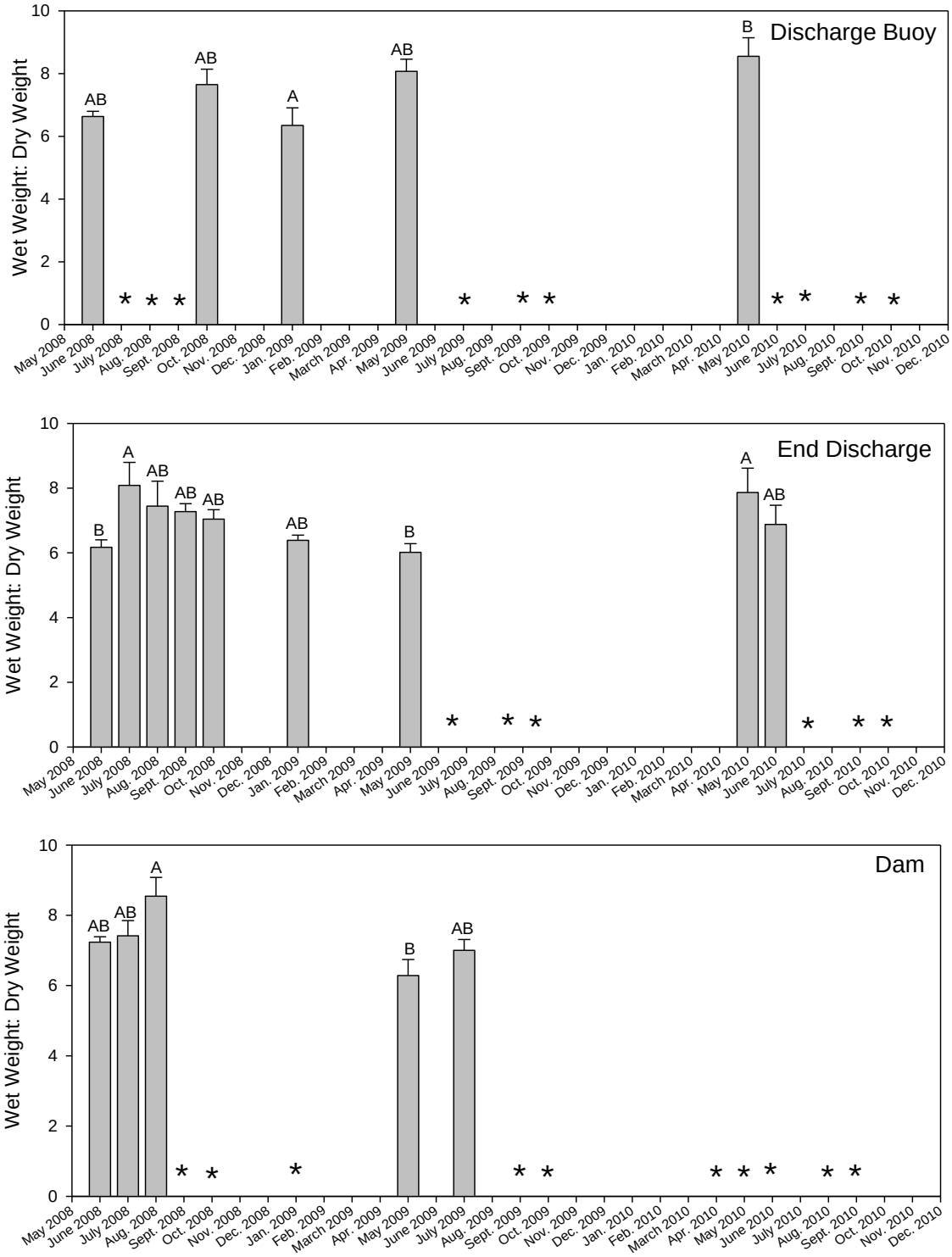

Figure 4.4. Mean wet: dry weight ratios for zebra mussels suspended at the Discharge Buoy, End Discharge and Dam locations. * indicates death of mussels before end of trial. Error bars represent standard error of the mean. Bars sharing a common letter are not significantly different.

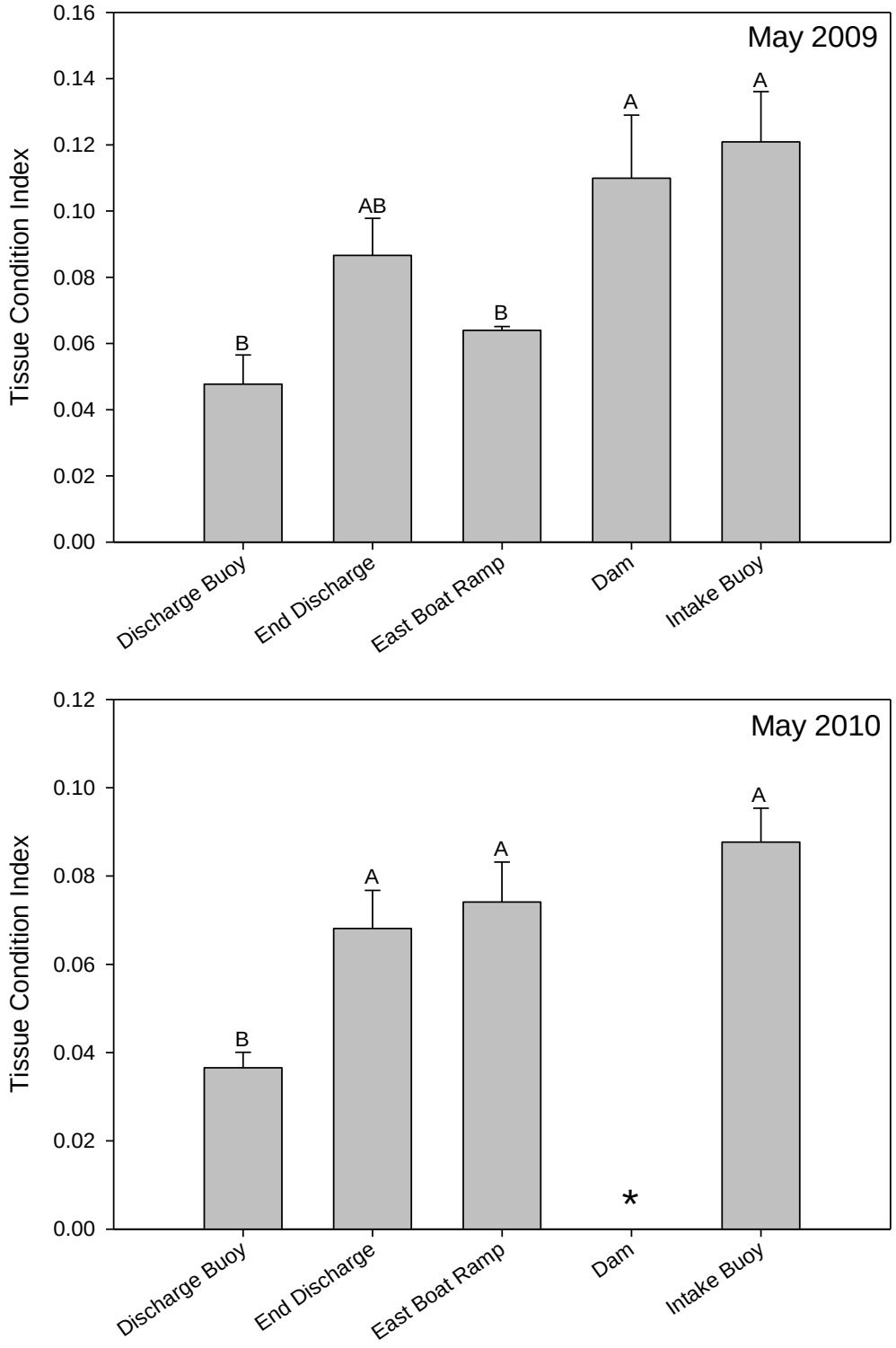

Figure 4.5. Mean tissue condition index for zebra mussels suspended at the various locations during May 2009 and 2010. * indicates death of mussels prior to end of trial. Error bars represent standard error of the mean. Bars sharing a common letter are not significantly different.

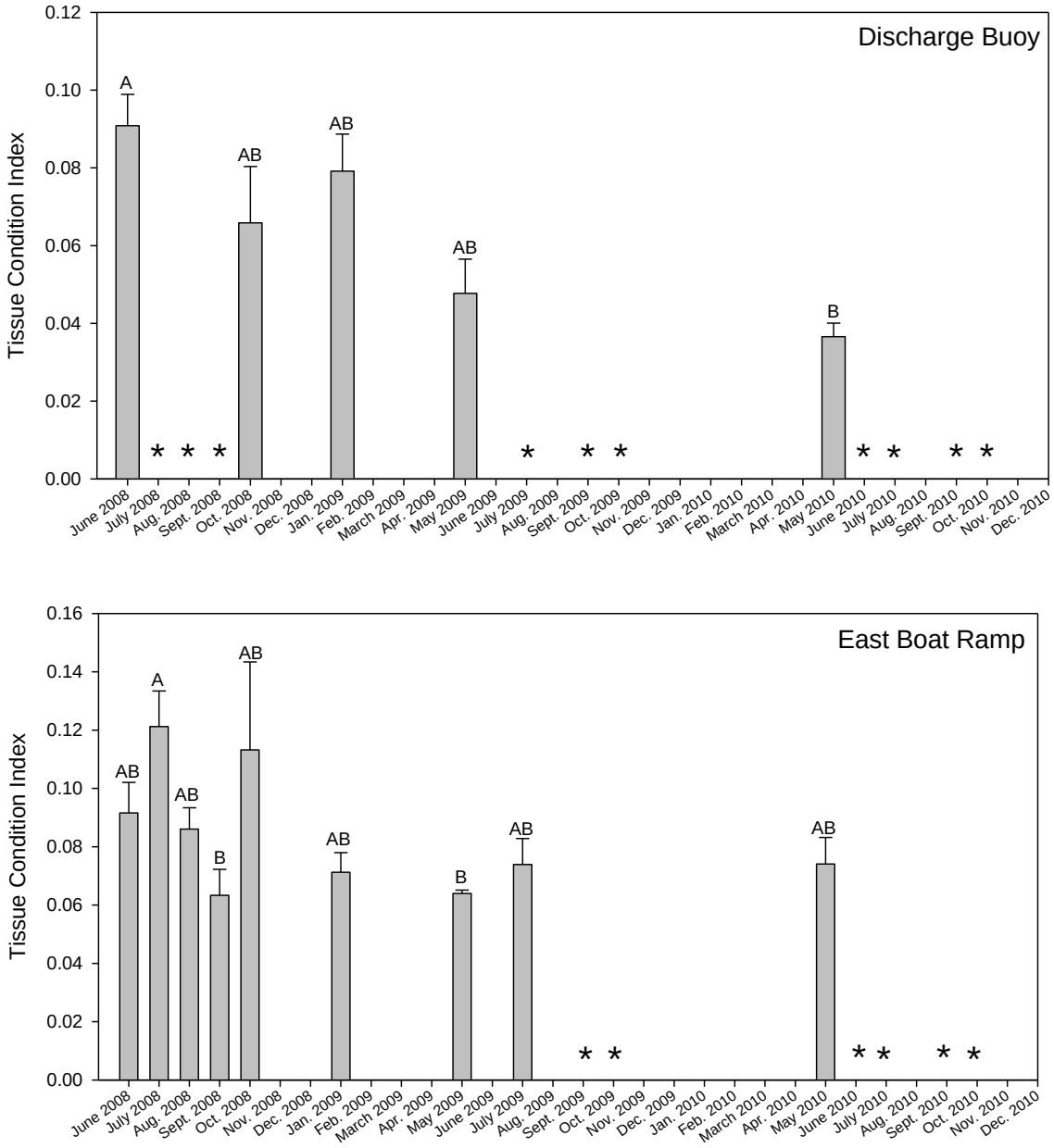

Figure 4.6. Mean tissue condition index for zebra mussels suspended at the Discharge Buoy and East Boat Ramp. * indicate death of mussels prior to end of trial. Error bars represent standard error of the mean. Bars sharing a common letter are not significantly different.

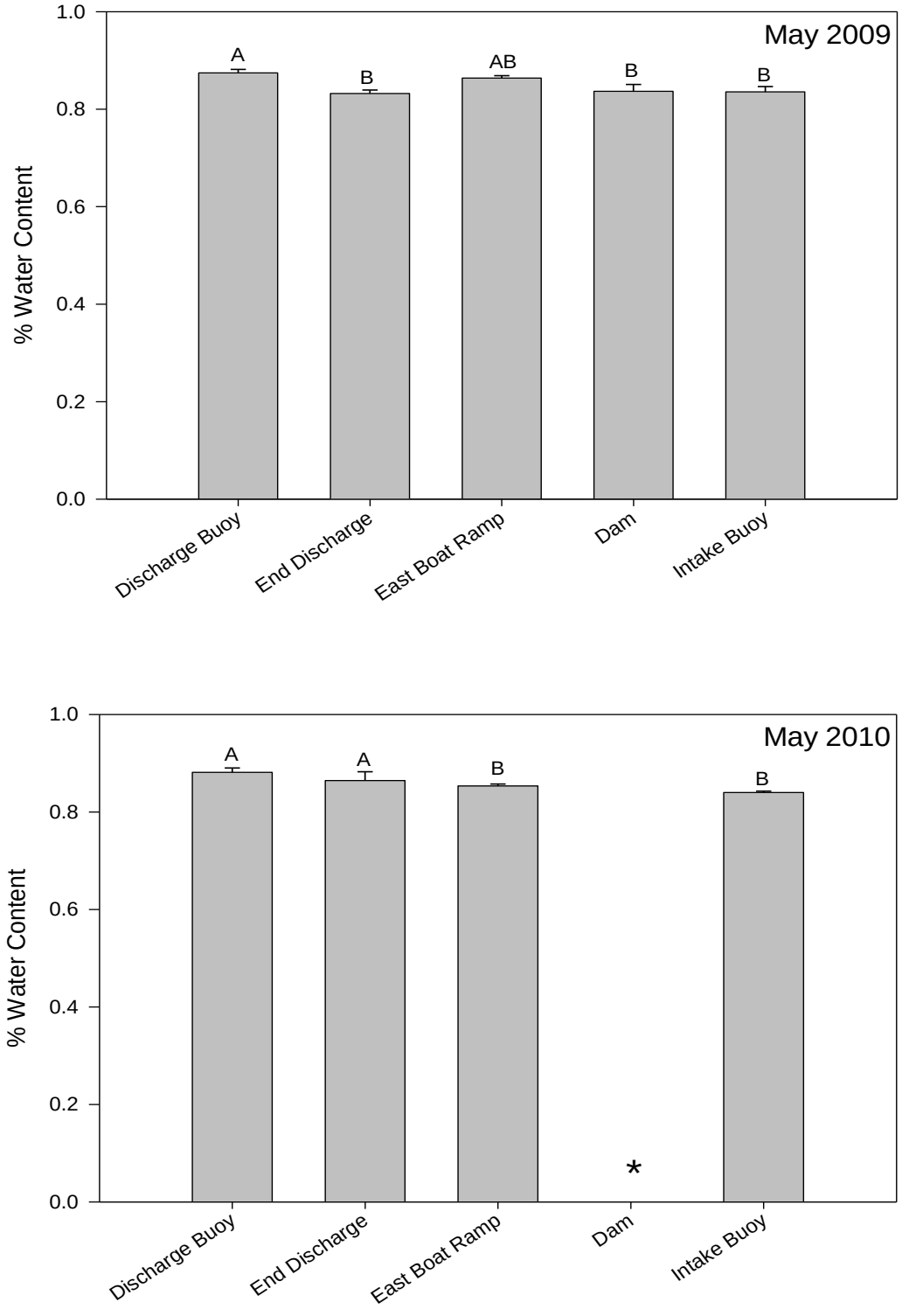

Figure 4.7. Mean percent water content for zebra mussels suspended at the various locations in May 2009 and 2010. * indicates death of mussels prior to end of trial. Error bars indicate standard error of the mean. Bars sharing a common letter are not significantly different.

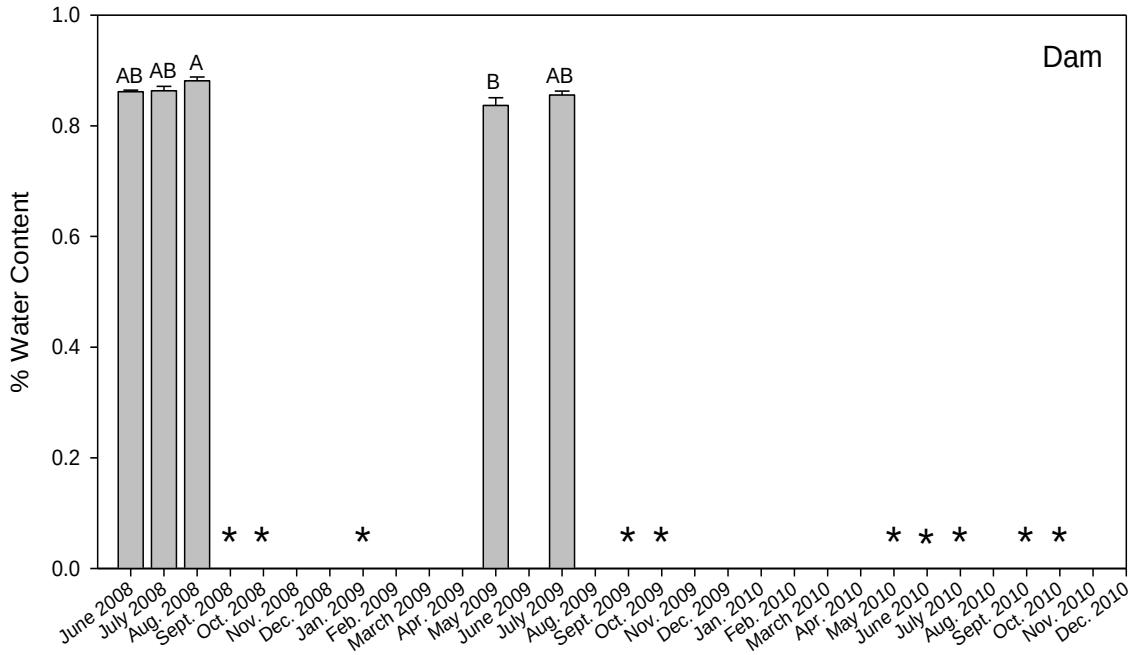

Figure 4.8. Mean percent water content for zebra mussels suspended at the Dam. * indicates death of mussels prior to end of trial. Error bars represent standard error of the mean. Bars sharing a common letter are not significantly different.

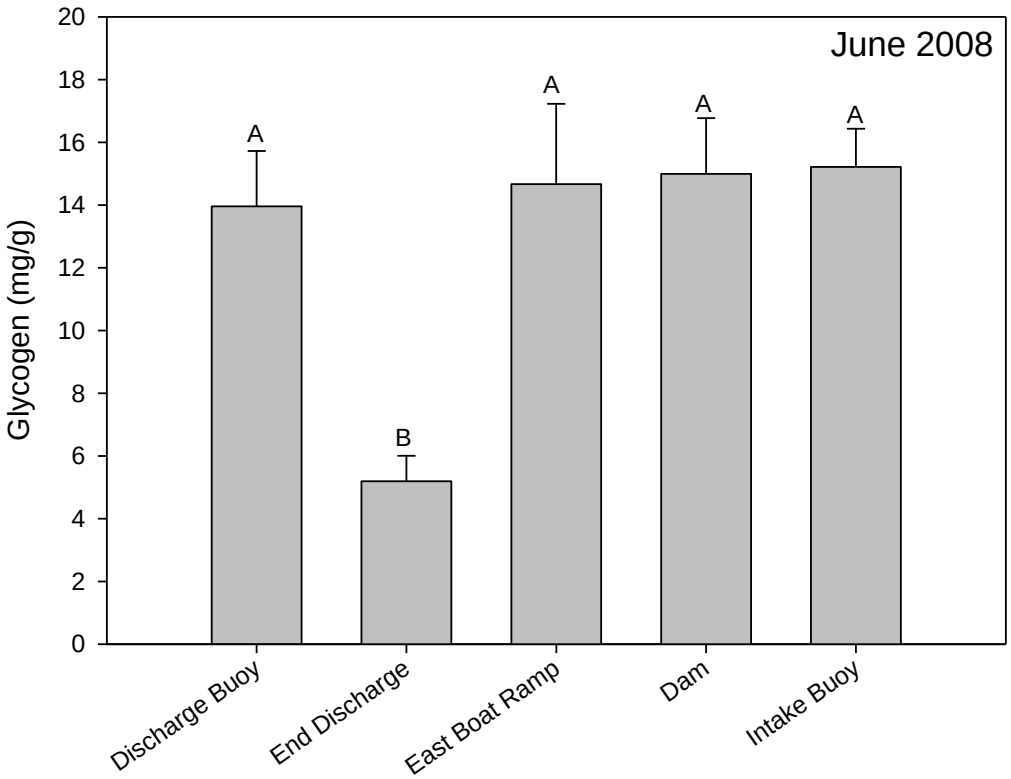

Figure 4.9. Mean glycogen concentrations for zebra mussels suspended at the various locations in June 2008. Error bars represent standard error of the mean. Bars sharing a common letter are not significantly different.

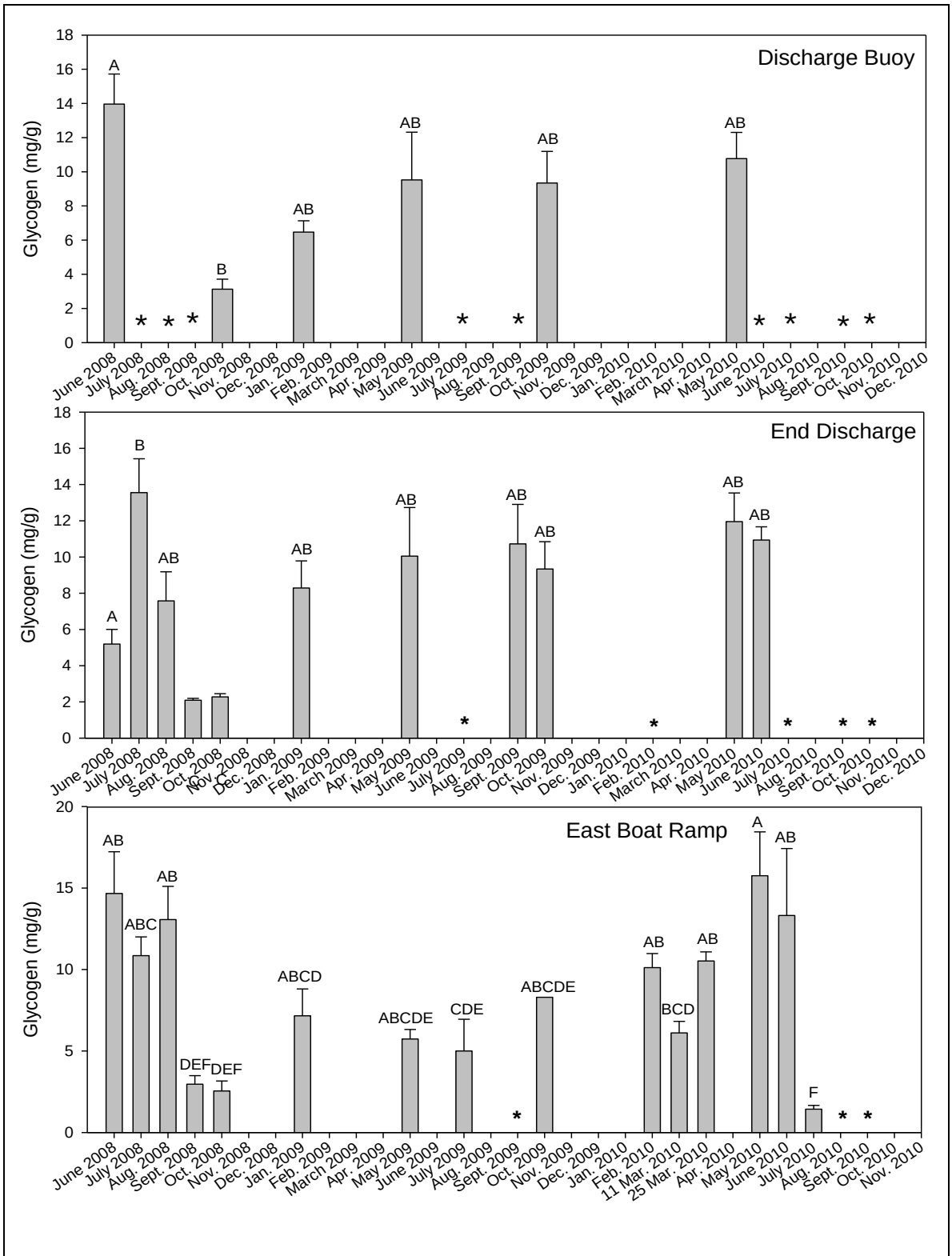

Figure 4.10. Mean glycogen concentrations for zebra mussels suspended at the Discharge Buoy, End Discharge and East Boat Ramp locations. * indicates death of mussels prior to end of trial. Error bars indicate standard error of the mean. Bars sharing a common letter are not significantly different.

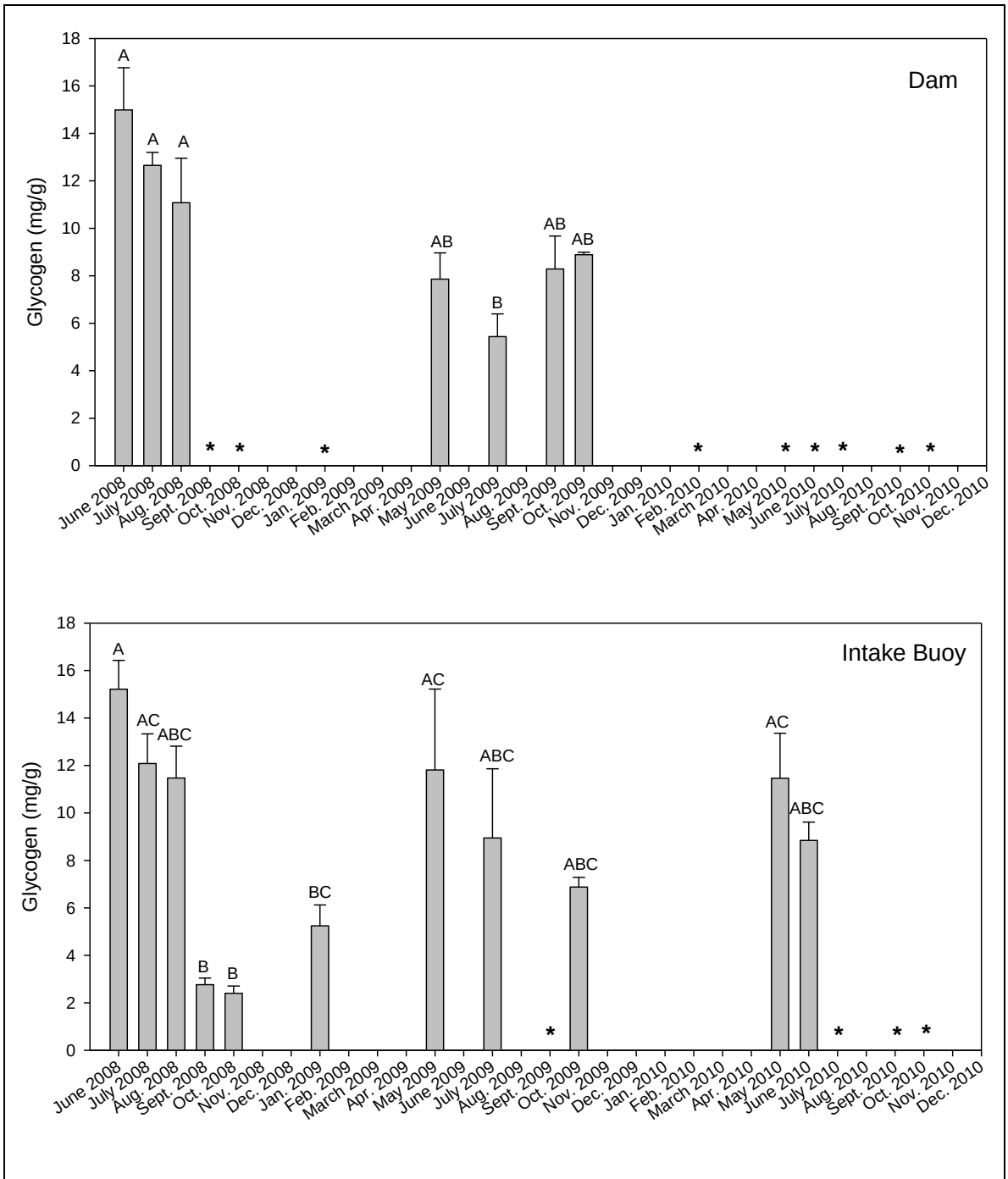

Figure 4.11. Mean glycogen concentrations for zebra mussels suspended at the Dam and Intake Buoy locations. * indicates death of mussels prior to end of trial. Error bars represent standard error of the mean. Bars sharing a common letter are not significantly different.

CHAPTER V

ASSESSEMENT OF ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) PHYSIOLOGICAL CONDITION IN OKLAHOMA RESERVOIRS II. THERMAL TOLERANCE AND PHYSIOLOGICAL RESPONSES

ABSTRACT

It has been hypothesized that zebra mussel (*Dreissena polymorpha*) distributions within the United States may be limited by warm water temperatures in southern latitudes, however few studies have addressed mussel thermal tolerance and physiological condition of southern populations. This study describes upper thermal tolerance, oxygen consumption and ammonia excretion for zebra mussel populations located near their hypothesized southern limits. Additionally, one mussel population assessed in this study was located in a reservoir that receives a warm-water discharge and consequently has an altered thermal regime, exposing mussels to artificially high temperatures. There was no difference in thermal tolerance of mussels derived from this warm water reservoir as compared with other populations in the region. However, as a group zebra mussels exposed to 34˚C in this study had greater time to death than published values of mussels

from Buffalo, NY. These effects were attributed to differences in laboratory handling time between studies or temperature acclimation within mussels used in this study. Oxygen consumption and ammonia excretion concentrations determined in this study were also greater than literature published values. O:N ratios generated in this study indicated protein catabolism was the primary source for metabolism at temperatures of 28˚C and above, in agreement with more northern populations. Consequently, zebra mussel populations used in this study, as of yet, do not appear to have any enhanced upper thermal tolerance as compared with more northern populations and therefore, warm water temperatures may continue to influence mussel populations within southern North America.

INTRODUCTION

Since the discovery of zebra mussels (*Dreissena polymorpha*) in North America (Hebert et al. 1989), studies have attempted to estimate their potential distribution (Strayer 1991, Buch and McMahon 2001, Drake and Bossenbroek 2004). Some of the focus of this work has been on regional water chemistry parameters and others on thermal tolerance of *D. polymorpha* (Aldridge et. al 1995, McMahon and Ussery 1995, McMahon et al. 1995). For example, early range predictions considered the Oklahoma-Texas border to be the extent of the southern distribution of *D. polymorpha* in the United States (Strayer 1991), although this study relied on temperature data from the European distribution of zebra mussels. Since then, thermal tolerance experiments conducted with North American *D. polymorpha* have demonstrated zebra mussels in the United States are more tolerant of

warm temperatures than are European populations (e.g. Hernandez et al. 1995, McMahon and Ussery 1995, McMahon et al. 1995) which could indicate the species is capable of colonization further south than initially reported by Strayer (1991).

The issue of selection for enhanced upper thermal tolerance of zebra mussels has been raised in several studies (Hernandez et al. 1995, McMahon et al. 1995, Elderkin and Klerks 2005). Enhanced upper thermal tolerances may allow zebra mussels to spread further south to even warmer climates. To date, the result of these studies has been equivocal with some studies indicating enhanced tolerance (eg. Elderkin and Klerks 2005) and others not supporting this idea (Hernandez et al 1995, McMahon et al. 1995). Zebra mussels in Oklahoma may be exposed to stressful conditions during summer months since reservoirs across the state may have late summertime water temperatures that exceed 30˚C for several weeks, often under low flow conditions (OWRB 2007). Consequently, these populations provide a unique opportunity to assess the effects of these high temperatures on thermal tolerance and physiological condition. Additionally, field monitoring of zebra mussel populations in Oklahoma have described repeated die-offs associated with warm water temperatures in several reservoirs (Boeckman and Bidwell in review), which provides support for the idea that zebra mussels experience significant physiological stress in Oklahoma. These data suggest that temperature may in fact provide an important environmental control on zebra mussel population densities in Oklahoma and may serve to alleviate some of the negative ecological and industrial impacts associated with their presence in more northern systems.

As zebra mussels are exposed to elevated temperatures, oxygen consumption and filtration rate increase and energy reserves are mobilized to help meet the increasing physiological demands (Aldridge et al. 1995). Lipids and glycogen appear to be utilized first, followed by protein sources as a last resort (Sprung 1995). With increased reliance on protein substrates, nitrogen is released, primarily in the form of ammonia. Evaluation of oxygen consumption and ammonia excretion rates allows for calculation of O:N ratios which can highlight the relative proportion of substrates used in metabolism (Mayzaud and Conover 1988, Quigley et al. 1993). For example Mayzaud and Conover (1988) suggest O:N ratios greater than 60 indicate high lipid use, with values less than 50 indicative of protein catabolism, and less optimal environmental conditions. Quigley et al. (1993) describe reductions in O:N ratios for *D. polymorpha* collected from Lake St. Claire during August, implicating greater reliance on protein derived energy sources during the warmest months. Similarly, Aldridge et al. (1995) demonstrated *D. polymorpha* acclimated to 28 and 32˚C exhibited O:N ratios below 40, which indicated a reliance on protein catabolism to meet physiological demands, which is unsustainable at these temperatures.

This study evaluated the upper thermal tolerance and oxygen consumption/ammonia excretion of *D. polymorpha* from several populations in Oklahoma and Kansas. Given *D. polymorpha* often occur in very high densities and in aggregated clumps (Boeckman and Bidwell in review), two exposure methods were used for the thermal tolerance assays.

Burks et al. (2002) have shown nitrate concentrations to be significantly greater at the base of aggregations of zebra mussels (druses) and dissolved oxygen is also reduced in this microhabitat. Therefore, thermal tolerance assays were conducted on isolated, individual mussels as well as on clumps of mussels to evaluate the potential interactive effects of declining water quality and high temperatures and the effect that may have on the biological response to those temperatures.

D. polymorpha used in this study were collected from several locations from Oklahoma, Kansas and Texas. Sooner Lake, OK, is a reservoir constructed to provide cooling water for a coal-fired power plant, and consequently has elevated temperatures and a temperature gradient in part of the lake. Texoma Lake, OK-TX, is located on the Oklahoma-Texas border and represents the southern-most distribution of zebra mussels within the state of Oklahoma. Thermal tolerance and physiological condition of *D. polymorpha* from these two reservoirs were compared with the response of zebra mussels from a more northern source Cheney Lake, KS.

Evaluations into zebra mussel physiological condition were made assessing glycogen concentrations on individuals chronically exposed to temperatures above 28˚C within Sooner Lake. Additionally, oxygen consumption and ammonia excretion was characterized for mussels from Texoma and Cheney Lakes at four different temperatures ranging from 20 to 32˚C. Results from this experiment were used to determine if zebra mussels from Texoma Lake had an enhanced ability to tolerate these high temperatures

relative to the Cheney Lake population, given the relatively more southern location and warmer water temperatures of Texoma Lake.

METHODS

Study area

Sooner Lake is an impoundment of Greasy Creek, a tributary of the Arkansas River, and was constructed in 1976 to provide cooling water for a coal-fired electricity generating facility stationed on the lake (OWRB 1990). Sooner Lake has a capacity of 149,000 acre-feet at normal pool with an average depth of 8.5 m and a maximum depth of 27 m (Angyal et al. 1987). Additionally, make-up water can be withdrawn from the Arkansas River with overflow returning to the river. The power plant on the lake releases a heated effluent which is directed from the discharge area to the main body of the lake by a series of dikes that facilitate cooling prior to the water being taken into the plant again via an intake channel. This creates a series of discrete temperature zones in the lake that range from 10-15˚C above ambient at the discharge to ambient in the main lake. Water temperatures in the discharge channel ranged from 5 to 38˚C in 2010 with a range of 0 to 32˚C outside the zone of influence. Water temperatures outside the discharge channel remained at 30˚C from mid-July to mid-August. Zebra mussels were first reported in Sooner Lake in 2006. While introduction due to boating/fishing activities is a possibility, the presence of established mussel populations in the Arkansas River above and below Sooner Lake (Bidwell 2010) suggest make-up water taken from the Arkansas River was the most likely source of mussels in the system.

Lake Texoma, OK-TX is a 2.6 million acre-ft. impoundment of the Red and Washita Rivers in southern Oklahoma. Zebra mussels were discovered in the lake in April 2009, the result of an overland transportation event as no upstream source of mussels existed at the time. In monitoring of five sites during 2010, Texoma Lake, water temperature at one meter depth ranged from a low of 5˚C in January to a high of 35˚C in July. Water temperatures remained above 30˚C from mid-July until the end of August (Boeckman and Bidwell 2010). In contrast, in the same year at Cheney Lake, KS, water temperature at one meter depth ranged from 0 in January to 31˚C in July, and temperatures persisted above 30˚C for less than one week (Boeckman and Bidwell unpublished data). Zebra mussels were discovered in Cheney Lake in 2007 which is a 150,000 acre-ft impoundment on the north fork of the Ninnescah River.

Upper thermal tolerance

D. polymorpha used in thermal tolerance assays were collected in Sooner Lake from just outside the influence of the heated water discharge. Mussels were also collected from Kaw Lake, OK, and Cheney Lake, near Wichita, KS, for comparison. One additional collection occurred Texoma Lake, OK-TX, for a single comparison experiment between the Cheney, Sooner and Texoma populations. *D. polymorpha* were transported back to the laboratory in 500 mL polyethylene containers placed in coolers. Ten mussels from each population were used to obtain initial glycogen or wet: dry weight ratios to determine initial physiological condition. In the laboratory, mussels were isolated by

cutting the byssal threads with a scapel and 10 individuals were placed into each of five replicate 500 mL glass beakers containing moderately-hard reconstituted water (MHW) (USEPA 2002). Additionally, clumps (druses) of mussels were also collected and a single clump from each population containing approximately 50 mussels was placed into a 1 L glass beaker, also containing MHW. Each beaker was continuously aerated using aquarium pumps and air stones. All beakers were placed into an environmental chamber (Percival Scientific, Perry, IA) and housed at the ambient lake temperature and light/dark ratio for four days to allow mussels to attach inside the beaker. On day five, the temperature was increased 1˚C every 10 minutes until 34˚C was achieved (Hernandez et al. 1995). After reaching 34˚C, mussels were assessed for survival by gently tapping the valves with a blunt probe. Survival assessments were conducted hourly for the first 10 hours and every 6 hours thereafter. Mussels that did not respond by closing the valves were considered dead, removed from the beaker and total length and time-to-death was recorded. Mussels exposed in a druse however, were not removed as the objective here was to assess the effect of expired mussels on water quality. Time-to-death was recorded for the druse when all mussels had expired. At the end of each bioassay, a 100-mL water sample was collected from the druse beaker and one beaker containing 10 individuals for ammonia, nitrite, and dissolved oxygen determinations.

Transplant study

To determine what effect a long-term exposure to elevated temperatures has on zebra mussel glycogen levels under natural conditions in the field, a transplant study was

initiated within the discharge zone at Sooner Lake in June 2010. In order to ensure the mussels used in this experiment were not acclimated to warmer temperatures, *D. polymorpha* were collected from Cheney Lake, KS, and from the ambient temperature zone at Sooner Lake. Ten mussels from each population were used for initial glycogen determination. Groups of 100 mussels were placed in wire mesh cages and suspended 1 m below the surface at one of four locations, discharge buoy, mid-discharge, end discharge, or intake buoy. Mussels suspended at the intake buoy served as a control as water temperature at this site is not affected by the warm-water discharge. Approximately every 10 days, 10 mussels from each population and from each location were collected and returned to the laboratory for glycogen analysis. To determine glycogen concentration, the entire soft tissue mass was removed with a small spatula, blotted dry, and wet weight recorded. Each tissue mass was then transferred to 2-mL screw-top cryogenic vials (Fisher Scientific, Pittsburg PA), and flash frozen in liquid nitrogen. Tissue samples were then transferred to an ultra cold freezer and stored at -80˚C until the glycogen assay was conducted.

The procedure for determining tissue glycogen is described by Naimo et al. (1998) and Herod et al. (2001). In summary, tissue samples were thawed and 500 µL of 30% KOH (potassium hydroxide) was added to each vial. Vials were then heated in a 100˚C water bath for 20 min. Each vial was then vortexed for 30 seconds and placed on crushed ice for 5 min. Next, 750 µL of 95% ethyl alcohol (ETOH) was added and the vial was again vortexed for 5 s. Each vial was then placed back into the 100˚C water bath for another 15 min. The glycogen extraction, now complete, was analyzed immediately or frozen for no

more than one month. Glycogen determinations were conducted using a Molecular Devices Spectra max 190 plate reader (Molecular Devices, Sunnyvale, CA), and analyzed in triplicate for absorbance at 490 nm, with appropriate standard curves, and matrix blanks (reagent grade water + KOH + ETOH). Standard curves were constructed from serial dilution of a 2000 mg/L glycogen stock solution using glycogen type VII (*Mytilus edulis*) (Sigma-Aldrich, Saint Louis MO). Each standard was subjected to the digestion procedure outlined above and final standard concentrations ranged from 0.5 to 8.3 µg glycogen in the well plate. Unknown samples exceeding these concentrations were diluted with reagent grade water until glycogen concentrations were brought to within range of the standard curve. Final glycogen concentrations were expressed as mg glycogen/g wet tissue weight.

Oxygen consumption/ammonia excretion

Methodology for the oxygen consumption/ammonia excretion experiment largely followed that described by Aldridge et al. (1995), with a few exceptions as noted below. *D. polymorpha* were collected from Cheney Lake, KS, and Texoma Lake, OK-TX, in September 2010. Zebra mussels were not collected from Sooner Lake for this experiment since densities there were not sufficient to acquire enough organisms. Mussels were transported back to the laboratory in clean 500 mL polyethylene containers placed in coolers. Upon arrival at the laboratory, mussels from each population were sorted into groups of 50 organisms and placed into one of four 40-L aquaria (8 aquaria total) containing de-chlorinated tap water from Oklahoma State University and maintained at

20˚C for one week. Aquaria were continuously aerated and filtered with small aquarium filters containing activated carbon. Light-dark cycles were maintained at ambient conditions. On a daily basis, mussels were fed a slurry of dried *Chlorella sp.* (SunChlorella, Los Angeles, CA). that had been ground and rehydrated in reagent grade water, at a concentration of 3.2 g *Chlorella sp.* for every 1000 organisms over 10 mm total length (Nichols 1993).

After the initial one-week acclimation period, each aquarium was assigned to one of four target temperatures, 20, 24, 28, or 32˚C. Water temperature was then raised 2˚C per day using aquarium heaters, until target temperatures were achieved. Once the target temperatures in all aquaria had been achieved, they were maintained for 30 days. On day 31, four replicates of five organisms per tank were transferred to 300 mL biological oxygen demand (BOD) bottles filled with water from each tank. Bottles were housed within each tank for 24 h to allow mussels to attach inside. To remove *Chlorella sp.* from within each bottle, after 24 h, bottles were removed from the tanks and the water within each was replaced with clean dechlorinated tap water, at a temperature that matched acclimation temperatures. Initial dissolved oxygen concentrations were recorded using a YSI 5000 dissolved oxygen meter and a YSI 5010 BOD probe (Yellow Springs Instruments Inc., Yellow Springs, OH). Bottles were then transferred to environmental chambers set at each acclimation temperature and incubated for 5 h after which the bottles were removed and dissolved oxygen was again determined. One BOD bottle, filled with water but containing no zebra mussels, served as a blank for each temperature and each population (8 total controls). Oxygen loss in these blank bottles

was subtracted from the observed oxygen consumption in each experimental bottle. After assessing oxygen consumption, a 50 mL water sample was collected from each bottle for determination of total ammonia concentration using an ion-specific probe and an Accumet AR-25 ammonia meter (Fisher Scientific Pittsburg, PA). Again, ammonia concentrations observed in each blank bottle were subtracted from the experimental bottles for each temperature and population.

After determination of oxygen consumption and ammonia excretion, zebra mussels were removed from the BOD bottles and analyzed for total length and wet: dry weight ratios. All soft tissue was removed from each mussel by prying open the valves and using a small spatula to scrape tissue onto paper towels. Byssal threads were cut away from the tissue if needed. Soft tissue was then blotted dry using lint free Kimwipes® and placed in pre-dried and pre-weighed aluminum weigh pans. Wet weight of both soft tissue and shell material was recorded separately to the nearest 0.00001g using a Mettler Toledo AT261 analytical balance (Columbia, MD) and weigh pans containing the tissues were placed in drying ovens at 60˚C for 24-48 hr. Tissue and shell dry weights were recorded using the same balance. Wet: dry weight ratios were calculated by dividing soft tissue wet weight by soft tissue dry weight. Oxygen consumption was expressed as mg O_2 consumed per g dry weight per hr and ammonia excretion expressed as mg NH_4 excreted per g dry weight per hr. Finally, O:N ratios were calculated by dividing the number of moles of oxygen consumed by moles N excreted during the 5 h incubation.

Statistics

Thermal tolerance experiments were analyzed using a median lethal time-to-death statistic (LT_{50}) which was calculated using the probit method with Comprehensive Environmental Toxicity Information System software (CETIS McKinleyville, CA). LT_{100} values reflect the time at which all mussels had expired in the bioassay. The effect of zebra mussel length on time-to-death was analyzed using linear regression within SigmaPlot version 11.0 (Systat Software Inc. San Jose, CA).

Glycogen concentrations from the transplant study were analyzed using a one-way repeated measures analysis of variance (ANOVA) assessing for potential effects of date, site, and population. Data not meeting assumptions of normality or heterogeneity of variance were natural log transformed and reanalyzed. If significant differences, at $\alpha = 0.05$ level were noted, a Holm-Sidak multiple comparison procedure was initiated with appropriate Bonferroni style α-level corrections (Zar 1999).

Oxygen consumption and NH_4 excretion data were analyzed in SAS 9.2 (SAS Institute Inc. Cary, NC) using both a multiple linear regression procedure and analysis of covariance (ANCOVA), controlling for dry weight and population. Oxygen consumption data were linearized by natural log transformation and regressed against temperature, dry weight, and population, using vif and collin as colinearity diagnostics within the SAS code. ANCOVA's with Tukey's multiple comparison procedure were then used to

compare natural log normalized oxygen consumption and NH_4 excretion results separately among temperatures, controlling for dry weight and population.

RESULTS

Upper thermal tolerance

As indicated by the time to death statistics (LT_{50}'s and LT_{100}'s), sensitivity to the thermal challenges varied based on season for mussels from all three reservoirs (Tables 5.1 through 5.3). *D. polymorpha* tested during the summer months had higher LT_{50}'s than those assessed during times when water temperature was colder. For instance, at Sooner Lake during August 2008, an LT_{50} of 7000 minutes was observed as compared with only 300 minutes in March 2010. A significant positive correlation was noted between LT_{50} and LT_{100} values and water temperature at time of collection for Sooner Lake *D. polymorpha* ($P = 0.003$, $r^2 = 0.34$ and $P = 0.04$, $r^2 = 0.18$, respectively) with mussels collected during warmer water temperatures having greater upper thermal tolerances. Similar correlations were not observed for Cheney Lake ($P = 0.079$, $r^2 = 0.43$) and at Kaw Lake water temperature was not recorded during each collection event.

In 2009, zebra mussels collected from Kaw Lake were used to compare thermal tolerance times with the population in Sooner Lake. LT_{50} values generated with Sooner Lake *D. polymorpha* exceeded those from Kaw Lake mussels in all but one assay (Figure 5.1), with non-overlapping confidence intervals in 5 of the 8 trials. After September 2009, the

zebra mussel population density in Kaw Lake was not sufficient to support further collections for thermal tolerance trials, therefore mussels were collected from Cheney Lake, KS, which had a more dense population. *D. polymorpha* from Cheney Lake showed similar tolerance times as compared with Sooner Lake mussels (Figure 5.2). Additionally, in June 2010, mussels were collected from Texoma, Cheney and Sooner Lakes for a single comparison of upper thermal tolerances between the three populations. LT_{50} values ranged from 1222 min for Sooner-derived mussels, to 4784 min for Cheney collected mussels. The mussels collected from Texoma Lake exhibited an LT_{50} of 1388 min, similar to the Sooner-derived mussels.

Exposure method also affected zebra mussel time to death statistics, with those mussels in a druse more sensitive to high temperatures. Druses of approximately 50 individuals were selected for bioassays to match the 50 mussels (5 replicates of 10) that were exposed as separate individuals. In all trials and all populations, LT_{100}'s from druses were less than the LT_{100} generated for individual zebra mussels (Table 5.3). Greater sensitivities of druse-exposed mussels was perhaps enhanced by increased NH_4 and NO_2 concentrations as shown in Table 5.4. Mean ammonia concentrations for all individual exposures was near 1 mg/L compared with 8.5 mg/L in the druse. Similarly, nitrite concentrations averaged 0.3 mg/L in the druse versus 0.15 mg/L in individual exposures. Dissolved oxygen concentration remained above 4 mg/L in all assays in both the druse and individual exposures as beakers were continuously aerated.

As zebra mussel length has been previously shown to have an effect on thermal tolerance (McMahon et al. 1995), linear regression was used to determine if correlations existed between zebra mussel length and time to death. In 23 bioassays with Sooner Lake *D. polymorpha*, only three showed significant length by time to death interactions. One assay with Kaw Lake zebra mussels and again three trials with Cheney Lake mussels showed significant effects of length on time to death with small mussels having greater thermal tolerance (Table 5.5).

Glycogen and tissue water content were examined for possible correlation with LT_{50} values. Glycogen was examined to determine if mussel energy reserves influenced time to death, and tissue water content to assess if loss of osmoregulatory ability influenced time to death in thermal tolerance assays. There was no significant correlation between mussel tissue glycogen concentrations and LT_{50} values ($P = 0.78$, $R^2 = 0.017$). Tissue water content did not change over the course of the four day incubation period, but increased from 88% to 90% during the first 16 hours after temperature was increased to 34˚C (Figure 5.3). As mussels began to expire, variability increased and tissue water content decreased slightly.

Transplant study

To determine how zebra mussel tissue glycogen concentrations would respond to a long-term incubation at elevated temperatures in the field, *D. polymorpha* were collected from Cheney and Sooner Lakes and suspended at four sites within Sooner Lake. Within one

week, all mussels from the Sooner and Cheney populations died at the two warmest sites, with water temperatures between 33 and 35˚C at these sites. Of the two remaining sites, water temperatures at the end discharge location ranged from 28 to 32˚C over the one month deployment, with a range of 25 to 30˚C at the intake buoy.

Over the 30 day incubation, glycogen concentrations did not differ among any of the collection dates for the Cheney population suspended at the end discharge location (P=0.29) or intake buoy (P=0.16). Similarly, glycogen concentrations did not change for the Sooner Lake populations across sampling dates for mussels at the end discharge (P=0.06) or intake buoy (P=0.27). Overall, glycogen concentrations ranged between 5 and 15 mg/g wet weight for both populations and both locations (Figure 5.4). Given there was no difference in glycogen concentrations among sampling dates, all data were combined within a population to test for differences between sites and between populations. There was also no difference in glycogen concentrations for mussels at the end discharge vs. intake buoy locations for the Cheney (P=0.07) or Sooner populations (P=0.12). When comparing between populations, Cheney mussels at the intake buoy had significantly greater glycogen stores than did Sooner derived mussels (11.7 and 7.4 mg/g, respectively, P=0.04). Mussels suspended at the end discharge however were not significantly different between populations (P=0.19). By June 30, 2010 all mussels had expired at all locations and the experiment was terminated.

Oxygen consumption/ammonia excretion

During the 30 day acclimation of mussels for the oxygen consumption/ammonia excretion experiment, significant mortality of large (> 15 mm) *D. polymorpha* occurred in the 32˚C treatment. Consequently, there were significant differences among dry weights of zebra mussels used at each temperature treatment (Figure 5.5). Within the Cheney population, mussels acclimated to 20˚C had significantly higher dry weights than the 24, 28, and 32˚C treatments ($P < 0.005$ for all comparisons). Additionally, mussels acclimated to 24˚C had higher dry weights than the 32˚C treatment ($P = 0.007$). There was no difference in dry weight among any of the Texoma Lake treatments ($P = 0.057$). In comparing between populations within a temperature treatment, Cheney mussels at 20˚C had greater dry weight than Texoma mussels at 20˚C ($P < 0.001$) and Cheney mussels at 24˚C also had greater dry weight then Texoma mussels at 24˚C ($P = 0.012$). These differences may be an artifact of the availability of zebra mussels during initial collection within each lake. A broad range of sizes was available from Cheney, while the size structure at Texoma Lake was restricted due to a summer die-off of the reproductively mature mussels (Boeckman and Bidwell 2010). Dry weight of *D. polymorpha* used in trials conducted at 28 and 32˚C did not differ between populations ($P = 0.074$ and 0.522, respectively).

Using multiple linear regression and colinearity predictors, dry weight and population were found to have no significant effect on natural log normalized oxygen consumption or ammonia excretion as variance inflation numbers were below 3 for all variables. Therefore, an ANCOVA was used to determine if significant differences existed in oxygen consumption and ammonia excretion among temperatures, controlling for dry

weight and population. Population had no affect on oxygen consumption ($P = 0.69$) or ammonia excretion ($P = 0.078$) so data were pooled for further analyses. As would be expected, temperature had a significant effect on natural log normalized oxygen consumption ($P < 0.0001$, Figure 5.6), with greater respiration at higher temperatures. Oxygen consumption was significantly greater at 32˚C than at 20 and 24˚C ($P < 0.0001$) and at 28˚C ($P = 0.0004$). Additionally, oxygen consumption was higher in mussels acclimated at 28˚C than at 20 ($P = 0.016$) and 24˚C ($P = 0.027$). Ammonia excretion was also significantly affected by temperature ($P = 0.0038$) with mussels acclimated to 32˚C excreting more ammonia than those tested at 20, 24 and 28˚C ($P = 0.009$, 0.004, and 0.024, respectively) (Figure 5.7).

O:N ratios were not significantly different between Cheney and Texoma mussels ($P = 0.21$), therefore data were combined and analyzed for significant differences among temperatures. Mussels acclimated to 20˚C had a significantly greater O:N ratio than mussels tested at 24, 28 and 32˚C ($P = 0.0002$, < 0.0001, and < 0.0001, respectively) (Figure 5.8). Additionally, mussels acclimated to 24˚C had significantly greater O:N ratio than mussels at 28˚C ($P = 0.022$) and 32˚C ($P = 0.005$).

DISCUSSION

Upper thermal tolerance

Acclimation temperature has been previously shown to have a significant effect on zebra mussel thermal tolerance times (Iwanyzki and McCauley 1993, Hernandez et al. 1995, McMahon et al. 1995). Therefore a seasonal difference in thermal tolerance of *D. polymorpha* is not unexpected. However, the LT_{50} values derived for zebra mussels in this study were higher than those reported in previous studies. For example, McMahon et al. (1995) found mussels acclimated to 30˚C had an LT_{50} of 730 minutes when exposed to 34˚C test temperatures. Zebra mussels used in this study from all three populations exhibited summertime LT_{50} values routinely well over 1000 minutes with some reaching 4000 to 7000 minutes. Additionally, mussels acclimated to 5-15˚C and tested at 34˚C had an LT_{50} of 100 to 150 minutes as reported by McMahon et al. (1995), where mussels in this study, collected when ambient lake temperatures were 5-15˚C, exhibited LT_{50}'s of approximately 350 to 400 minutes. These differences may represent different thermal tolerances of mussels used in the present study and those used by McMahon et al. (1995) since the latter tested organisms from Buffalo, NY which presumably would have experienced lower seasonal temperatures than zebra mussels from Oklahoma. However, Hernandez et al. (1995) investigated thermal tolerance differences between zebra mussels from Buffalo, NY, and those collected from the Mississippi River at Baton Rouge, LA, and found no difference. They suggested this may have been due to short residence times of mussels in the Mississippi River and constant inflow of new organisms from upstream populations that originated in cooler waters. Additionally, the mussels collected at Baton Rouge, LA, were housed at 21-23˚C for several weeks prior to testing, which may have negatively affected thermal tolerance results if physiological condition declined during this holding period. Also in assays with the Buffalo, NY, population both McMahon et al

(1995) and Hernandez et al. (1995) had one collection event and mussels were used within four months of collection. In the present study, mussels were collected seasonally, and only held in the laboratory for a four day acclimation at lake temperature to allow for byssal thread attachment. This difference in laboratory handling time may also account for increased thermal tolerance times observed in this study.

Elderkin and Klerks (2005) also assessed zebra mussel thermal tolerance in the Mississippi River and concluded that mussels collected from Louisiana did have a higher thermal tolerance than those collected from Minnesota. This supports the idea that the observed differences in LT_{50} values between Oklahoma populations and those reported for more northern sources are in fact real and not due to differences in experimental design.

Given the altered thermal regime in Sooner Lake, *D. polymorpha* from Kaw and Cheney reservoirs were used to compare thermal tolerance times to determine if differences in thermal tolerance existed between populations on a finer spatial scale. With water temperatures in Sooner Lake reaching 38˚C in the discharge channel, zebra mussels in this system are potentially subjected to temperatures greater than those in reservoirs that do not receive a heated discharge. Sustained water temperatures at Texoma Lake of over 30˚C for more than one month during the summer suggest mussels there are also subjected to relatively higher temperatures than those in Cheney Lake where 30˚C temperatures were only observed for one week during this study.

Initial zebra mussel collections were made from Kaw Lake, OK, where summer water temperatures are similar to areas unaffected by the warm-water discharge in Sooner Lake. In concurrent assays, Sooner-derived mussels had greater LT_{50} values than Kaw mussels. Unfortunately, zebra mussel numbers in Kaw Lake were low and only a limited number of thermal tolerance trials could be conducted with individuals from this system. There was no difference in LT_{50} values between Sooner and Cheney Lake zebra mussels. In fact, on one occasion, zebra mussels were collected from Cheney, Sooner, and Texoma Lakes for concurrent thermal tolerance assays and Cheney-derived mussels had greater LT_{50} values than did mussels from Texoma and Sooner Lakes. These mussels were collected and tested in late June 2010 and differences in mussel physiological condition may explain these results since mussels at Cheney had been subjected to water temperatures ranging from 25 to 27˚C in the month prior to collection. Mussels at Sooner and Texoma were subjected to much greater temperatures, ranging from 26-30˚C and from 28 to 31˚C, respectively. The Cheney population experienced temperatures below 28˚C, and the Sooner and Texoma mussels were exposed to temperatures above 28˚C and as Aldridge et al. (1995) demonstrated *D. polymorpha* are unable to meet physiological requirements at temperatures above 28˚C, therefore mussels collected from Sooner and Texoma may have been in a poorer physiological condition which resulted in decreased LT_{50}'s. Additionally, since zebra mussels were first found in Sooner in 2006 and mussels in Texoma were discovered in 2009, there may not have been enough time for selection to occur within the lake. Furthermore, observed adult mussel dieoffs in Sooner Lake are similar to other dieoffs of larger reproductively mature mussels in other systems in the

area (Boeckman and Bidwell in review), therefore both laboratory and field observations suggest no enhancement of thermal tolerance within the Sooner Lake *D. polymorpha* population.

Mussels exposed in a druse were more sensitive to high temperatures than *D. polymorpha* exposed as separate individuals. Burks et al. (2002) have shown nitrate concentrations to be significantly greater, and dissolved oxygen concentrations lower, at the base of these druses when compared with the overlying water. It appears high temperatures in association with declining water quality combine to reduce thermal tolerance times of mussels exposed in a group. Similarly, Tuchman et al. (2004) demonstrated zebra mussels on the interior of these druses are exposed to restricted food availability under low flow conditions. While these assays were conducted without food additions, the increased ammonia and nitrite concentrations demonstrated in this study, combined with restricted food availability, may help explain summertime dieoffs observed in several Oklahoma *D. polymorpha* populations (Boeckman and Bidwell in review). Additionally, lower LT_{100} values when exposed in a druse form suggest that thermal tolerance assays conducted on individuals may over estimate the true environmental tolerances given mussels typically occur as dense aggregations in the field.

McMahon et al. (1994) has shown small zebra mussels are more tolerant of high temperatures than larger mussels, while Bayne and Newell (1983) suggest small mussels may be better at conserving energy stores during periods of stress. This observation was

also noted in long-term monitoring studies of zebra mussels in Oklahoma (Boeckman and Bidwell in review). In thermal tolerance trials conducted in this study, small mussels generally had greater time to death than large mussels which supports the hypotheses above.

Since reproductively mature *D. polymorpha* can lose as much as 30% of their body weight after spawning (Sprung 1991, 1993), adult mussels collected and tested after spawning may have been in poorer physiological condition than smaller sub-adult mussels that did not spawn. Furthermore, even prior to spawning, reproductively mature mussels should have been investing more energy into reproductive tissues than sub-adults (Stoeckmann and Garton 1997, 2001) which may have conferred an energetic advantage to small mussels that facilitated their higher thermal tolerance. Under stressful environmental conditions, *D. polymorpha* have been shown to allocate energy into reproduction at the expense of growth and survival (Stoeckmann and Garton 2001). Therefore, sub-adult mussels would not have the reproductive energetic demands which may have allowed for a greater reserve of energy to be available to meet the physiological demands of exposure to the higher temperatures.

In an attempt to correlate physiological condition with mussel time to death, initial focus was placed on glycogen concentrations, under the assumption that mussels with higher glycogen reserves would be able to tolerate high temperatures longer than mussels with relatively lower glycogen stores. However, the test temperature of 34˚C is greater than

what would normally be encountered in the mussel's "natural" environment, which lead to relatively short exposure times before death occurred. While there appears to be a lack of studies investigating glycogen reduction rates in zebra mussels, Patterson et al. (1997) demonstrated a 70-85% reduction in glycogen concentrations of unfed native mussels over a 30 day period. While glycogen is the primary storage form of energy in native unionid mussels (Giese 1959), lipids appear to fill this role in zebra mussels (Sprung 1995) and would be utilized first under similar stressful environmental conditions. This suggests glycogen reductions in bivalves are a long-term event, on the order of days to months, and may not occur as readily under the more acute thermal challenges described here.

Another possibility is that the specific mode of action causing death in *D. polymorpha* under elevated temperatures was a loss of osmoregulatory ability associated with increasingly leaky cell membranes. Dietz et al. (1996) have described *D. polymorpha* as having rather leaky epithelial tissues even under optimal environmental conditions. Additionally, Dietz et al. (1996) have shown various ionic challenges induce zebra mussel death more acutely, within hours to days, which provide support for this hypothesis. To further evaluate this, tissue water content was assessed during one of the thermal tolerance assays and while tissue water did increase within the first 16 h of exposure to 34˚C, variability throughout the experiment was high. This was probably driven by only assessing five mussels at each time period. In order to more accurately assess the change in tissue water content, more individuals should be sacrificed at each time period.

Transplant study

To determine if glycogen concentrations would be reduced in a more chronic exposure to high temperatures, mussels from Cheney and Sooner Lakes were suspended at several locations within the discharge channel at Sooner Lake. Mussels in two of the four locations died within one week and there was essentially no change in glycogen concentrations over the course of the one month deployment. Lauer and Spacie (2000) described significant declines in glycogen concentrations of sponge-covered *D. polymorpha* as compared with unfouled individuals. Similarly, Bidwell et al. (1995) showed significant reductions in whole-body glycogen concentrations of zebra mussels exposed to a surfactant based molluscicide. Therefore, other studies have shown reductions in zebra mussel tissue glycogen concentrations in both acute and chronic exposure to stress.

Variability in the glycogen concentrations observed in this experiment were high (derived from 10 individuals each period), making detection of any decrease difficult during the month long exposure. Alternatively, increased water temperature within the discharge channel may have elevated filtration rates in the zebra mussels in order to keep pace with metabolic requirements. This may have resulted in *D. polymorpha* being able to maintain current glycogen concentrations throughout the month long exposure. As mentioned above, while most bivalves utilize glycogen, zebra mussels appear to use lipids as a primary storage form (Sprung 1995). Physiologically stressed *D. polymorpha* may

preferentially deplete lipids first and then switch to glycogen and protein sources, (Sprung 1995) consequently lipid analysis may be a more appropriate biochemical metric for future studies involving zebra mussels.

Oxygen consumption/ammonia excretion

In the present study, mortality during the 30 day acclimation period in the O_2/NH_4 experiment was much greater than reported by Aldridge et al. (1995). While Aldridge et al. (1995) selected mussels in the 13-17 mm size class, there was no specific size selection in this study and large individuals, greater than 18 mm did not survive acclimation at 28 or 32˚C. Zebra mussel oxygen consumption rates measured at 20 and 24˚C were as high as those tested at 28 and 32˚C in Aldridge et al. (1995). Oxygen consumption in this study ranged from 4 to 17 mg/g/hr which was also somewhat greater than reported by Fisher et al. (1993) and Alexander and McMahon (2004) (0.5 to 3.5 mg/g/hr). Ammonia excretion was also greater in this study than that reported by Aldridge et al. (1995) (0.19 to 4.11 mg/g/hr vs. 0.039 to 0.191 mg/g/hr, respectively). As with oxygen consumption, ammonia concentrations at 28 and 32˚C reported by Aldridge et al. (1995) were similar to that which occurred at 20 and 24˚C in this study. Conroy et al. (2005) however, found 20-25 mm mussels incubated at 22-24˚C excreted 0.36 mg NH_4/g/hr, which is more similar with values determined in this study.

O:N ratios can give an indication of the relative proportion of energy sources used for metabolism (Bayne and Widdows 1978, Mayzaud and Conover 1988). For example

Mayzaud and Conover (1988) suggest O:N ratios greater than 60 indicate high lipid use with values less than 50 indicative of protein catabolism. *D. polymorpha* in this study appear to be utilizing primarily lipids at 20˚C with O:N ratios above 50 at this temperature. At 24˚C, mussels appear to use about equal portions of lipid and protein, associated with O:N ratios between 30 and 40. As reported by Aldridge et al. (1995), zebra mussels at 28 and 32˚C appear to be catabolizing primarily protein resources as indicated by O:N ratios between 18 and 25. Again, the catabolism of protein resources at these high temperatures is not sustainable in the long term.

Temperature increased both oxygen consumption and ammonia excretion. Aldridge et al. (1995) observed no decline in O:N ratio from 20 to 24˚C, where in this study, O:N ratio at 20˚C was significantly greater than at 24, 28 and 32˚C. For Cheney-derived mussels O:N ratio declined from 87 at 20˚C to 40 at 24˚C, and Texoma mussel O:N ratios declined from 50 to 31 over the same change in temperature. Organisms for this study were collected in late September 2010, after peak summertime water temperatures and reproduction had occurred. Those organisms used in Aldridge et al. (1995) were collected in early June from Buffalo, NY, prior to reproduction and peak summer water temperatures (Fraleigh et al. 1993) which indicates mussels used in this study may have already been in a compromised physiological condition prior to acclimation to the four temperature regimes. This may have resulted in the relatively lower O:N ratios at 24˚C observed in this study and may explain the greater mortality rates observed here. Overall, there was no evidence of any enhanced ability to cope with elevated summer water temperatures in mussels utilized in this study. The reliance on protein substrates for

metabolic demands at 28 and 32˚C is the same result found by Aldridge et al. (1995), in a similar experiment with *D. polymorpha* from Buffalo, NY.

Summary

The primary objective of this study was to determine if zebra mussels from the southern United States exhibited differences in thermal tolerance and physiological response to temperature than mussels used in previous studies that were derived from higher latitudes, and also to determine if zebra mussels from a thermally-influenced lake had different responses to elevated temperatures than mussels from systems that were not thermally influenced. There was no difference in thermal tolerance between zebra mussels derived from a thermally-influenced reservoir and other populations in the region with different thermal regimes. However, as a group, zebra mussels in this study appeared to have greater time to death than was reported by McMahon et al. (1995) and Hernandez et al (1995) for a Buffalo, NY, population. These differences may be attributed to ambient temperature at time of collection, or temperature acclimation by zebra mussels used in this study. Alternatively, *D. polymorpha* near their predicted southern ranges may have acquired an enhanced upper thermal tolerance. However, results of the oxygen consumption and ammonia excretion experiment conducted in this study do not support that hypothesis. The change from lipid and carbohydrate metabolism to protein catabolism was noted at 28˚C, which is similar to the results of Aldridge et al. (1995) who used mussels from Buffalo, NY. One may hypothesize that if *D. polymorpha* had evolved any enhanced upper thermal tolerance, the point at which

they enter the unsustainable protein catabolism may occur at a higher temperature. As the zebra mussel introductions into Sooner and Texoma reservoirs are still relatively recent, 2006 and 2009, respectively, consequently there may not have been enough time for selective forces to shape the metabolic response of *D. polymorpha* in these systems.

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TABLES AND FIGURES

Table 5.1. Mean (±S.E.) seasonal LT_{50} and LT_{100} values (minutes) for zebra mussels from two populations exposed as individuals to 34˚C. Temperature range reflects minimum and maximum lake water temperatures for indicated period.

Lake	January – March	April – June	July – September	October – December
Sooner LT_{50}	436 (93)	1095 (361)	2460 (883)	1580 (1221)
Sooner LT_{100}	2715 (782)	4555 (1890)	5273 (1101)	6435 (5325)
Kaw LT_{50}	236 (137)	481 (222)	961 (204)	1429 (936)
Kaw LT_{100}	880 (440)	1828 (661)	2520 (784)	6675 (4965)
Temperature range (˚C)	0 - 14	12 - 29	22 - 32	4 - 24

Table 5.2. Mean (±S.E.) seasonal LT_{50} and LT_{100} values (minutes) for zebra mussels from Cheney Lake exposed as individuals to 34˚C. Temperature range reflects minimum and maximum lake water temperatures for indicated period.

Lake	January – March	April – June	July – September	October – December
Cheney LT_{50}	1874 (1403)	2851 (1932)	4201	5726
Cheney LT_{100}	8430 (4140)	7917 (3252)	7690	14400
Temperature range (˚C)	0 - 12	12 - 29	21 - 31	0 - 22

Table 5.3. Mean (±S.E.) seasonal LT_{100} values (minutes) for zebra mussels from two populations exposed in a druse to 34˚C.

Lake	January – March	April – June	July – September	October – December
Sooner	820 (198)	1289 (465)	3463 (750)	1065 (45)
Cheney	3330 (2160)	3260 (2120)	6250	4070

Table 5.4. Summary of ammonia and nitrite concentrations for all thermal tolerance challenges comparing individual vs. druse exposures.

	NH_4 mean (mg/L)	**NH_4 range (mg/L)**	**NO_2 mean (mg/L)**	**NO_2 range (mg/L)**
Individual	0.94	0.1 – 2.6	0.156	0.005 – 0.34
Druse	8.53	0.3 – 35.6	0.307	0.006 – 1.85

Table 5.5. P and r^2 values for significant length by time-to-death interactions in zebra mussel thermal tolerance challenges at 34˚C.

Date collected	Lake	P	r^2
9/20/2008	Sooner	0.038	0.086
1/12/2009		0.011	0.126
5/4/2010		<0.001	0.263
4/6/2009	Kaw	0.037	0.107
3/15/2010	Cheney	0.048	0.080
5/4/2010		<0.001	0.207
10/7/2010		0.023	0.103

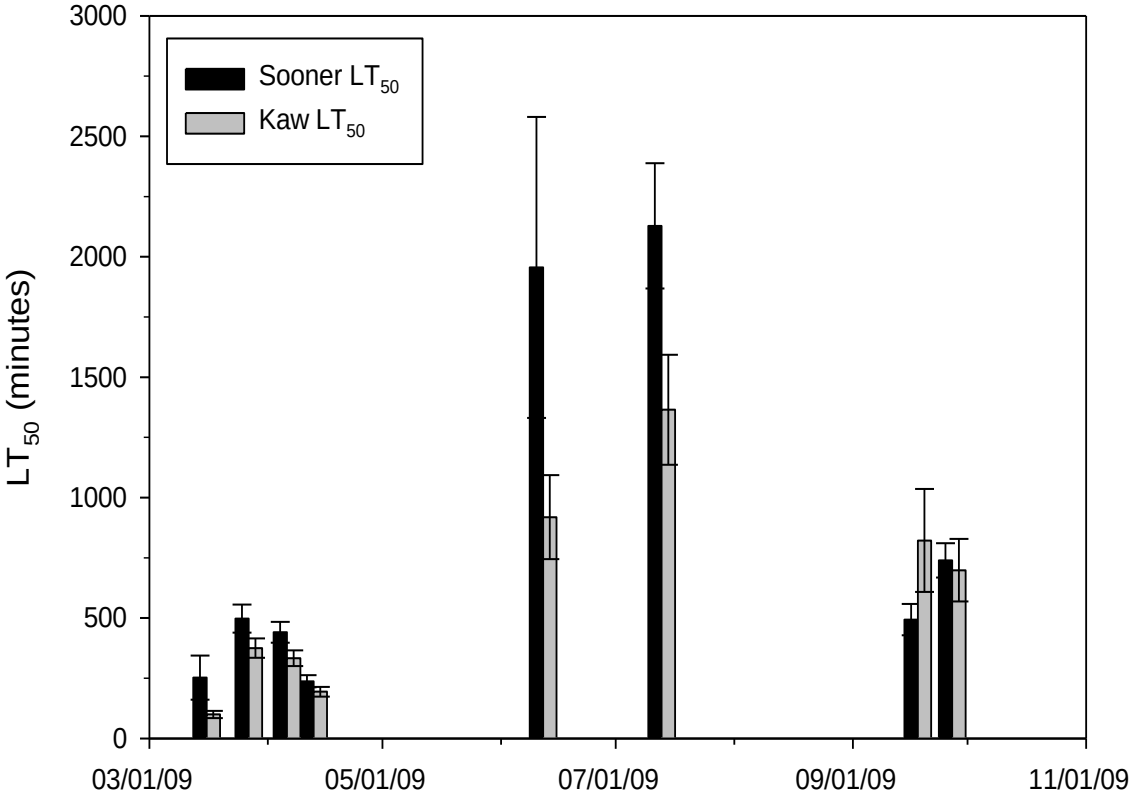

Figure 5.1. Zebra mussel LT_{50} values from thermal tolerance assays conducted at 34˚C with mussels from Sooner and Kaw Lakes.

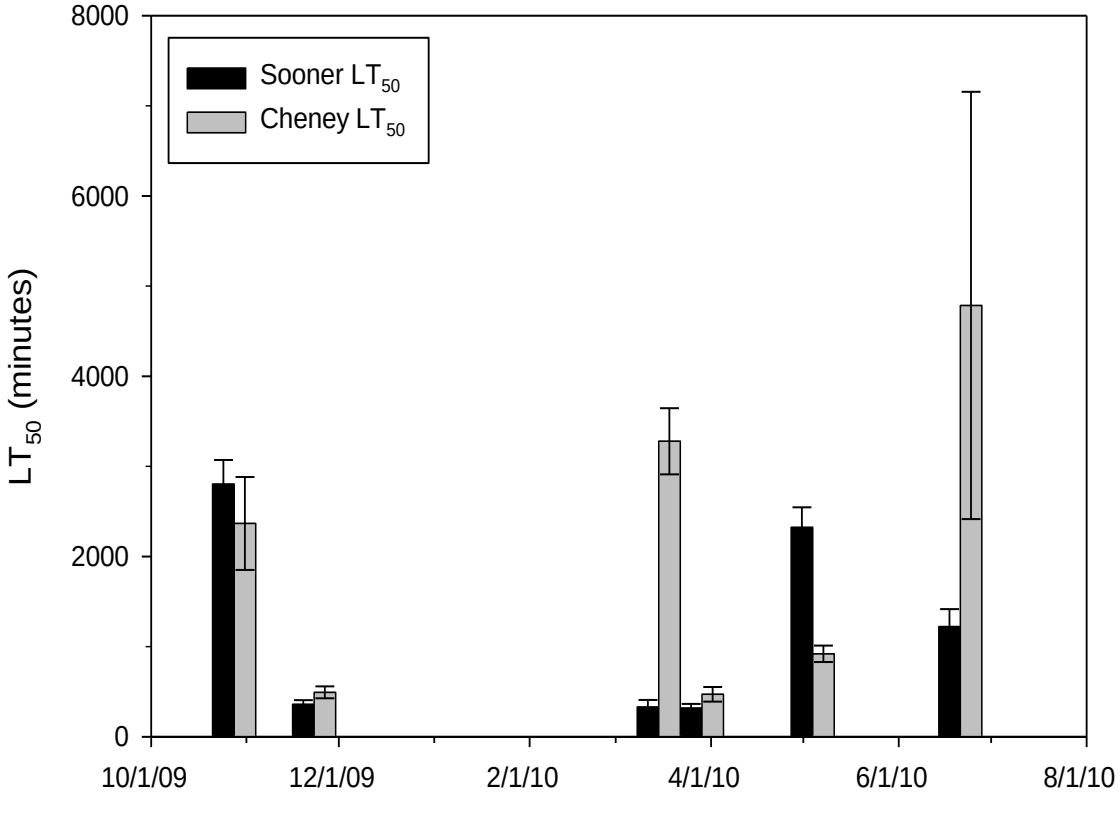

Figure 5.2. Zebra mussel LT_{50} values from thermal tolerance assays conducted at 34˚C with mussels from Sooner and Cheney Lakes.

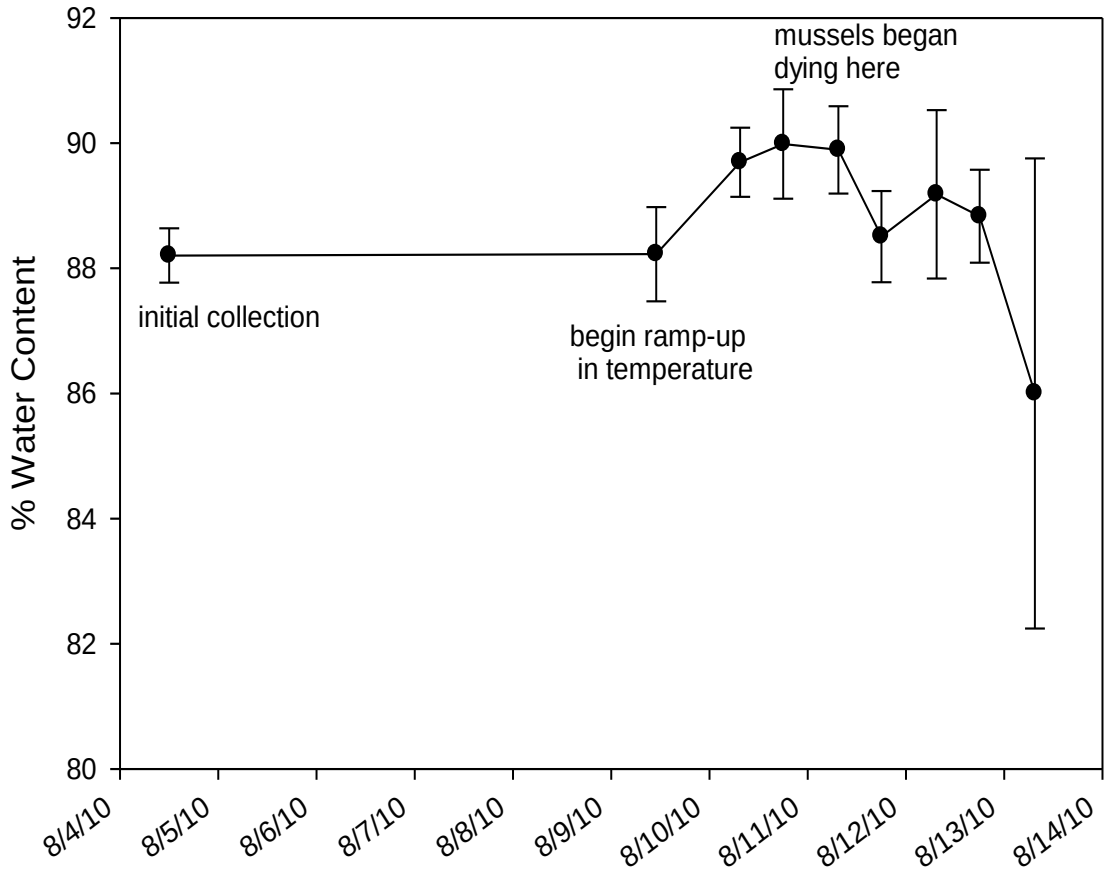

Figure 5.3. Zebra mussel tissue water content prior to and during exposure to 34˚C.

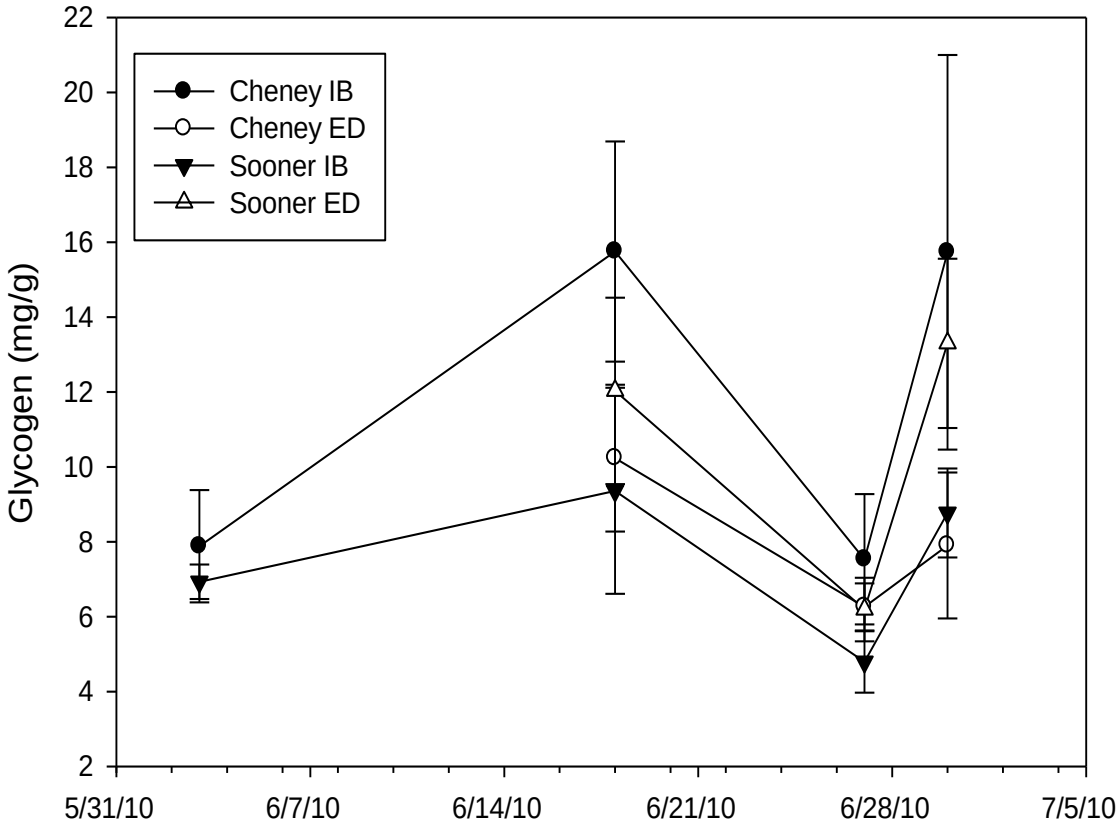

Figure 5.4. Glycogen concentrations of zebra mussels from Sooner and Cheney Lakes deployed at two sites within Sooner Lake. IB is an ambient temperature site with ED representing a site with elevated water temperature.

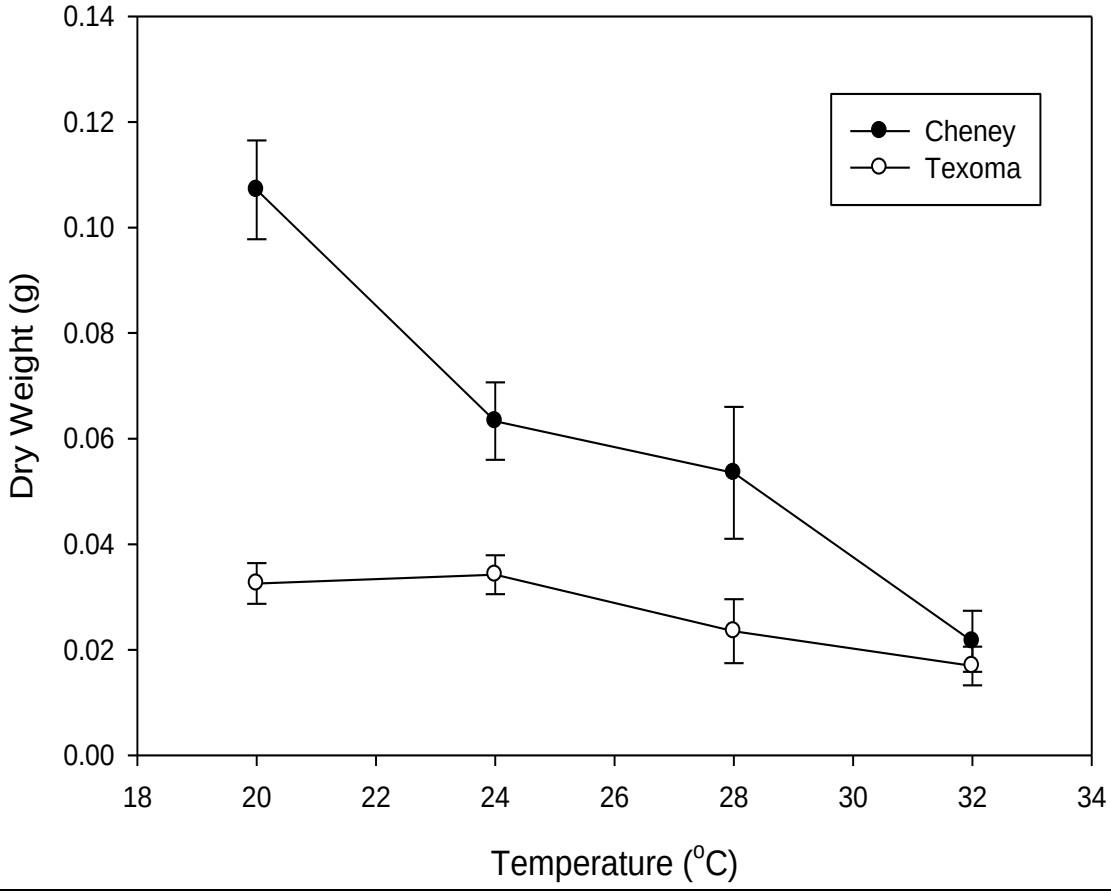

Figure 5.5. Mean and standard error dry weight of zebra mussels in O_2/NH_4 experiment.

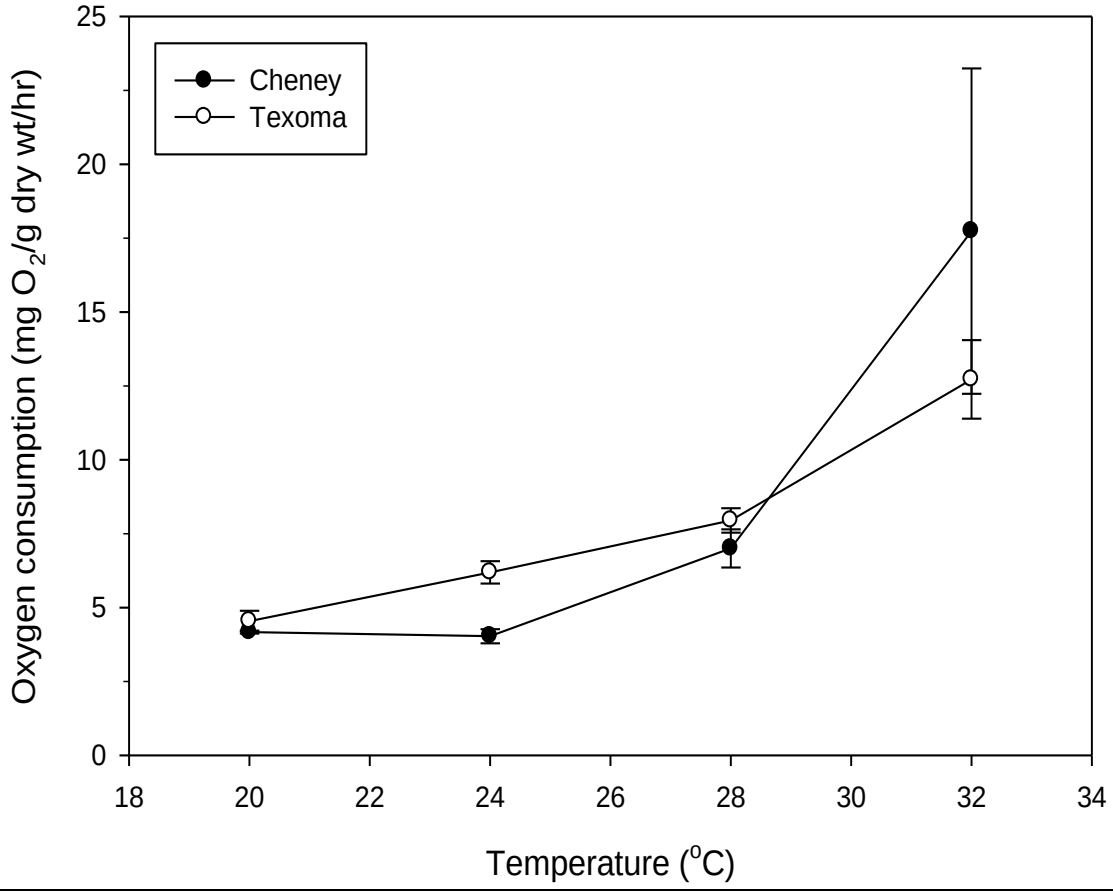

Figure 5.6. Dry weight corrected oxygen consumption of zebra mussels from two populations assessed at four temperatures.

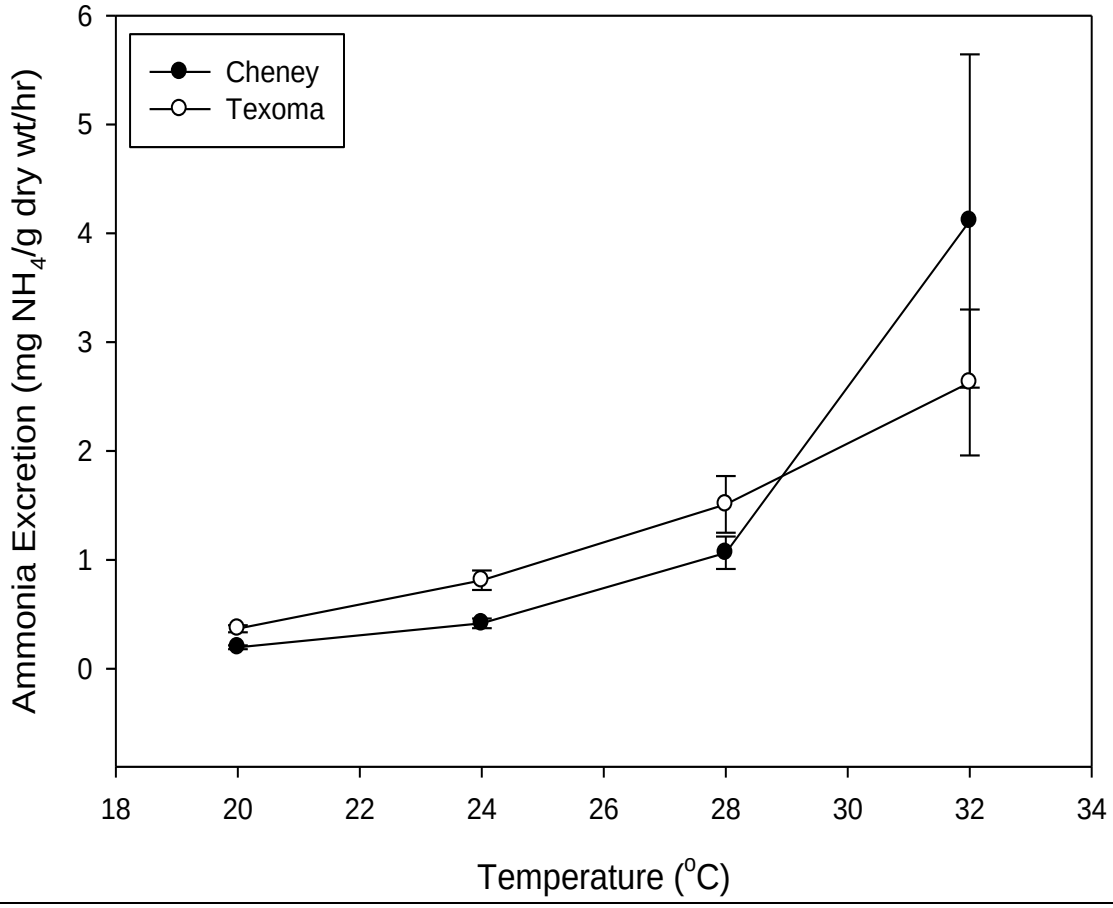

Figure 5.7. Dry weight corrected NH_4 excretion of zebra mussels from two populations assessed at four temperatures.

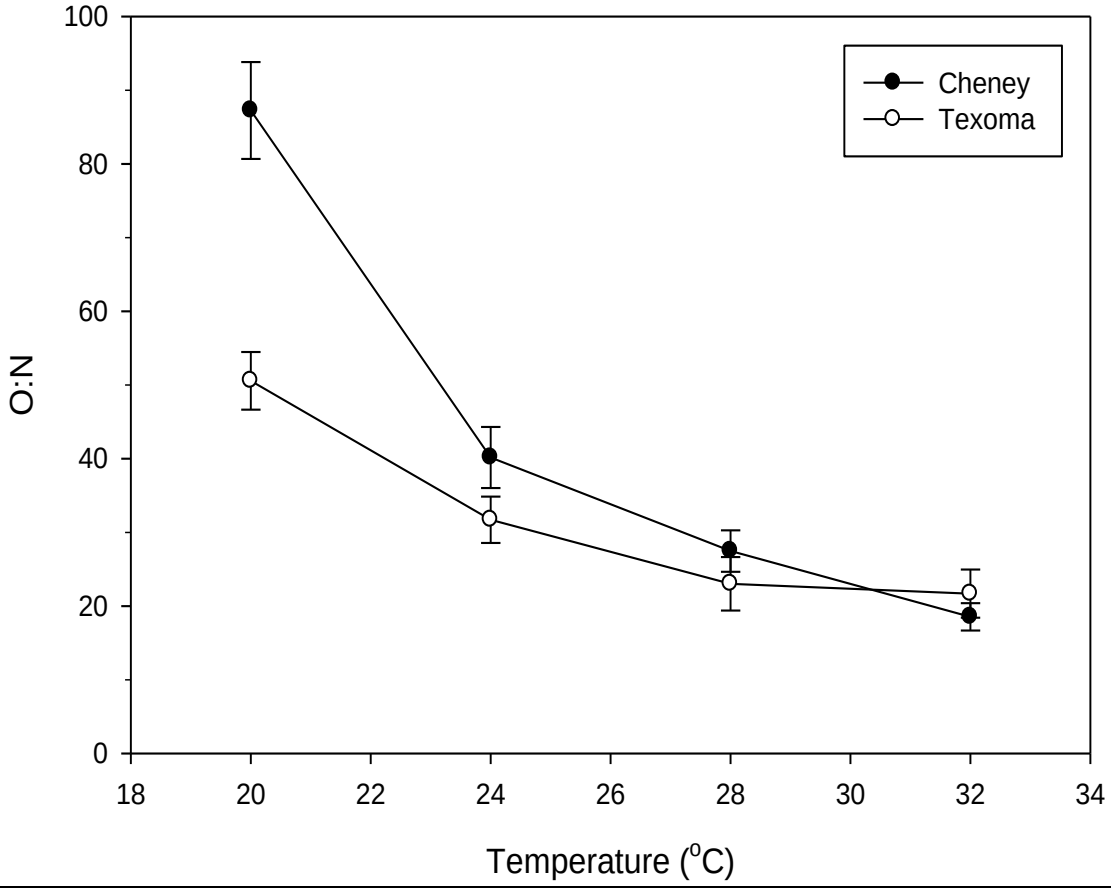

Figure 5.8. Dry weight corrected moles oxygen consumed versus moles nitrogen excreted for zebra mussels from two populations assessed at four temperatures.

VITA

Chad Jarrod Boeckman

Candidate for the Degree of

Doctor of Philosophy

Thesis: CHARACTERIZING ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) CONDITION, POPULATIONS, AND COMMUNITY EFFECTS IN OKLAHOMA HABITATS

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Completed the requirements for the Doctor of Philosophy in Zoology at Oklahoma State University, Stillwater, Oklahoma in May, 2011.

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: CHARACTERIZING ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) CONDITION, POPULATIONS, AND COMMUNITY EFFECTS IN OKLAHOMA HABITATS

Pages in Study: 185 Candidate for the Degree of Doctor of Philosophy

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Scope and Method of Study: Zebra mussels (*Dreissena polymorpha*) are one of the most widely recognized aquatic invasive species in North America. Their negative effects on native species, ecosystems, and industry have been widely documented within the scientific literature, yet their ultimate distribution is still widely debated. As Oklahoma was initially considered their southern distributional limits due to warm summer water temperatures, an evaluation of their population characteristics, growth and physiological condition within Oklahoma's reservoirs may provide insight into the potential for infestations further south. This study sought to characterize *D. polymorpha* density and reproduction in several Oklahoma reservoirs, assess their physiological condition on a seasonal scale, and determine if they have had a negative effect on native mussel communities in the Verdigris River.

Findings and Conclusions: An 8-year study at Oologah Lake, OK revealed peak zebra mussel densities near 150,000/m^2 within two years of discovery, however during 2006 a dieoff associated with 30˚C water temperatures and flooding events in subsequent years reduced these densities significantly. Native mussel surveys within the Verdigris River suggested there had been no significant decline in richness or abundance of native species since introduction of the zebra mussel, however reductions may still occur in the future particularly if zebra mussel densities remain high for several consecutive years. Similar zebra mussel densities were observed in Sooner Lake, OK, during a 4-year study despite an altered thermal regime in that system. Summer dieoffs of reproductively mature zebra mussels were recorded, however the population was able to recover in each subsequent year driven by young of the year mussels, perhaps better able to tolerate warm summer water temperatures. Multiple condition indices revealed *D. polymorpha* are in poorest physiological condition during July and August and are in best condition in May and June. Thermal tolerance bioassays indicated zebra mussels in Oklahoma have not developed any enhanced upper thermal tolerance and exhibit similar oxygen consumption and ammonia excretion ratios as compared with mussels from more northern populations.

ADVISER'S APPROVAL: Dr. Joseph R. Bidwell