THERMAL TOLERANCE, PHYSIOLOGIC CONDITION, AND POPULATION GENETICS OF DREISSENID MUSSELS (*DREISSENA POLYMORPHA* AND *D. ROSTRIFORMIS BUGENSIS*) RELATIVE TO THEIR INVASION OF WATERS IN THE WESTERN UNITED STATES

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ABSTRACT

THERMAL TOLERANCE, PHYSIOLOGIC CONDITION, AND POPULATION GENETICS OF DREISSENID MUSSELS (*DREISSENA POLYMORPHA* AND *D. ROSTRIFORMIS BUGENSIS*) RELATIVE TO THEIR INVASION OF WATERS IN THE WESTERN UNITED STATES

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Zebra mussels, *Dreissena polymorpha*, and quagga mussels, *D. rostriformis bugensis*, Eurasian freshwater bivalves, invaded the North American (NA) Great Lakes in the mid 1980's. Subsequently, they invaded water bodies east of the Rocky Mountains. Their more recent expansion into warm, isolated, southwestern U.S. water bodies suggests that both species are developing thermally tolerant physiological lineages. Chronic upper thermal tolerance limits were determined for *D. polymorpha* populations from Winfield City Lake, KS (WCL), Lake Oologah, OK (LO) and Hedges Lake, NY (HL), and the *D. rostriformis bugensis* population from Lake Mead, NV/AZ (LM). Individuals from WCL had 12-d and 28-d incipient upper thermal limits of 31.7°C and 30.7°C, respectively, greater than any other *D. polymorpha* population in

NA. In contrast, specimens from the warmer LO had a 12-d incipient upper thermal limit of 26.6°C, unexpectedly lower than that for individuals from the cooler HL at 29.0°C.

To examine the basis for the low thermal tolerance of LO mussels, the relationship between dry tissue weight (DTW) and shell length was assessed for WCL samples of *D. polymorpha* collected during summer and fall 2008. Adults experienced significant DTW loss at ambient water temperatures >25°C. DTW loss was even greater at LO where adults on 29 June 2007 had a DTW roughly one third that of WCL individuals on the same date in 2008. The LO population experienced nearly twice the number of degree-days >25°C than the WCL population, exacerbating their DTW loss by prolonged and intensified temperature-induced negative energy balance, and reducing their energy stores below those required to tolerate prolonged exposure to high temperatures experienced in chronic thermal tolerance testing. Prolonged negative energy balance may have been responsible for their possible extirpation from LO in 2007.

Specimens of *D. rostriformis bugensis* from LM had a 28-d incipient upper thermal limit of 27.2°C which was approached or exceeded by the lake's summer ambient water temperatures at depths <12 m. However, their recent expansion into the surface waters of LM and the species' dispersal into warmer, shallow southwestern reservoirs indicate that, like the WCL *D. polymorpha* population, this species may be adapting to the warm waters of the southwestern U.S.

The adaptation of southwestern U.S. dreissenid populations to elevated temperatures could reduce the efficacy of thermal mitigation treatments in this region and potentially require higher temperatures for hot-water spray mitigation of mussel fouling on recreational boats and submerged equipment.

This study's genetic evaluation of both long and recently established populations of NA *Dreissena polymorpha* and *D. rostriformis bugensis* by Amplified Fragment Length Polymorphisms indicated a lack of founder effects and genetic bottlenecks in newly established populations, strongly suggesting that successful invasion of a new habitat requires introduction of a large number of individuals. Therefore, dreissenid prevention and containment measures that are <100% effective may still allow uninfested western NA water bodies to remain free of these mussels if coordinated, integrated, and region-wide prevention, containment and management plans are adopted.

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CHAPTER 1

INTRODUCTION

1.1 General Biology

Zebra mussels, *Dreissena polymorpha*, and quagga mussels, *Dreissena rostriformis bugensis*, are freshwater members of the family Dreissenidae (Phylum Mollusca, Class Bivalvia, Subclass Heterodonta, Order Veneroida, Superfamily Dreissenoidea). Both species are native to the Caspian and Black Sea regions and their associated drainages in Eastern Europe and Western Asia (Zhulidov *et al*. 2004). Dreissenid mussels in North America attain a maximum size of approximately 4 cm (Mills *et al*. 1996). Lifespans and growth rates in *D. polymorpha* are variable and correlated with habitat primary productivity. In highly productive habitats, individuals of *D. polymorpha* tend to grow more rapidly and have shorter life spans than individuals in less productive habitats (Morton 1969). In North America, individuals of *D. polymorpha* typically have lifespans of 2–4 y (Chase and Bailey 1999, Karatayev *et al*. 2006) with an average lifespan of 3.3 y based on cohort shell length analysis (Karatayev *et al*. 2006). Lifespan estimates for European *D. polymorpha* populations are more variable ranging from 1–9 y (Mackie and Schloesser 1996), however, Morton (1969) presented evidence that the lifespans of European populations of *D. polymorpha* have been overestimated and that the majority have maximum life spans of 3–5 years, similar to that recorded for North American populations.

There are much fewer reports on individual growth rates and lifespans for *D. rostriformis bugensis* in North America or Europe. In North America, MacIsaac (1994) reported no difference in the growth rates of caged individuals of *D. polymorpha* and *D. rostriformis bugensis* in Lake Erie, nor did Orlova and Shcherbina (2002) in the upper Volga River Basin, and Schonenberg and Gittenberger (2008) in the Rhine-Meuse Estuary, The Netherlands. The maximum age of sampled individuals of *D. rostriformis bugensis* in the Danube River, Bulgaria, was 2–4 y (Hubenov and Trichkova 2007) and >2 y in three Russian reservoirs (Lvova 2004), indicative of lifespans similar to that of *D. polymorpha*. Although limited, these data suggest that the population dynamics of *D. rostriformis bugensis*, like *D. polymorpha*, are similar in North America and Europe.

Both *D. polymorpha* and *D. rostriformis bugensis* are gonochoristic with males and females releasing sperm and eggs into the water column. Female specimens of *D. polymorpha* are highly fecund, capable of releasing over one million eggs in a single spawning event (Mackie and Schloesser 1996, McMahon 2002, Stoeckmann 2003, Keller *et al*. 2007). Females of *D. rostriformis bugensis* appear less fecund than those of *D. polymorpha*. When collected from the same site in western Lake Erie (OH), females of *D. polymorpha* released 3–4 times more eggs than females of *D. rostriformis bugensis* (Stoeckmann 2003).

Males and females of both species typically reach sexual maturity within one year (Mackie and Schloesser 1996, McMahon 2002, Keller *et al*. 2007). Following external fertilization, zygotes metamorphose through several pre-settlement larval stages (i.e., trochophore and veliger) which are planktonic, enabling rapid downstream

dispersal into previously uninhabited waters (Ackerman 1995). However, neither larval forms nor adult mussels are generally capable of moving against water currents. A unique feature of dreissenid mussels compared to native North American freshwater bivalves (i.e., the Unionidae) is that adults possess a specialized organ for secretion of proteinaceous byssal attachment threads which allows attachment to hard substrates (Bonner and Rockhill 1994, Clarke and McMahon 1996). Dreissenid mussels byssally attached to ships and boats, or aquatic vegetation entangled on vessels, may be moved upstream or overland to uninfested bodies that were otherwise unlikely to be invaded through these species' natural dispersal mechanisms (Johnson and Carlton 1996).

1.2 Impact of Invasion

Specimens of *D. polymorpha* can attain densities greater than 750,000 individuals m^{-2} (Schloesser *et al*. 1996), impacting infested bodies of water both abiotically and biotically. A single adult individual of *D. polymorpha* can filter more than 5 L of water per day (Horgan and Mills 1997). Dreissenids, like most bivalves, filter feed using stiffened stereo-cilia (cirri) on their greatly enlarged and modified ctenidia (gills) to capture microscopic particles carried on the respiratory/feeding currents flowing between adjacent gill filaments maintained by the beating of the lateral cilia (reviewed for dreissenid mussels in McMahon and Bogan 2001). Along with their planktonic micro-zooplankton, micro-phytoplankton, bacterial, and mircodetrital food sources, dreissenid mussels filter suspended fine sediments and other non-ingestible organic detritus from the water column. Filtered material is mechanically sorted on the labial palps with food particles (i.e., micro-zooplankton, micro-phytoplankton, bacteria

and ingestible micro-detritus) being carried to the mouth for ingestion. Nonfood particles (i.e., fine sediments, and larger organic micro-detritus) are consolidated with mucus on the labial palps into dense particles (i.e., pseudofeces) that are released from the tips of the palps into the mantle cavity where they are carried on ciliated pathways to the inhalant siphon and ejected externally by periodic shell valve clapping (reviewed for dreissenid mussels in McMahon and Bogan 2001). In the gut, undigested and unassimilated food particles are also consolidated with mucus into dense feces in the rectum and released through the anus into the dorsal exhalent mantle cavity to be carried externally on water flow through the exhalent siphon (reviewed for dreissenid mussels in McMahon and Bogan 2001). Accumulation of consolidated, dense pseudofeces and feces can greatly increase sedimentation rates and the organic detrital content of surface sediments (Botts *et al*. 1996, Thayer *et al.* 1997, for a review see McMahon and Bogan 2001)

At high densities, it is possible for dreissenid mussel populations to filter the entire volume of a water body daily, greatly increasing clarity and drastically reducing phytoplankton and micro-zooplankton densities (MacIsaac 1996). A decrease in phytoplankton and zooplankton abundance may negatively impact energy transfer to higher trophic levels (MacIsaac 1996), members of which generally do not feed on adult dreissenid mussels. Additionally, increased light penetration in clarified water bodies leads to increased growth and diversity of submerged macrophytes and an increased depth of the macrophyte euphotic zone (Zhu *et al*. 2006). Selective filtration and ingestion of eukaryotic phytoplankton over cyanobacteria (Vanderploeg *et al*. 2001) and

dreissenid-induced reduction in N:P ratios (Bykova *et al*. 2006) can result in environmentally damaging, toxic, cyanobacterial blooms (Vanderploeg *et al*. 2001, Bykova *et al*. 2006).

Dreissenid mussels attach to hard substrata, including native unionid bivalve shells, many species of which are already imperiled. Unionid bivalves infested with dreissenid mussels have restricted valve function, food availability, and mobility, which eventually results in starvation (Byrne *et al*. 1995, Schloesser *et al*. 1996). Thus, dreissenid infestations have been reported to result in substantial unionid bivalve mortalities and even extirpations in North America (for a review see McMahon and Bogan 2001).

Despite the negative ecological impacts of *D. polymorpha* and *D. rostriformis bugensis* infestations, these species' impacts on industrial raw-water systems have received more attention and public visibility. Dreissenid mussel fouling is of great concern to industrial, electrical (particularly nuclear) power-generating and municipal facilities that rely on raw-water intake and distribution systems for cooling and other internal processes. Individuals of these species byssally attach to and foul piping, screens and other raw-water system components, occluding flow. Thus, mussel fouling greatly impacts the operations of these systems and is often difficult and costly to control or mitigate (Claudi and Mackie 1994, O'Neill 1996). When molluscicides are used for control and mitigation in once-through systems, this also raises environmental, water quality, and human health concerns (Claudi and Mackie 1994, O'Neill 1996). Thus, it has been estimated that as much as $500 million dollars has been spent annually

in the Great Lakes region alone for mitigation and control of dreissenid macrofouling in raw-water-using facilities (Claudi and Mackie 1994, O'Neill 1996, 1997, USGS Great Lakes Science Center 2000). With the recent introduction to, and spread of, dreissenids in the waters of the southwestern and western United States (USGS 2009), the costs for management, control and mitigation of dreissenid mussels in water bodies and raw-water-using facilities are likely to continue to increase in the foreseeable future.

1.3 North American Invasion

In North America, specimens of *D. polymorpha* were first discovered on natural gas wellheads in Lake Erie (Ontario, Canada) during the summer of 1986 (Carlton 2008), although their presence was not formally published until they were discovered in Lake St. Clair on June 1, 1988 (Hebert *et al*. 1989). Subsequently, specimens of *D. rostriformis bugensis* were first discovered in North America near Port Colborne (Ontario, Canada) in Lake Erie in September 1989 (Mills *et al*. 1996). It is generally accepted that commercial, transoceanic ships accidentally transported dreissenid mussel veligers in ballast water taken on in European freshwater ports and released them by dumping that ballast water into the Laurentian Great Lakes before entering a port (Hebert *et al*. 1989). As a result, United States Federal legislation was passed requiring transoceanic vessels to exchange ballast water at sea to destroy potential aquatic hitchhikers prior to entering the St. Lawrence Seaway and the Great Lakes (Nonindigenous Aquatic Nuisance Prevention and Control Act, 104 Stat. 4761, 16 U.S.C. 4701, enacted November 29, 1990). Others suggest that it is equally plausible that dreissenids were introduced to the Great Lakes as adults byssally attached to the

anchors and/or anchor chains of transoceanic vessels (Ram and McMahon 1996), particularly as adult dreissenids can survive emersion for long periods in cool moist conditions (McMahon *et al.* 1993, Ricciardi *et al.* 1995). Changes in legislature and economics have been shown to affect invasion patterns of *D. polymorpha*. Irish water bodies were recently colonized by *D. polymorpha* following abolishment of the Value-Added Tax on boats, which stimulated the importation of recreational barges formerly plying mussel-infested British freshwaters for use on canals in Ireland without prior decontamination of their mussel infested hulls (Pollux *et al*. 2003).

The rapid spread of *D. polymorpha* through the interconnected, navigable waterways of North America has been largely due to downstream hydrological transport of planktonic larval stages and upstream anthropomorphic movement of adults attached to watercraft, particularly barge hulls (Johnson and Carlton 1996). Within one year of being reported in Lake St. Clair (MI) in 1988 (Hebert *et al.* 1989), *D. polymorpha* populations were found in all of the Great Lakes. By 1991 they were recorded in the Illinois and upper Mississippi Rivers. Currently, the North American distribution of *D. polymorpha* includes the majority of freshwater river drainages and lakes in the United States east of the 100th Meridian (100° west longitude) with newly introduced populations in Offutt Air Force Base Lake (NE) and San Justo Lake (CA) (Figure A1).

The dispersal of *D. rostriformis bugensis* throughout the Laurentian Great Lakes was considerably slower than that of *D. polymorpha* (Mills *et al*. 1996). *D. rostriformis bugensis* populations have generally been restricted to the lower Great Lakes, the St. Lawrence River, and several drainages associated with the Erie Barge Canal (NY).

Between 1995 and 2005, populations of *D. rostriformis bugensis* have also been recorded in the Mississippi River near St. Louis (MO) and at several sites in the lower Ohio River. In January 2007, *D. rostriformis bugensis* was discovered in the lower Colorado River at Lake Mead (NV/AZ) and, soon afterward, downstream in Lakes Mohave (NV/AZ) and Havasu (CA/AZ) where veliger larvae from Lake Mead were presumed to have been hydrologically dispersed (USGS 2009). Soon after their discovery in the Lower Colorado River, *D. rostriformis bugensis* populations were reported in numerous southern California reservoirs that received raw water, the presumed transport vector for this species' veliger larvae, diverted into these distant water bodies through piping and canals from Lakes Mead, Havasu and Mohave (Figure A1). Because of the abundance of such artificial interconnections and the heavy recreational boater traffic among drainage systems, *D. rostriformis bugensis* will likely continue to expand its range in the southwestern United States unless rigorous efforts are made to manage and control its anthropomorphic dispersal vectors (Western Regional Panel on Aquatic Invasive Species 2009).

Further distribution of *D. polymorpha* and *D. rostriformis bugensis* to uninfested North American drainage systems and isolated water bodies will require overland transport where they are not connected to infested waters by raw-water conveyance systems and navigable canals. While long distance overland transport events are probably rare (<1% of boater movements) they do represent a threat to non-infested water bodies (Buchan and Padilla 1999). Britton and McMahon (2005) analyzed data from boater surveys conducted in the western United States. Their study recorded 793

separate water bodies visited by boaters. Of these, 93 water bodies not harboring *D. polymorpha* and *D rostriformis bugensis* populations in 16 different states and a Canadian province were visited by boaters who had previously launched in areas infested with one or both species. Their analysis identified ten lakes that were particularly at risk of dreissenid introduction based on the level of boater traffic they received from infested reservoirs. Of these ten identified, at-risk lakes, six now harbor confirmed adult *D. polymorpha* populations. Dreissenid veligers (but not adults) have been found in tail waters below a seventh (Lake Francis Case, SD). The *D. rostriformis bugensis* population recently discovered in Lake Mead (NV/AZ) was, at that time, more than 2,000 km from the nearest known population dominated by this species in Lake Michigan, further suggesting that long distance, overland, anthropomorphic dispersal of dreissenids is not only possible, but likely to occur, even though successful establishment of new populations may be a rare event. However, the intensity of overland transport through recreational boating is likely to assure eventual dispersal of dreissenids into the majority of suitable, presently uninfested freshwater habitats in North America if efforts are not made on a cooperative and integrated interstate and Federal basis to regulate, manage and prevent the overland transport of mussels by this vector.

The research reported here provides insight into the population genetics and thermal biology of *D. polymorpha* and *D. rostriformis bugensis* populations of recently invaded warm water bodies of the southwestern United States. These studies included the chronic and acute upper thermal limits of southwestern populations of *D.*

polymorpha and *D. rostriformis bugensis* (Chapter 2), the impact of nutritional/physiologic condition in southwestern populations of *D. polymorpha* on thermal tolerance and survival in warm southwestern U.S. waters (Chapter 3), and the genetic characterization of *D. polymorpha* and *D. rostriformis bugensis* in both past, and recently invaded U.S. water bodies (Chapter 4). The implications presented by the resulting data for further dreissenid invasion of North American water bodies, particularly warm southwestern U.S. water bodies, were reviewed in regard to the potential for natural selection of increased thermal tolerance, long-term population persistence, and the genetic basis for successful establishment of new populations in previously uninfested waters. General conclusions were then drawn on the invasion dynamics of both species in regard to the potential effectiveness of present methods and regulations for preventing their establishment in previously uninfested water bodies by overland transport through recreational boating or other anthropomorphic vectors.

CHAPTER 2

ACUTE AND CHRONIC UPPER THERMAL LIMITS OF *DREISSENA POLYMORPHA* AND *D. ROSTRIFORMIS BUGENSIS* IN THE SOUTHWESTERN UNITED STATES

2.1 Introduction

2.1.1 Dreissenid Thermal Biology

The upper thermal tolerance limits of *D. polymorpha*, the zebra mussel, and *D. rostriformis bugensis*, the quagga mussel, appear to be among the most important physiological factors affecting their eventual North American distribution and potential impact on southern inland waters (Strayer 1991, Neary and Leach 1992, Ramcharan *et al.* 1992, Cohen and Weinstein 1998, Drake and Bossenbroek 2004). Dreissenid mussels can survive in liquid water at 0°C for extended periods. However, growth and fertilization does not generally occur in *D. polymorpha* below 10°C (Sprung 1987, Karatayev *et al*. 1998) and spawning is generally initiated at 12°C and maximized above 18°C (McMahon 1996). In contrast, approximately 76% of female individuals of *D. rostriformis bugensis* collected from Lake Erie (ON, CA), when water temperatures were just 4.8°C, had mature eggs in their gonads or had recently spawned indicating that critical reproductive temperatures for this species may be considerably lower than those for *D. polymorpha* (Roe and MacIsaac 1997). Most, if not all, temperate North America water bodies seasonally attain temperatures capable of supporting dreissenid growth and reproduction. Therefore, the upper thermal tolerance limits of *D.*

polymorpha and *D. rostriformis bugensis* are more likely to restrict their eventual North American distributions rather than these species' lower thermal limits or temperatures for reproduction and larval settlement.

The chronic, upper thermal limit for dreissenids has been a matter of some debate. Some authors report that specimens of *D. polymorpha* can be held at 30°C for extended periods (McMahon *et al*. 1995, Spidle *et al.* 1995), while others report high levels of mortality at this temperature (Stoeckmann and Garton 2001). Stoeckmann and Garton (2001) found that mussels fed a suspension of dried algae experienced high levels of mortality after three weeks at 30°C. Generally, North American populations of *D. rostriformis bugensis* are reported to have lower chronic thermal tolerances than populations of *D. polymorpha* (Domm *et al*. 1993, Spidle 1994, Spidle *et al*. 1995, Thorp *et al*. 1998). In contrast, individuals of *D. rostriformis bugensis* within their native Eurasian range are reported to have an upper thermal tolerance limit greater than that of *D. polymorpha* (Antonov and Shkorbatov 1990, as cited in Mills *et al*. 1996, Orlova *et al*. 2005). Long-term seasonal temperature acclimatization (i.e., long-term seasonal variation in thermal tolerance that cannot be eliminated by laboratory acclimation to a constant temperature) (Hernandez 1995) and short-term laboratory temperature acclimation (McMahon *et al*. 1994, McMahon and Ussery 1995, McMahon *et al.* 1995, Rajagopal *et al*. 2005a) were positively correlated with chronic upper thermal tolerance limits of *D. polymorpha* and may account for the variation in thermal tolerance limits reported for this species (McMahon 1996). Reproductive effort is also reported to reduce the body mass (i.e., body energy stores) of adult *D. polymorpha* by

as much as 30% (Sprung 1991) and has been associated with major reductions in chronic thermal tolerance in this species (Rajagopal *et al*. 2005a).

2.1.2 Thermal Adaptation in Invasive Dreissenids

There are growing concerns that, as populations of *D. polymorpha* and *D. rostriformis bugensis* disperse into the warm water bodies of the southwestern United States, they will thermally adapt to elevated water temperatures, which could facilitate further spread into warm southwestern water bodies from which they have previously been presumed to be thermally excluded. Selection for thermal tolerance has not been well documented in most aquatic species. However, eastern Pacific abalone (*Haliotis* spp.) from warmer climates reportedly have adapted to thermal stress such that there are latitudinally correlated differences in cytosolic malate dehydrogenase activity of populations under varying thermal stresses (Dahlhoff and Somero 1993a, 1993b). Similarly, cellular-level adaptations to thermal stress have been reported for congeneric species of the eastern Pacific barracuda (*Sphyraena* spp.) (Lin and Somero 1995). In contrast, Elderkin and Klerks (2005) could find no evidence for *D. polymorpha* population genetic differentiation along a latitudinal gradient in the Mississippi River (LA) even though there was strong selection pressure for increased thermal tolerance among those individuals where they experienced substantial late summer mortality after ambient water temperatures exceeded tolerated incipient upper thermal limits.

Despite strong selection pressures, thermal adaptation may not occur in *D. polymorpha* populations in the lower Mississippi River (LA) due to high levels of gene flow via downstream hydrological transport of veliger larvae from the cooler, more

northern portions of its drainage (Stoeckel *et al.* 1997, 2004), where ambient temperatures do not approach the upper thermal limit of this species. Additionally, much of the reproductive output of dreissenids in the lower Mississippi River is carried on water currents to the Gulf of Mexico where they cannot survive. Elderkin and Klerks (2005) also suggest that there may be insufficient genetic variation in thermal tolerance among North American *D. polymorpha* populations for thermal adaptation to occur. Alternatively, Hernandez (1995) reported elevated, chronic thermal tolerance times on exposure to 33°C for a *D. polymorpha* population in the lower Mississippi River (LA) relative to a northern population in the Niagara River (NY). His results indicated that only thermally tolerant individuals survived annual late-summer thermal selection events leading to a thermally tolerant adult population, despite substantial downstream gene flow from the cooler, northern reaches of the Mississippi drainage.

If extreme thermal selection can annually generate thermally tolerant populations of *D. polymorpha* in the lower Mississippi River (LA) in spite of substantial downstream gene flow from the cool northern portions of its drainage (Hernandez 1995), it is possible that the recent establishment of dreissenid mussels in some of the isolated warm southwestern water bodies of the United States (i.e., not subjected to substantial downstream gene flow from cooler northern portions of their drainage) could drive even more rapid and extensive thermal selection of thermally resistant lineages of dreissenids than may have occurred in the lower Mississippi River (LA) (Hernandez 1995). Indeed, Huey *et al*. (2000) reported that the Old World fruit fly, *Drosophila subobscura,* evolved a geographic cline in wing size over a relatively short

period after being introduced to North America, indicating that evolution of selectively advantageous traits following invasion can be rapid. Similarly, the European wild rabbit, *Oryctolagus cuniculus*, evolved heritable morphologies related to thermal tolerance within 30 y of introduction to Australia (Williams and Moore 1989). Allen *et al*. (2006) showed that mutation rates, and hence the ability to evolve, in aquatic ectotherms increased exponentially with increasing temperature, suggesting that dreissenid populations on the warm, southwestern periphery of their North American range may be more likely to undergo mutation than those in relatively cooler, higher latitude, water bodies.

Allozyme analysis of *D. polymorpha* has shown that there is a high degree of individual heterozygosity (Hebert *et al.* 1989) and, therefore, this species should have high potential to respond to selection pressures and adapt to environmental change (Marsden *et al.* 1996). The overland transport and introduction of dreissenid mussels into isolated, uninfested water bodies via trailered boats and other transported objects previously submerged in mussel infested waters may, through founder effects, form thermally tolerant lineages capable of dispersing to new, warmer locations in North America if, by chance, these populations are founded by a relatively few, thermally tolerant individuals (Lee 2002). Given the strong thermal selection pressures, increased mutation rates, and documented cases of rapid adaptation in other introduced animal species, the possibility that dreissenid mussels recently established in warm-water bodies in the southwestern United States (United States Geological Service 2009) are undergoing rapid evolution of increased thermal tolerance should be investigated.

To investigate the possibility that dreissenid mussel populations recently established in southwestern U.S. water bodies are undergoing rapid evolutionary change in their thermal tolerance, the thermal tolerance of populations of *D. polymorpha* first discovered in Lake Oologah (OK) in 2003 and Winfield City Lake (KS) in 2006 were assessed and compared to that of a northern population in Hedges Lake (NY) (United States Geological Service 2009). In addition, the chronic thermal tolerance limits of a *D. rostriformis bugensis* population first discovered in Lake Mead (NV/AZ) in 2007 were determined and compared to those previously published for this species and to the result attained for the two southwestern *D. polymorpha* populations. The results were compared to published thermal tolerance estimates for both species in order to assess the potential for rapid thermal tolerance adaption in these recently established populations. The potential ecological and economic consequences from development of thermally-tolerant lineages of both species are also discussed.

2.2 Methods

2.2.1 Collection Sites

2.2.1.1 *Dreissena polymorpha*

Specimens of *D. polymorpha* were collected for thermal tolerance determinations from three different sites including Hedges Lake (NY), Lake Oologah (OK) and Winfield City Lake (KS). The three sites are described in greater detail below.

Lake Oologah transects Rogers and Nowata Counties approximately 35 km northeast of Tulsa (OK) (Figure A2). It was created during the early 1960s by

impoundment of the Verdigris River and has a surface area of approximately 125.5 km^2. The lake's water is moderately hard and borderline mesotrophic/eutrophic with wind action and low residence time preventing strong thermal stratification or formation of an anoxic hypoliminion (U.S. Army Corps of Engineers 2001). Zebra mussels, *D. polymorpha*, were first recorded in Lake Oologah (OK) in 2003, but they were likely to have been introduced in 2000 or 2001. The collection site for this study was located at the Redbud Bay Marina (36.4202°N, 95.6664°W; Figure A3) along the southern edge of the lake near State Route 88.

Hedges Lake is located in Washington County (NY) approximately 7 km north of Cambridge (NY) along State Route 22 (Figure A2). The coordinates for the collection site were 43.1068°N, 73.3838°W (Figure A4). The lake covers approximately 0.5 km^2 and little is known about its physical parameters including trophic condition, temperature profile, or time of *D. polymorpha* introduction.

Winfield City Lake is located in Cowley County (KS) approximately 14 km northeast of Winfield (KS) (Figure A2). The coordinates for the collection site were 37.3454°N, 96.8959°W (Figure A5). The lake has a surface area of approximately 5 km^2. Specimens of *D. polymorpha* were first discovered in Winfield City Lake (KS) in December 2006 (Nance 2008) but were likely introduced in 2004 or 2005. Like Hedges Lake (NY), there is little information available on Winfield City Lake's physical parameters including trophic condition or temperature profiles.

2.2.1.2 *Dreissena rostriformis bugensis*

Specimens of *D. rostriformis bugensis* were collected from Sentinel Island in the Boulder Basin of Lake Mead (36.0575°N, 114.7455°W) which is located on the border of Clark County (NV) and Mohave County (AZ). Lake Mead is approximately 58 km east of Las Vegas (NV) (Figures A2 and A6). It was created in 1935 by impoundment of the Colorado, Muddy and Virgin Rivers by Hoover Dam. The lake's water is very hard, mesotrophic and has a surface area of approximately 660 km^2 (USGS 1998). Lake Mead has an average depth of 69 m, a maximum depth of 180 m, and a retention time of approximately 3.9 y. The lake thermally stratifies in the summer but does not form a hypoxic hypolimnion. Specimens of *D. rostriformis bugensis* were first discovered in Lake Mead (NV/AZ) in January 2007 (Stokstad 2007). Analysis of the size distributions of samples of *D. rostriformis bugensis* from four sites in the Boulder Basin of Lake Mead indicated that the mussels were probably introduced to the lake in 2003–2004 (McMahon 2007).

2.2.1.3 Water Temperature Monitoring at Collection Sites

Temperature dataloggers (Onset Corp® model# UA-002-64) were immersed at depths of 1, 2, 4, and 8 m on a weighted rope suspended from docks at the Redbud Bay Marina, Lake Oologah, *D. polymorpha* collection site. Dataloggers recorded ambient water temperatures hourly from 30 June 2006 to 31 August 2008. Dataloggers placed at 2 m and 4 m below the surface of the water represented the approximate range of depths from which samples were collected. The lake bottom was approximately 8 m from the water's surface under typical hydrological conditions (i.e., non-flood or dry season).

Similarly, ambient water temperature was recorded hourly with dataloggers suspended from the Winfield City Lake (KS) *D. polymorpha* collection site at depths of 1, 2, and 4 m from 30 June 2008 to 15 October 2008. The datalogger placed 1 m below the surface of the water represented the approximate depth from which specimens were collected. The lake bottom was approximately 4 m from the water's surface at the collection site under normal hydrological (i.e., non-flood or dry season) conditions.

Temperature dataloggers could not be deployed at the collection site in Hedges Lake (NY). However, ambient lake water temperature and dissolved oxygen (DO) profiles at depths of 0, 4.5, 9, 18 and 23 m were recorded on 22 June 2006, 23 August 2006, 13 March 2007, and 20 August 2007 and provided to the author by Steve Butz, an earth and environmental science teacher from the Cambridge High School in the Cambridge, NY, Central School District.

Ambient temperature and dissolved oxygen (DO) profiles taken for Lake Mead (NV/AZ) from 1 January 2006 to 23 January 2007 at the United States Geological Survey (USGS) station at Sentinel Island were provided by Ronald Veley of the USGS. Ambient water temperature data through time was extracted from the USGS data set for depths of 1, 12, 15, 20, 30 and 70 m.

2.2.2 Collection and Holding Conditions

Specimens of *D. polymorpha* from Lake Oologah (OK) were collected on 29 June 2006 and 29 June 2007 by hauling submerged, dead, trees infested with mussels to the surface. Submerged trees were typically suspended between 2–4 m below the surface of the water from the docks at the Redbud Bay Marina. Mussel-infested

branches were trimmed from trees with anvil loppers and immediately placed into a large cooler (82 cm x 36 cm x 38 cm) filled to a depth of approximately 2.5 cm with lake water taken at the site. Branches with emersed mussels were wrapped with lake-water soaked newspaper to buffer them from high temperature stress and to increase humidity levels during transport. Plastic bags filled with ice were placed in the cooler to prevent heat stress during specimen transportation to The University of Texas at Arlington (UT Arlington), which occurred within twelve hours of collection. Upon arrival, specimens were carefully removed from the branches by cutting their byssal threads with a scalpel.

Individuals from Hedges Lake (NY) were collected on 6 July 2006 and 26 June 2007 by scraping mussels with a single-sided razor or sharp-edged trowel from hard, submerged substrata (i.e., rock and metal surfaces). As with specimens collected in Lake Oologah (OK), specimens were wrapped in paper towels or newspaper soaked with lake water and emersed in 500-ml, plastic, Nalgene® containers. The containers were shipped to UT Arlington overnight in a cooler with several icepacks to prevent heat stress. A minimum/maximum thermometer was placed in the shipping container in order to assess the levels of temperature stress experienced by the specimens during shipment. Maximum temperature during shipment was 16°C in 2006 and 19°C in 2007.

Specimens from Winfield City Lake (KS) were collected on various dates throughout the summer and fall of 2008 (16 June, 29 June, 19 July, 8 August, 31 August and 15 October 2008) by scraping the bottom of floating docks maintained on the lake shore by the City of Winfield. Once removed from the substratum, specimens were

wrapped in newspaper saturated with lake water taken on site and stored in an insulated container (30 cm x 18 cm x 28 cm). The container was compartmentalized such that the bottom was filled with ice and the specimens were stored above. Samples were then transported back to UT Arlington within twelve hours of collection.

Specimens of *Dreissena rostriformis bugensis* were collected from Sentinel Island, Lake Mead (NV/AZ) on 16 May 2007 by the National Park Service. Mussels were scraped off of hard substrates by divers and wrapped in paper towels soaked in lake water. Sampled individuals were placed into an insulated container and layered with wet paper towels to buffer temperature and maintain high relative humidity. Frozen plastic water bottles were placed in the insulated container to prevent high temperature stress. Containers were shipped overnight to UT Arlington.

Upon arrival to the laboratory at UT Arlington, samples from all collecting sites were inspected for damage before being placed into square, 1-L plastic containers containing dechlorinated, City of Arlington, Texas, tap water (CADTW) which was initially warmed or cooled to match the air temperature inside the shipping container, preventing temperature shock. The containers were then placed on the laboratory bench where they were allowed to reach room temperature (≈20°C) before being covered with a 3.5-mm nylon mesh to prevent mussel escape. Samples of individuals (n > 1000) were placed in six to ten 1-L plastic containers and emersed in round, 1.1-kL fiberglass acclimation tanks containing 800 L of constantly circulated, aerated, CADTW initially held at 20°C. Acclimation tanks were fitted with a gravel filter capable of providing both biological and mechanical filtration with an approximate 1-h turnover rate and

were held in a constant-temperature, aquatic holding facility with an air temperature of 20°C. Acclimation tanks containing specimens were then brought to temperatures of 5, 10, 15, 20, 25 and 30°C at a rate < 3°C h^{-1}, although not all acclimation temperatures were used in each study. Specimens were then held at their respective acclimation temperatures for at least 14 d and no more than 17 d prior to thermal tolerance testing. Acclimation temperatures of 5–15°C were maintained with a circulating constant temperature chiller (Frigid Units, Inc.® model BHL-1089-3) while acclimation temperatures of 25–30°C where maintained with a 1500 W submersible stainless steel heating element and digital controller (Clepco® model QDMMML15). The 20°C acclimation tank did not require temperature regulation as it remained ± 1°C of the 20°C holding room air temperature. Containers with mussels were placed on submerged plastic shelving 5–12 cm below the surface of the water and were individually aerated to maintain internal oxygen levels and water circulation.

2.2.3 Chronic Upper Limit Testing

Following acclimation, specimens were removed from each acclimation holding tank and separated into groups of 14–32 individuals each, without size bias, which were then placed into 300-ml plastic testing containers filled with water from their acclimation tank. Containers were covered with 3.5-mm nylon mesh to prevent mussel escape and maintained in the laboratory until contained water reached air temperature (20°C). Thereafter they were randomly placed into plastic experimental tanks (56 cm x 34 cm x 21 cm) (Figure A7) containing 34 L of 20°C CADTW to avoid temperature shock to specimens. Each test tank contained a holding container, each with a sample

of individuals from each acclimation temperature and concurrently tested population. Tanks were fitted with a circulating heater (Haake® models C1 or D1, Techne® model TE-8J or TE-10A) whose outflow was passed via Tygon® tubing through a 1-L biological filter containing small pebbles, crushed coral and filter floss, allowing the entire water volume of the testing tanks to be circulated once every 3 min. Additionally, each experimental tank was constantly aerated through both a sponge filter (Lee's Aquarium and Pet Products, Inc.® model# 13390) and an air-stone (Figure A7). Thermostats on the circulating heaters were then set to the appropriate test temperatures that were reached within approximately 1 h. Tests of chronic upper thermal limits were performed from 2006–2008 on specimens from different collection sites and collection dates as described below.

2.2.3.1 *D. polymorpha* from Lake Oologah and Hedges Lake

Specimens of *D. polymorpha* collected from both Hedges Lake (NY) on 6 July 2006 and Lake Oologah (OK) on 29 June 2006 were held in a 20°C acclimation tank for 33 d and 40 d, respectively before being divided into six different acclimation groups and acclimated to temperatures of 5, 10, 15, 20, 25 and 30°C for 14 d as described above. Specimens were not fed during temperature acclimation or thermal tolerance testing. Their chronic thermal tolerance limits were determined from constant exposures to test temperatures of 28–34°C in 1°C increments as described above (Table B1). Acclimation/population groups (n = 29–32) were held at test temperatures until 100% sample mortality was recorded or for 28 d if 100% sample mortality did not occur (McMahon and Ussery 1995).

Specimens of *D. polymorpha* from both Hedges Lake (NY) (collected 26 June 2007) and Lake Oologah Lake (OK) (collected 29 June 2007) were acclimated to 20, 25, and 30°C for 17 d as described above. Prior to the collection of Lake Oologah specimens and the onset of acclimation, individuals from Hedges Lake (NY) were held in a 20°C acclimation tank for 3 d. During acclimation, specimens were fed a 50/50 (v/v) solution of Phytoplex® and Micro-Vert® (Kent Marine® #PPX64 and #MV64) daily by using a glass pipette to add 5.0 ml volume of feeding solution directly into each mussel container. One hour after feeding water in holding containers was decanted into the acclimation tank and the containers immediately re-immersed. During the 1-h feeding period, both feces and pseudofeces accumulated on the bottom of holding containers indicative of mussel filtering and consumption of the food particles introduced with the feeding solution. Treatment test temperatures were 26–34°C in 1°C increments. Two lower test temperatures of 26 and 27°C were utilized in this experiment in order to assure that the tested temperature range included non-lethal temperatures because significant mortality was unexpectedly recorded at the lowest test temperature of 28°C in the 2006 testing (previous chronic thermal testing with this species had indicated that 30°C was the incipient upper thermal limit for *D. polymorpha*; McMahon *et al*. 1994, 1995) For each acclimation/population group (n = 16–23), two containers were placed into test tanks (Table B2).

During temperature tolerance testing, specimens in one of the two acclimation/collection group containers were fed every two days for 1 h as carried out during acclimation holding periods with the exception that after feeding, container

water was decanted outside of the test tank and test tank water replenished with fresh CADTW. In contrast, the second acclimation/collection sample was not fed during the entire 28-d testing period (Table B2).

During chronic temperature tolerance testing, test specimens were inspected for viability approximately every 3 h for the first 12 h of exposure, every 4 h for next 12 h, every 6 h for the next 24 h, every 12 h for the next 8 d and at least once a day for the remaining 20 d of the 28-d exposure period using methodology modified from that of McMahon *et al*. (1994, 1995). Viability checks involved inspecting containers for any specimens with widely gaping valves. If specimens were found to exhibit gaping valves, containers (still containing water) were removed from the test tank and gaping individuals gently prodded on the external surfaces of their shell valves with a pair of blunt-end forceps. Specimens not responding to this initial stimulus by immediate shell valve closure were then gently stimulated in the area of their inhalant and exhalent siphons with the tips of the forceps. If individuals did not respond to this latter stimulus by immediate valve closure, the forceps were then used to forcibly close their shell valves. If closed shell valves widely gaped immediately after release from the forceps the specimen was considered to be dead. Previous testing by McMahon *et al*. (1995) indicated that non-responsive, widely-gaping mussels exposed to thermal stress are dead and do not recover when returned to water at room temperature (20°C). Dead individuals were removed from the container and their time to death and shell length (i.e., the greatest distance from the anterior tip of the umbos to the posterior shell valve margins measured to the nearest 0.1 mm with dial calipers) recorded. If an individual's

shell valves remained adducted or did not widely gape after being forcibly closed, the mussel was considered to be viable and returned to the testing container which was them re-immersed in the test tank. Testing continued until all individuals in all samples either died or until the experiment was terminated after the 28-d exposure period.

After each viability test, water from all test containers was decanted outside of the test tank before being re-immersed in the test tank. Water levels in the test tank were replenished as needed during the course of the 28-d testing period. One-third of water in test tanks was periodically exchanged for fresh CADTW during periods of high sample mortality in order to prevent water fouling. At the end of the 28-d experimental period, any surviving specimens had their shell lengths measured to the nearest 0.1 mm with dial calipers and were recorded as having survived the treatment.

2.2.3.2 *D. polymorpha* from Winfield City Lake

Specimens of *D. polymorpha* were collected on 16 June and 31 August 2008, from Winfield City Lake (KS). As described above, specimens from both collections were acclimated to 20°C without feeding for 14 d. For each collection, acclimated specimens were subjected to chronic thermal tolerance testing as described above with the exception that specimens were randomly assigned to three different testing containers, each holding 15 individuals, to avoid overcrowding. Treatment test temperatures were 26–34°C in 1°C increments. Specimens were not fed during the experiment (Table B3). Viability testing procedures during the course of the 28-d exposure period were the same as that described for test specimens in Section 2.2.3.1 above.

2.2.3.3 *D. rostriformis bugensis* from Lake Mead

Specimens of *D. rostriformis bugensis* were collected on 16 May 2007. The sample was divided into two subsamples with one subsample being acclimated to 20°C and the second to 25°C. Specimens were fed daily during the acclimation period as described above for *D. polymorpha* (see Section 2.2.3.1 above). Acclimation to 25°C resulted in 100% mortality leaving only 20°C-acclimated individuals to be used for chronic thermal tolerance testing. Because 100% mortality occurred at 25°C the initial range of treatment temperatures tested ranged from 20–27°C at 1°C intervals. For each test temperature, specimens were randomly placed into subsamples (n = 19–20) in separate 300-ml containers which were fed or not fed as described above for *D. polymorpha* in Section 2.2.3.1 (Table B4). Fed mussel treatments received 0.5 ml of food solution pipetted into the testing containers for 1 h every 2 d over the 28-d exposure period as described for *D. polymorpha* thermal testing in Section 2.2.3.1. *D. rostriformis bugensis* survival was high (> 70%) over the 28-d exposure to these initial test temperatures, suggesting that the previously observed mortality at the 25°C acclimation temperature was not the result of thermal stress. Thus, specimens surviving this initial 28-d experimental treatment were subsequently re-acclimated to 20°C with daily feeding for 14 d after which their chronic thermal tolerance times to a higher range of temperatures (i.e., 28–33°C at 1°C intervals) were tested. As with prior testing, paired subsamples (n = 14–16) of fed and unfed individuals were placed in thermal test tanks at constant water temperatures ranging from 28–33°C in 1°C increments with one of the two subsamples being fed every 2 d during the testing period

(Table B4). Viability testing of individuals in the subsamples was carried out over the same schedule during the course of the 28-d exposure period as described for chronic thermal testing of *D. polymorpha* in Section 2.2.3.1 above.

2.2.4 Acute Upper Lethal Temperatures in Dreissena polymorpha

Acute, upper lethal temperatures were determined for specimens of *D. polymorpha* collected from Winfield City Lake (KS) on 29 June, 19 July, 8 August, 31 August and 15 October 2008 using the methodology of McMahon and Ussery (1995). Individuals from each collection were acclimated to 20°C for a period of 12–21 d as described in Section 2.2.2. Following acclimation, 21 subsamples (n = 20) each were placed into separate, 280-ml glass containers covered with 3.5-mm nylon mesh to prevent escape. Containers with mussel samples were immersed in a 34-L plastic test tank containing CADTW at 20°C as described in Section 2.2.2.3 above. Water in the test tank was continuously aerated with an airstone and water temperature regulated with a circulating heater (Techne® model TE-8J). Water temperature in the test tank was monitored by an Omega® 866C digital thermistor. After immersing the 21 containers with contained individuals in the test tank, the circulating water heater was manually manipulated to raise the tank water temperature at a rate of 1°C every 5 min (0.2°C min^{-1}) from the initial 20°C acclimation temperature. A container holding a subsample of mussels and water from the test tank was withdrawn at 30°C and subsequently at every 1°C increase in water temperature resulting in the last subsample being removed at a water temperature of 50°C. Test containers were then placed on the bench top and allowed enough time for the water they contained to equilibrate with

room temperature (≈20°C). One hour after water in test containers attained 20°C, subsample viability was assessed and shell length measured as described for chronic, upper thermal tolerance testing in Section 2.2.3.1.

2.2.5 Statistics

Chronic thermal tolerance data contained information on sampled individuals that died at a given time or survived the 28-d experimental exposure period. Surviving individuals were treated as censored observations that provided partial information when combined with observed survival times of individuals dying during the experiment. Survival times of individuals that did not die during the 28-d experimental period could not be determined and were thus treated as "right justified" data (Lawless 2003). Survival times of individuals dying during experimentation were not known exactly, but, instead fell within an interval, I_j ($I_j = (a_{j-1},a_j)$) equivalent to the time between successive mortality checks, a_j, the time at any one observation and a_{j-1}, the time of the immediately prior observation.

The proportional hazard model (PHM) procedure from SAS® Proc PHREG was used to analyze survivorship data while accounting for size differences by treating shell length as a covariate (SAS®, Cary, North Carolina). PHREG can be used to estimate survivorship from discrete time series data where imprecise lifetime data leads to tied times of death at the end of each observation interval. In this study, identical times of death were not truly discrete but appeared to be so as a result of imprecise event time measurement. Accordingly, PHREG was modified to include a TIES=EXACT statement that calculates partial likelihoods of the model parameters (i.e., test

temperature, acclimation temperature, collection site, shell length, feeding regime and their interaction terms) after calculating the probabilities of all possible ordering of tied data. This method results in unbiased parameter estimates when there are many tied times of death. Survival models were constructed using an alpha level of 0.2 for inclusion of the model parameters into the final model. Interaction terms were added in a stepwise manner (e.g., single terms, second-order interaction, third-order interactions, etc.) keeping all subsets of higher order interactions within the model.

Following model creation, the proportional hazard (PH) assumption was assessed by examining plots of the Schoenfeld residuals (Lawless 2003) against times of death, examining plots of log negative log survival against model parameters and by using the ASSESS statement within Proc PHREG. In the event that the PH assumption was violated due to time-dependency of model parameters the analysis was stratified across that parameter. Stratified analyses were performed using an identical model to evaluate survival of data subsets defined by that variable (i.e., each treatment temperature was analyzed separately). The stratified model was based on the most complete model necessary to fit all of the stratified datasets, and thus may have included insignificant model terms for some of the separate data sets. Inclusion of time by term interactions within the model was avoided due to PHREG's inability to utilize the BASELINE statement in the presence of time-dependent terms.

Estimates of the survival function with a 95% confidence interval (CI), for standard-sized individuals at various treatment levels, were created using the BASELINE statement. These survival functions were used to graphically estimate

median survival times by determining at what time the model estimated survival function had a survival probability of 0.5 (Allison 1995). The 95% CI for any particular estimated median survival time under any set of testing conditions (i.e., test temperature, acclimation temperature, collection site, shell length, feeding regime) was estimated by determining the time at which the upper and lower 95% CIs of the survival function had a survival probability of 0.5 (Figure A8). The confidence intervals for median survival times determined by this method are highly dependent on the shape of the survival curve and were occasionally asymmetrical in this study.

The importance of factors such as shell length, collection site, temperature, acclimation temperature, and feeding regime on the estimation of the survivorship was determined by testing the General Linear Hypothesis for all terms included in the model that contained the factor in question via PHREG TEST statements. The importance of shell length, collection site, test temperature, acclimation temperature, and feeding regime on median survival times were assessed by testing the null hypothesis (H_0) of no treatment effects using the Wald Chi-square statistic to compare estimated median survival times across these factors. Pairwise Wald Chi-Square comparisons of median survival times with asymmetrical CIs were calculated by utilizing the standard error (SE) that logically approached (higher or lower) the estimate to which they were being compared (Computed from a SAS® program provided by Dr. Doyle L. Hawkins of the Department of Mathematics at UT Arlington) and corroborated by visual interpretation of the confidence intervals by the methods of Cumming *et al*. (2007). In other words, if a median time was compared to one which was greater than the upper SE of the lesser

and the lower SE of higher estimate would be utilized and vice-versa (Figure A9). Wald comparisons comparing more than two median survival time estimates with asymmetrical CIs were calculated by treating the highest and lowest survival estimates as above and calculating the SE of intermediate estimates as the arithmetic mean of its upper and lower SE values (Figure A9).

A logistic model, using SAS® Proc LOGISTIC for estimating the LT_{50} value (i.e., the estimated temperature of 50% sample mortality) was constructed using the same criteria as described above for chronic survival models, except that survivorship was modeled as time against the dependent variable of temperature for any given observation period over the 28-d exposure period. Proc LOGISTIC determination of LT_{50} values required re-coding of chronic thermal mortality data for each time of observation as a new dataset with a binary mortality term (1 = dead, 0 = alive) as the dependent variable for each individual in the study. Logistic survival curves were used to estimate survivorship as the dependent variable versus treatment temperature for any single observation period against a set of treatment variables including collection site, exposure temperature, acclimation temperature, and feeding regime for individuals with a standard shell length (SL) of 15 mm. The 15-mm standard SL was chosen because it was close to the median shell lengths of samples of *D. polymorpha* from Hedges Lake (NY), Lake Oologah (OK), and Winfield City Lake (KS) and the sample of *D. rostriformis bugensis* from Lake Mead. Bonferroni corrected Wald Chi-square tests were used to make pair-wise comparisons of treatments on LT_{50} values.

Logistic regression analysis using SAS® Proc LOGISTIC was used to calculate LT_{50} values (i.e., estimated temperature for 50% sample survival) from acute thermal tolerance data for individuals of *D. polymorpha* from individual samples taken throughout the summer and fall of 2008 at Winfield City Lake. The analysis was also utilized to perform independent contrasts of estimated survival curves across different collection dates. The logistic model was prepared with mortality as the dependent variable with an alpha value of 0.2 used for inclusion of model parameters (i.e., temperature, shell length, and collection date) as independent variables. Model fit was assessed using the LACKFIT option in SAS for application of the data to the Hosmer and Lemeshow Chi-square goodness-of-fit test (Hosmer *et al*. 1997, Hosmer and Lemeshow 2000) and the SAS RSQUARE option to determine the maximum re-scaled R^2 approximation (Nagelkerke 1991). Pairwise Wald Chi-square tests were used to test differences between LT_{50} values across various treatments.

2.3 Results

2.3.1 Chronic Upper Thermal Limits of D. polymorpha

2.3.1.1 Lake Oologah and Hedges Lake in 2006

Residuals from the Proportional Hazard Model analysis suggested that exposure temperature had a time-dependent effect on survivorship; therefore, model parameters were analyzed separately for each treatment temperature. In the model, shell length and acclimation temperature were continuous variables, while collection site was a single categorical variable. To ensure that analyses were preformed similarly across all treatment temperatures, higher order polynomials for acclimation temperature and

interactions of acclimation temperature and collection site were retained in the final model even though some were not significant at all exposure temperatures (Tables C1–C7 in Appendix C).

A test of the General Linear Hypothesis for all model terms containing the collection site variable indicated that collection site significantly affected the survival function, either directly or through interactions with acclimation temperature and/or shell length, at all treatment temperatures except 32°C (Tables C1–C7 in Appendix C). Adult specimens from Hedges Lake (NY) acclimated to 20°C, with a standard SL of 15 mm, experienced near-linear mortality at temperatures ranging from 28–30°C throughout the 28-d exposure period with mortality rate increasing with increased temperature. At temperatures >30°C mortality was much more rapid with survivorship decreasing exponentially with time of exposure (Figure A10A). In contrast, the Proportional Hazards Model estimated that 100% sample mortality was not achieved within the 28-d treatment duration at temperatures <31°C (Figure A10A). For Lake Oologah (OK) in 2006, estimated mortality rates for a 20°C acclimated, standard 15-mm SL individual at 28–30°C were greater than those of individuals from Hedges Lake (NY) (Figure A10B). Further, estimated sample morality was less than 100% at temperatures <30°C after the 28-d exposure period compared to <31°C for Hedges Lake (NY) mussels (Figures A10A and A10B). At 28–30°C, standard 15-mm SL Hedges Lake (NY) individuals acclimated to 20°C survived significantly longer than Lake Oologah (OK) specimens with median survival times of 490h vs 230h at 28°C, 413h vs 262h at 29°C, and 326h vs 143h at 30°C (28°C: Wald Chi-square = 22.26, $df = 1$, $P <$

0.0001; 29°C: Wald Chi-square = 7.23, *df* = 1, *P* = 0.0071; 30°C: Wald Chi-square = 12.75, *df* = 1, *P* = 0.0004) (Figure A11). Conversely, survivorship was not significantly different ($P > 0.05$) among Hedges Lakes (NY) and Lake Oologah (OK) mussels at test temperatures ≥31°C, with the exception that Lake Oologah specimens had a significantly increased survivorship relative to Hedges Lake individuals at 33°C (Wald Chi-square = 43.49, *df* = 1, $P < 0.0001$; Figure A11).

Testing the General Linear Hypothesis for all model terms containing acclimation temperature indicated that it significantly affected survivorship at all treatment temperatures (i.e., 28–34°C), either directly or through interactions with collection site and/or shell length (Tables C1–C7 in Appendix C). Standard 15-mm SL adults from Lake Oologah (OK) displayed a tendency for increased median survival time with increasing acclimation temperature at all treatment temperatures while standard-sized individuals from Hedges Lake (NY) displayed a similar tendency except that median survival times were maximized among 25°C acclimated individuals and reduced among 30°C acclimated mussels at test temperatures ranging from 28–32°C (Figure A12). Generally, a standard 15-mm SL Hedges Lake (NY) individual survived longer than a standard Lake Oologah (OK) mussel when acclimated to ≤20°C and exposed to test temperatures of ≤30°C. Thus, following Bonferroni correction, after acclimation to 5°C and subsequent exposure to 28°C, standard 15-mm SL Hedges Lake (NY) individuals had significantly higher ($P < 0.008$) median survival times than did individuals from Lake Oologah (OK) (Wald Chi-square = 30.1259, *df* = 1, $P < 0.0001$) (Figure A12). Similarly, Hedges Lake (NY) individuals acclimated to 10°C were more

tolerant of exposure to 28°C (Wald Chi-square = 27.3134, $df = 1$, $P < 0.0001$), 29°C (Wald Chi-square = 29.0698, $df = 1$, $P < 0.0001$), and 34°C (Wald Chi-square = 10.7532, $df = 1$, $P = 0.001$). Hedges Lake (NY) individuals acclimated to 15°C were also more tolerant of exposure to 28°C (Wald Chi-square = 24.4749, $df = 1$, $P < 0.0001$), 29°C (Wald Chi-square = 18.5911, $df = 1$, $P < 0.0001$), and 34°C (Wald Chi-square = 46.2278, $df = 1$, $P < 0.0001$), after acclimation to 20°C were more tolerant of exposure to 28°C (Wald Chi-square = 22.2617, $df = 1$, $P < 0.0001$), 29°C (Wald Chi-square = 7.2367, $df = 1$, $P = 0.0071$), and 30°C (Wald Chi-square = 12.7559, $df = 1$, $P = 0.0004$), after acclimation to 25°C were more tolerant of exposure to 29°C (Wald Chi-square = 9.9357, $df = 1$, $P = 0.0016$), and after acclimation to 30°C were more tolerant of exposure to 34°C (Wald Chi-square = 46.2278, $df = 1$, $P < 0.0001$) (Figure A12).

In contrast, a standard 15-mm Lake Oologah (OK) individual survived as long, or longer, than a standard-sized Hedges Lake (NY) mussel when acclimated to temperatures >25°C and exposed to temperatures ≥28°C (Figure A12). Thus, a standard 15-mm SL Lake Oologah (OK) individual survived longer than a standard-sized specimen from Hedges Lake (NY) when acclimated to 15°C and exposed to 33°C (Wald Chi-square = 16.6420, $df = 1$, $P < 0.0001$), when acclimated to 20°C and exposed to 33°C (Wald Chi-square = 42.4528, $df = 1$, $P < 0.0001$), and when acclimated to 25°C and exposed to 33° (Wald Chi-square = 14.3611, $df = 1$, $P = 0.0002$) and 34°C (Wald Chi-square = 46.2278, $df = 1$, $P < 0.0001$) (Figure A12).

Comparisons between acclimation temperatures within collection sites (using a Bonferroni-corrected pair-wise Wald Chi-square Test) showed that median survival

times among standard 15-mm SL specimens were generally not significantly different among the 5, 10, and 15°C acclimation groups for both the Hedges Lake (NY) (Table B5) and Lake Oologah (OK) samples (Table B6) at all test temperatures (Figure A12). In contrast, the median survival times of a 15-mm SL standard-sized specimen from either population acclimated to 20, 25 and 30°C were generally greater than those of individuals acclimated 5, 10 and 15°C (Figure A12, Tables B5 and B6). The data for Lake Oologah (OK) specimens indicated a significant tendency for increased temperature tolerance with increasing acclimation temperature from 20–30°C on exposure to test temperatures ≥30°C (Figure A12, Table B6). Similarly, although less apparent, there was a significant tendency for increased thermal tolerance among Hedges Lake (NY) specimens with increasing acclimation temperature from 20–30°C on exposure to test temperatures ranging from 31–34°C (Figure A12, Table B5). In contrast, at test temperatures ≤30°C, acclimation temperature had no effect on the thermal tolerance of a standard-sized Hedges Lake (NY) mussel with the exception of 25°C acclimated individuals having a significantly higher median survival time than 5°C and 15°C acclimated individuals at a test temperature of 29°C and of 5°C acclimated individuals having a significantly lower median survival time than 15, 20, and 25°C acclimated individuals at a test temperature of 30°C (Figure A12 and Table B5).

Wald Chi-square comparisons indicated that estimated median survival times for Hedges Lake (NY) individuals with standard SL of 10, 15 and 20 mm, increased significantly with increasing SL only at test temperatures of 28°C (Wald Chi-square =

6.80, *df* = 2, *P* = 0.0333; Figure A13A and A13B). Similarly, Lake Oologah (OK) specimens had significantly greater median survival times with increased SL only at test temperatures of 29°C and 33°C (29°C: Wald Chi-square = 8.79, *df* = 2, *P* = 0.0123; 33°C: Wald Chi-square = 10.02, *df* = 2, *P* = 0.0067) (Figure A13C and A13D). Somewhat surprisingly, when data for the two populations were combined, the General Linear Hypotheses for all model terms containing SL indicated that it significantly affected survivorship, either directly or through interactions with acclimation temperature and/or collection site, at the majority of test temperatures including 28°C (Wald Chi square = 16.16, *df* = 4, *P* = 0.0028), 29°C Wald Chi square = 22.59, *df* = 4, *P* = 0.0002), 30°C (Wald Chi square = 10.05, *df* = 4, *P* = 0.0396) and 32°C (Wald Chi square = 10.68, *df* = 4, *P* = 0.0305) (Tables C1–C7 in Appendix C). The increased power of the General Linear Hypothesis analysis of the combined data set (*df* = 4) suggested that chronic thermal tolerance increases with increasing SL in *D. polymorpha*, but this tendency was less apparent in Wald Chi-square comparisons among standard-sized individuals at a lower power (*df* = 2) for specific collection sites.

2.3.1.2 Lake Oologah and Hedges Lake in 2007

Residuals from the Proportional Hazard Model analysis suggested that treatment temperature had a time-dependent effect on survivorship; therefore, model parameters were analyzed separately for each treatment temperature. A separate Proportional Hazard Model was constructed for each treatment temperature, as with the 2006 dataset. Shell length (SL) was a continuous variable, while population source, feeding regime, and acclimation temperature were categorical variables. To ensure that analyses were

preformed similarly across all treatment temperatures, higher order polynomials for acclimation temperature and interactions of acclimation temperature, collection site and feeding regime were retained in the final model even though some were not significant at all treatment temperatures (Tables C8–C16 in Appendix C).

A test of the General Linear Hypothesis for all model terms containing the collection site variable indicated that population source significantly affected the survival function, either directly or through interactions with acclimation temperature, feeding regime and/or shell length, at all treatment temperatures except 34°C (Tables C8–C16 in Appendix C). A standard, 15-mm SL Hedges Lake (NY) individual, acclimated to 20°C, and starved throughout the treatment experienced nearly linear mortality with exposure time at test temperatures of 28°C and 29°C, similar to that of individuals collected in 2006 (Figure A14A, Figure A10A). Unlike the 2006 Hedges Lake (NY) sample in which 100% mortality was recorded during the 28-d exposure period at test temperatures ≥31°C, standard 15-mm SL Hedges Lake (NY) mussels in 2007 experienced 100% mortality at ≥30°C (Figures A14A and A10A). Specimens collected from Lake Oologah (OK) in 2007 displayed 100% mortality at all treatment temperatures (26–34°C) within the 28-d exposure period (Figure A14B). At test temperatures ranging from 28–33°C, a standard 15-mm SL Hedges Lake (NY) specimen acclimated to 20°C survived significantly longer ($P < 0.05$) than Lake Oologah (OK) mussels with median survival times of 665 h vs 102 h at 28°C (Wald Chi-square = 42.41, $df = 1$, $P < 0.0001$), 296 h vs 82 h at 29°C (Wald Chi-square = 11.82, $df = 1$, $P = 0.0005$), 113 h vs 33 h at 30°C (Wald Chi-square = 26.21, $df = 1$, $P <$

0.0001), 72 h vs 19 h at 31°C (Wald Chi-square = 37.37, $df = 1$, $P < 0.0001$) , 40.5 h vs 18.5 h at 32°C (Wald Chi-square = 16.17, $df = 1$, $P < 0.0001$) and 8.5 h vs 5.5 h at 33°C (Wald Chi-square = 5.36, $df = 1$, $P = 0.0206$) (Figure A15). A standard 15-mm SL Hedges Lake (NY) specimen exposed to 27°C displayed only 33% mortality by the end of the 28-d exposure period, thus a median survival time was not directly estimable, although it must have been greater than 672 h (i.e., 28 d). In contrast, a standard 15-mm SL Lake Oologah (OK) mussel exposed to 27°C had a median survival time of 211 h. Hedges Lake (NY) and Lake Oologah (OK) specimens had lower median survival times at 26°C than at 27°C being 476 h vs >672 h and 88.5 h vs 211 h, respectively (Figure A14A and A14B). The low survivorship of specimens from both Hedges Lake (NY) and Lake Oologah (OK) at 26°C was likely not caused by thermal exposure but may have resulted from infiltration of tank media by an unidentified black fungus, although this was not experimentally assessed. Due to the inability to calculate a median survival time for the Hedges Lake (NY) samples at 27°C and the decrease in survivorship for both Hedges Lake and Lake Oologah (OK) mussels at 26°C, median survival times at these temperatures were not analyzed further.

A test of the General Linear Hypothesis for all model terms containing the feeding regime variable indicated that feeding regime (i.e., fed versus unfed) significantly affected ($P < 0.05$) survivorship, either directly or through interactions with acclimation temperature, collection site, and/or SL, only at treatment temperatures of 29, 30, and 33°C. Survivorship was reduced among fed individuals at all three of these test temperatures (Tables C8–C16 in Appendix C). After Bonferroni correction,

LT_{50} values for unfed individuals from Hedges Lake (NY) were not significantly different from those of fed specimens at any treatment duration over the entire 28-d exposure period (Figure A16A). Similarly, there was no significant effect of feeding regime on the LT_{50} values of Lake Oologah (OK) individuals at exposure durations of <12 d (Figure A16B). At exposure durations >12 d, LT_{50} values could not be estimated for both fed and unfed Lake Oologah (OK) specimens due to their extremely rapid mortality rates resulting in all survivorship values at any exposure temperature being <50% (Figure A14B).

A test of the General Linear Hypothesis for all model terms containing a categorical predictor for acclimation temperature indicated that acclimation temperature significantly ($P < 0.05$) affected survivorship, either directly or through interactions with collection site, feeding regime, and/or SL, at all treatment temperatures except 26°C (Tables C8–C16 in Appendix C). However, Bonferroni corrected Pair-wise Wald Chi-square comparisons indicated that there were no significant impacts of increasing acclimation temperature from 20–25°C on median survival time at all test temperatures (28–34°C) in mussels from either Lake Oologah (OK) or Hedges Lakes (NY) (Figure A17, Tables B7 and B8). In contrast, median survival times for standard 15-mm SL individuals from Hedges Lake (NY) acclimated to 30°C were significantly higher than that of either 20°C or 25°C acclimated individuals at test temperatures of 29, 31, 32, 33, and 34°C (Figure A17, Table B7). The impact of 30°C acclimation was less obvious in Lake Oologah (OK) specimens with the thermal tolerance of a standard-sized, 15-mm SL, 30°C acclimated individual being significantly greater than that of either a 20°C or

25°C acclimated mussel or of both groups only at test temperatures of 30, 33 or 34°C (Figure A17, Table B8). Specifically, the median survival time of a 30°C acclimated standard-sized specimen from Hedges Lake (NY) at 29°C was significantly greater than that of a 25°C acclimated individual (Wald Chi-square = 13.4544, $df = 1$, $P = 0.0002$), greater than that of a 20°C individual at 31°C (Wald Chi-square = 8.0090, $df = 1$, $P = 0.0047$), greater than that of 20°C and 25°C acclimated individuals at 32°C (Wald Chi-square = 7.6182, $df = 1$, $P = 0.0058$ and Wald Chi-square = 7.6182, $df = 1$, $P = 0.0058$, respectively), greater than that of 20°C and 25°C acclimated individuals at 33°C (Wald Chi-square = 59.3664, $df = 1$, $P < 0.0001$, and Wald Chi-square = 53.2339, $df = 1$, $P < 0.0001$, respectively) and greater than that of 20°C and 25°C acclimated individuals at 34°C (Wald Chi-square = 30.7574, $df = 1$, $P < 0.0001$ and Wald Chi-square = 18.6959, $df = 1$, $P < 0.0001$, respectively) (Table B7). Similarly, median survival times for 30°C acclimated standard 15-mm SL specimens from Lake Oologah (OK) at 30°C were significantly greater than that of a 25°C acclimated individual (Wald Chi-square = 15.1192, $df = 1$, $P = 0.0001$), greater than that of a 20°C acclimated individual at 33°C (Wald Chi-square = 11.2924, $df = 1$, $P = 0.0008$) and greater than that of both 20°C and 25°C acclimated individuals at 34°C (Wald Chi-square = 18.5208, $df = 1$, $P < 0.0001$ and Wald Chi-square = 18.2918, $df = 1$, $P < 0.0001$, respectively) (Figure A17, Table B8).

Size effects on survival were not analyzed for the Hedges Lake (NY) and Lake Oologah (OK) samples collected in 2007. The specimens collected from both populations in 2007 were very different in sample SL structure. The median SL for the

2007 Hedges Lake sample was 17.0 mm with 1st and 3rd quartiles of 14.1 and 19.7 mm, respectively, with 90% of the individuals ranging from 10.9 to 23.5 mm. In contrast to the Lake Hedges (NY) sample, which consisted of individuals from several preceding annual generations, all individuals in the 2007 Lake Oologah (OK) sample represented a single fall 2006 generation (see Chapter 3 for details) whose median SL was 10.1 mm with 1st and 3rd quartiles of 8.8 and 11.5 mm, respectively, and with 90% of individuals ranging in SL from 7.0 and 13.3 mm. Thus, there was only a very small overlap in the SL distributions of the Hedges Lake (NY) and Lake Oologah (OK) samples which prevented reliable analysis of size effects. In spite of this fact, all thermal tolerance estimations for the 2007 Lake Oologah (OK) sample were based on standard 15-mm SL individual in order to allow comparisons with data from the 2006 Hedges Lake (NY) and Lake Oologah (OK) samples and those from the 2008 Winfield City Lake (KS) samples (See Section 2.3.1.3 below). With this single exception of Lake Oologah (OK) mussels collected in 2007, all other *D. polymorpha* and *D. rostriformis bugensis* samples utilized in this study had size distributions in which median SL was relatively close to that of the standard 15-mm SL individual.

2.3.1.3 Winfield City Lake in 2008

Collection date was initially treated as a categorical variable in the Proportional Hazards Model used to analyze the combined thermal tolerance data for samples of *D. polymorpha* collected from Winfield City Lake (KS) on 16 June (early sample) and 31 August 2008 (late sample). However, these two datasets had to eventually be treated separately (resulting in the removable of collection date as a categorical variable)

because the initial combined model produced invalid estimates of survivorship as a result of time-dependent effects (Tables C17–C18 in Appendix C).

When analyzed separately across collection date, the independent early and late sample models indicated that the hazard associated with treatment exposure temperatures did not vary across treatment exposure durations as recorded in chronic thermal tolerance testing of the prior Lake Oologah (OK) and Hedges Lake (NY) mussel samples (See above). Data from the 27°C and 29°C treatment tanks were removed from the data set for the early trial and from the 27°C treatment tank for the late trial due to the presence of an unidentified black fungus in these tanks toward the end of the 28-d exposure period that may have confounded estimates of sample mortality.

A test of the General Linear Hypotheses for all model terms containing treatment temperature, as well as determination of the significance of individual model terms, indicated that treatment temperature significantly affected survivorship in both the early and late summer trials (Tables C17–C18 in Appendix C). In both trials, Winfield City Lake (KS) mussels did not achieve 100% sample mortality over the 28-d exposure period at test temperatures of 30°C and 31°C. In the early trial, survivorship after 28 d at 30°C and 31°C was 79% and 17%, respectively (Figure A18A), and in the late trial, 76% and 23%, respectively (Figure A18B) . A Wald Chi-square test indicated that a late-collected, standard 15-mm SL, Winfield City Lake (KS) individual had higher median survival times at 31°C (580.5 h) than did an early-collected specimen (469.0 h) (Wald *Chi-square* = 8.797, *df* = 1, *P* = 0.003). Similarly, at a test temperature

of 34°C, the median survival time of a late-collected standard individual (9.25 h) was significantly greater than that of an early-collected specimen (7.75 h) (Wald *Chi-square* = 13.798, *df* = 1, *P* = 0.0002) and, at 32°C, the median survival time of early-collected specimens (168.0 h) appeared lower than that of late-collected specimens (207.0 h) although the difference was not significant (Figure A19). In contrast, an early-collected, standard 15-mm SL, Winfield City Lake (KS) individual had a greater median survival time (30.5 h) at 33°C than a late-collected specimen (24.0 h) (Wald Chi-square = 8.774, *df* = 1, *P* = 0.003) (Figure A19). Least Squares Logistic Regression Analysis indicated that there were no significant differences between LT_{50} values (i.e., estimated temperature of 50% sample mortality) for early- and late-collected, standard 15-mm SL, Winfield City Lake (KS) specimens after Bonferroni correction at any exposure duration ranging from 1–28 d (Figure A20). The LT_{50} values for a standard 15-mm SL individual after 28 d of exposure was 30.7°C ± 0.34 for early-collected specimens and 30.41 ± 0.26°C for late-collected specimens (Figure A20).

A test of the General Linear Hypothesis for all model terms containing SL, as well as determination of significance for individual model terms, indicated that SL significantly affected chronic thermal tolerance in the late-collected specimens ($P < 0.05$), and, although not significant ($P < 0.05$), displayed a similar trend among early-collected specimens (Tables C17–C18 in Appendix C). In spite of this apparent relationship between chronic thermal tolerance and SL, Wald Chi-square comparisons of median survival times did not vary significantly ($P > 0.05$) with shell length in either the early or late collection (Figures A21A and A21B).

2.3.2 Chronic Upper Thermal Limits of D. rostriformis bugensis from Lake Mead

Chronic upper thermal tolerance data for *D. rostriformis bugensis* sampled from Lake Mead (NV/AZ) was analyzed as a single dataset because stratification by temperature was not necessary to alleviate violations of Proportional Hazard Model assumptions. Mortality was negligible at test temperatures ranging from 20–25°C which were essentially nonlethal to *D. rostriformis bugensis*. Thus, data at these temperatures were not included in the analysis because it resulted in a model with numerous polynomial terms required to fit minor variations in survivorship at these sublethal temperatures. Treatment temperature and shell length were treated as continuous variables while feeding regime was treated as a categorical predictor. Tests of the General Linear Hypotheses indicated that treatment temperatures ranging from 27–33°C impacted survivorship (Wald Chi-square = 197.2432, df = 3, $P < 0.0001$) (Table C19 in Appendix C; Figures A22 and A23). However, neither shell length (Wald Chi-square = 3.0253, df = 4, P = 0.5536) nor feeding regime (Wald Chi-square = 0.4731, df = 1, P = 0.4916) appeared to have a significant impact on *D. rostriformis bugensis* survival (Table C19 in Appendix C). Lake Mead (NV/AZ) specimens experienced 100% mortality within the 28-d exposure period at test temperatures ≥29°C while >50% mortality occurred at 28°C (Figure A22). In contrast, only 24.7% mortality occurred after 28 d at a test temperature of 27°C (Figure A22) which prevented median survival times from being estimated at this temperature. The median survival times associated with differences in SL were not significant ($P > 0.05$) at any treatment temperature (Figure A23). The median survival time (Lower CI ≤ Median ≤ Upper CI)

for a standard 15-mm SL unfed specimen of *D. rostriformis bugensis* from Lake Mead (NV/AZ) at 28°C was 324 h ≤ 454 h ≤ 646 h, at 29°C was 100 h ≤ 110 h ≤ 118 h, at 30°C was 32 h ≤ 43 h ≤ 53 h, at 31°C was 14 h ≤ 16 h ≤ 17.5 h, at 32°C was 6 h ≤ 7 h ≤ 8 h, and at 33°C was 2.5h ≤ 3.5 h ≤ 4 h (Figure A23).

Similar to the results for *D. polymorpha* from Lake Oologah (OK), and Hedges Lake (NY), after Bonferroni correction, LT_{50} values for a standard 15-mm SL, unfed specimen of *D. rostriformis bugensis* from Lake Mead (NV/AZ) were not significantly different from that of the fed sample (Figure A24). The LT_{50} values for a standard 15-mm SL, unfed specimen of *D. rostriformis bugensis* declined relatively rapidly with increasing exposure durations up to 7 d. Thereafter, the rate of decline was reduced such that the LT_{50} (± SE) value for the 28-d exposure period was 27.24 ± 0.18°C.

2.3.3 Comparison of Chronic Thermal Tolerances Among Collection Sites and Species

Bonferroni adjusted pair-wise comparisons using Wald Chi-square statistics indicated that a standard 15-mm SL specimen of unfed *D. polymorpha* from Winfield City Lake (KS) collected in early summer and acclimated to 20°C had significantly higher ($P < 0.0017$) median survival times at test temperatures ranging from 31–34°C than did those of the other tested populations of both *D. polymorpha* and *D. rostriformis bugensis* (Table B9, Figure A25). In contrast to the other tested populations of dreissenid mussels, a median survival time could not be calculated for Winfield City Lake (KS) samples at exposure temperatures ≤30°C because survivorship at these temperatures was >50% after the 28-d exposure period. In contrast, >50% mortality was recorded at 28–30°C for *D. polymorpha* from Lake Oologah (OK) and Hedges

Lake (NY) and *D. rostriformis bugensis* from Lake Mead (NV/AZ), suggesting that these populations were less thermally tolerant than specimens of *D. polymorpha* from Winfield City Lake (KS) (Figure A25). At a test temperature of 31°C, early-collected Winfield City Lake (KS), standard 15-mm SL specimens of *D. polymorpha* had a median survival time of 469 h which was 25 times greater than that of Lake Oologah (OK) specimens of *D. polymorpha* (= 19 h), seven times that of Hedges Lake (NY) specimens of *D. polymorpha* (= 72 h) and 29 times that of Lake Mead (NV/AZ) specimens of *D. rostriformis bugensis* (= 16 h) (Figure A25). Similarly, at a test temperature of 32°C, early-collected standard 15-mm SL specimens of *D. polymorpha* from Winfield City Lake (KS) had a median survival time of 168.0 h which was nine times greater than that of Lake Oologah (OK) specimens of *D. polymorpha* (= 18.5 h), four times that of Hedges Lake (NY) specimens of *D. polymorpha* (= 40.5 h) and 24 times that of Lake Mead (NV/AZ) specimens of *D. rostriformis bugensis* (= 7 h) (Figure A25). At a test temperature of 33°C, early-collected Winfield City Lake (KS) standard 15-mm SL specimens of *D. polymorpha* had a median survival time of 30.5 h which was six times greater than that of Lake Oologah (OK) specimens of *D. polymorpha* (= 5.5 h), three times that of Hedges Lake (NY) specimens of *D. polymorpha* (= 11 h) and nine times that of Lake Mead (NV/AZ) specimens of *D. rostriformis bugensis* (= 3.5 h) (Figure A25). Differences among the different collection sites in the median survival time of a standard 15-mm SL specimen were not as great at a treatment temperature of 34°C where 100% mortality was achieved within 18 h (Figure A25).

Bonferroni adjusted pair-wise comparisons using Wald Chi-square statistics indicated that standard 15-mm SL specimens of unfed, early-collected Lake Oologah (OK) specimens of *D. polymorpha* acclimated to 20°C had significantly lower ($P < 0.0017$) median survival times at test temperatures of 28–33°C than did individuals from Hedges Lake (NY) (Table B9, Figure A25, also Section 2.3.1.2 above).

When exposed to a test temperature of 28°C, a standard 15-mm SL, 20°C acclimated, unfed specimen of *D. rostriformis bugensis* from Lake Mead (NV/AZ) had a significantly greater median survival time (= 454 h) than a standard 15-mm SL specimen of *D. polymorpha* from Lake Oologah (OK) (= 102 h) (Wald Chi-square = 17.0446, $df = 1$, $P < 0.0001$) (Table B9, Figure A25). However, at temperatures ranging 29–33°C there were no significant differences ($P > 0.0017$) between the median survival times of early-collected Lake Mead (NV/AZ) quagga mussels and Lake Oologah (OK) zebra mussels (Table B9, Figure A25). In contrast, following Bonferroni correction, a standard 15-mm SL Lake Mead (NV/AZ) specimen of *D. rostriformis bugensis* had significantly lower ($P < 0.0017$) median survival times at test temperatures of 29–33°C than did standard-sized specimen of *D. polymorpha* from Hedges Lake (OK) (Table B9, Figure A25). At an exposure temperature of 28°C, the median survival times of a standard-sized, early-collected individual of *D. polymorpha* from Hedges Lake (NY) was not significantly different (Wald Chi-square = 3.135, $df = 1$, $P = 0.0766$) from that of a standard sized individual of *D. rostriformis bugensis* from Lake Mead (NV/AZ) (Table B9, Figure A25). The chronic thermal tolerance of Lake

Mead (NV/AZ) specimens of *D. rostriformis bugensis* was not determined at an exposure temperature of 34°C.

LT_{50} values for early-collected, standard 15 mm SL individuals from the four different collection sites reflected their median survival times (Figure A26). The LT_{50} values were compared by Bonferroni-corrected, pair-wise Wald comparisons among the four tested early collected populations after 12 d of exposure (the last day for which an LT_{50} value could be estimated for specimens of *D. polymorpha* from Lake Oologah (OK) as mortality was too great to estimate LT_{50} values thereafter) (Figure A26). The comparison revealed that after 12 days of exposure each population had significantly different ($P < 0.0083$) LT_{50} values from the others. A standard 15-mm SL unfed, early collected specimen of *D. polymorpha* from Winfield City Lake (KS) had a significantly higher LT_{50} value (31.68 ± 0.12°C) than a standard-sized specimen of *D. polymorpha* from both Hedges Lake (NY) (28.98 ± 0.26°C) (Wald Chi-square = 88.9024, $df = 1$, $P < 0.0001$) and from Lake Oologah (OK) (26.63 ± 0.36°C) (Wald Chi-square = 177.1007, $df = 1$, $P < 0.0001$). The LT_{50} value for a Winfield City Lake, standard 15-mm SL specimen of *D. polymorpha* was also significantly higher than that of a standard-sized specimen of *D. rostriformis bugensis* from Lake Mead (NV/AZ) (27.93 ± 0.19°C) (Wald Chi-square = 10.6316, $df = 1$, $P < 0.0011$). Additionally, the LT_{50} value of a standard 15-mm SL specimen of *D. polymorpha* from Lake Oologah (OK) (26.63 ± 0.36°C) was also significantly lower than that for a standard-sized specimen of *D. polymorpha* from Hedges Lake (NY) (28.98 ± 0.26°C) (Wald Chi-square = 28.0046, $df = 1$, $P < 0.0001$) and than that for a standard-sized specimen of *D. rostriformis bugensis*

from Lake Mead (NV/AZ) (27.93 ± 0.19°C) (Wald Chi-square = 10.1992, *df* = 1, *P* = 0.0014). A standard 15-mm SL specimen of *D. polymorpha* from Hedges Lake (NY) (28.98 ± 0.26°C) also had a significantly higher LT_{50} value than that of a standard-sized specimen of *D. rostriformis bugensis* from Lake Mead (NV/AZ) (27.93 ± 0.19°C) (Wald Chi-square = 10.6316, *df* = 1, *P* = 0.0011).

2.3.4 Acute Upper-Thermal Tolerance Limit of D. polymorpha from Winfield City Lake

When exposed to water temperatures increasing at 0.2°C min^{-1}, the acute thermal tolerance of a 20°C-acclimated, standard 15-mm SL individual of *D. polymorpha* from Winfield City Lake (KS) was significantly affected by test temperature (Wald Chi-square = 90.6164, *df* = 1, *P* < 0.0001), shell length (Wald Chi-square = 14.5158, *df* = 1, *P* = 0.0001) and collection date (Wald Chi-square = 16.1620, *df* = 4, *P* = 0.0029) (Tables B10 and B11). The survival probability of a standard-sized Winfield City Lake (KS) individual was ≥80%, for all sample collections at temperatures below 39°C, thereafter, rapid mortality was observed such that there was a <5% survival probability at temperatures ≥41°C (Figure A27). A sample of standard 15-mm SL, Winfield City Lake (KS) specimens collected on August 8 had an LT_{50} (±SE) value of 40.05 ± 0.11°C and experienced 100% sample mortality at 41°C. A standard-sized 15-mm SL Winfield City Lake (KS) specimen collected in August (August 8 and August 31) had LT_{50} values approximately 0.45°C higher than individuals collected both before and after August (June 29, July 19 and October 15; Wald = 14.4241, *df* = 1, *P* = 0.0001) (Figures A27 and A28). Survivorship of Winfield City Lake (KS) mussels exposed to acute thermal stress increased significantly (*P* <

0.05) with increasing SL for all collection dates (Figure A28), with 20-mm specimens having LT_{50} values approximately 0.5°C higher than 10-mm specimens and 0.25°C higher than 15-mm individuals on any one collection date (Figure A28).

2.3.5 Temperature Profiles of Sampled Lakes

Lake Oologah (OK) is well mixed throughout the year. Therefore, average daily ambient water temperatures at the Redbud Bay Marina, where sample collection occurred, were similar at the four monitored depths of 1, 2, 4, and 8 m. Thus, average daily, ambient water temperatures are only reported at a depth of 2 m, the shallowest water from which specimens were collected (Figure A29). At a depth of 2 m, Lake Oologah (OK), summer peak, average, daily, ambient water temperatures for 2006, 2007 and 2008 were 29.14°C, 29.14°C and 29.09°C, respectively (Figure A29). During the summer of 2006, there were 38 d with an average daily water temperature ≥28°C with 13 of those days averaging ≥29°C. The mean, daily, ambient, 2-m deep water temperature in Lake Oologah (OK) during the warmest consecutive 28-d period in 2006 (8 August–31 August) was 29.1°C. Average daily water temperatures during the summer of 2007 were similar with 32 d being ≥28°C and for seven of those days being ≥29°C. The mean daily water temperatures during the warmest, consecutive, 28-d period in 2007 (30 July–26 August) was also 29.1°C (Figure A29). A complete summer temperature profile of Lake Oologah (OK) in 2008 could not be provided due to the unexpected loss of the temperature dataloggers at the Redbud Bay Marina. However, from the limited data that was gathered, it appeared that in 2008, ambient water temperature was cooler at 2 m, with the warmest consecutive 28-d period having a daily

average water temperature of 28.9°C (7 July–8 August) and with 24 d having an average daily water temperature ≥28°C (seven of those days average ≥29°C).

Unlike Lake Oologah (OK), Hedges Lake (NY) stratifies during the summer, with a thermocline near a depth of 6 m. The lake is completely ice-covered through the winter. The surface water temperature was approximately 21°C in the third week of June 2006, approached 25°C in the third week of August 2006, and was 23°C in the third week of August 2007 (Figure A30). Surface water temperatures in Hedges Lake (NY) are likely to maximize in mid- to late August, although limited availability of temperature data make the duration of peak temperatures difficult to determine.

Temperature data recorded by data loggers at depths of 1, 2, and 4 m indicated that Winfield City Lake (KS) did not stratify at the collection site and briefly attained a maximum temperature of 29.4°C at a depth of 1 m (the approximate depth of mussel collection) in early August (Figure A31). Average, ambient, daily water temperatures >28°C occurred on only six days, one of which had an average >29°C. The highest overall mean of average daily water temperatures at a 1-m depth for the warmest 28-d period in the summer of 2008 (17 July–13 August) at Winfield City Lake (KS) was 27.4°C.

Lake Mead (NV/AZ) thermally stratifies during the summer. In the summer of 2006, the lake attained peak ambient water temperatures of 30.9, 29.8, 28.1, 26.6, 24.3, 21 and 13.5°C at depths of 1, 6, 12, 15, 20, 30 and 70 m, respectively (Figure A32). At a depth of 1 m there were 32 d with ambient water temperatures ≥28°C, of which nine were ≥29°C. At a depth of 6 m there were 28 d with ambient water temperatures

≥28°C, of which seven were ≥29°C. At a depth of 12 m there was only one day with an ambient water temperature ≥28°C. The mean daily ambient water temperatures for the warmest, consecutive 28 d in Lake Mead (NV/AZ) at 1 m was 28.8°C (12 July–8 August), 28.5°C at 6 m (13 July–9 August), 27.3°C at 12 m (14 August–10 September) and 24.9°C at 15 m (19 August–15 September) (Figure A32).

2.4 Discussion

2.4.1 Effects of Shell Length on Chronic Thermal Tolerance

In this study, specimens of *D. polymorpha* from Hedges Lake (NY) displayed a trend of increasing chronic thermal tolerance with increasing shell length (SL, a common surrogate for overall total body size) at exposure temperatures of 28°C and 29°C although the differences in median survival times were only significant at 28°C (Figure A13). Upon exposure to temperatures above 29°C, Hedges Lake (NY) specimens displayed no significant relationship between SL and chronic, upper thermal tolerance (Figure A13). Generally, SL and chronic, upper thermal tolerance showed a positive correlation in specimens of *D. polymorpha* from Lake Oologah (OK), although the differences were only significant at 29°C and 33°C (Figure A13). In contrast, neither early/late summer collected specimens of *D. polymorpha* from Winfield City Lake (KS) nor specimens of *D. rostriformis bugensis* from Lake Mead (NV/AZ) displayed a relationship between SL and chronic upper thermal tolerance (Figures A21 and A23). However, there was a significant positive relationship between SL and the acute thermal tolerance of tested individuals from Winfield City Lake (KS) (Figure A28).

As recorded for *D. polymorpha* in this study, the effects of SL on the physiological tolerances of bivalves are generally not consistent among or even within species. Samples of *D. polymorpha* collected from a small freshwater lake in the Netherlands and subsequently submerged at water temperatures of 34–38°C did not display significantly different times to 100% mortality among standard SL classes of 5, 10, 15 or 20 mm (Rajagopal *et al*. 1997). Similarly, samples of *D. polymorpha* collected from the brackish Noordzeekanaal in the Netherlands and subsequently submerged at water temperatures of 36–41°C also did not have significantly different times to 100% mortality among SL classes of ≈2.5, ≈10, and ≈20 mm (Rajagopal *et al*. 2005a). In contrast, Rajagopal *et al*. (2005a) found chronic thermal tolerance times to be positively correlated with SL in individuals of the mussels, *Mytilus edulis* and *Mytilopsis leucophaeata*, two other mussel species co-occurring with *D. polymorpha* in the Noordzeekanaal. Wallis (1975) also recorded a positive relationship between SL and chronic thermal tolerance in individuals of *Mytilus edulis*. Thermal tolerance was also positively correlated with SL in the invasive oyster, *Crassostrea gigas* (Rajagopal *et al*. 2005b). In contrast, Elderkin and Klerks (2005) found a significant negative effect of SL on upper thermal tolerance among three *D. polymorpha* populations from the Mississippi River when individuals were chronically exposed to 32°C. In addition, Elderkin and Klerks (2005) also reported interpopulation differences in the relationship between SL and chronic thermal tolerance among three geographically separated populations of *D. polymorpha* in the Mississippi River. The northernmost population (Minnesota) displayed a decrease in chronic thermal tolerance with increasing SL and

the southernmost population (Louisiana), an increase in thermal tolerance with increasing SL up to 25 mm, while at SL ≥25 mm chronic thermal tolerance decreased with increasing SL. Similarly, McMahon *et al*. (1995) also found a significant negative correlation between SL and chronic upper thermal tolerance among individuals of *D. polymorpha* exposed to 31–37°C. While there appears to be no consistent relationship between SL and chronic thermal tolerance in *D. polymorpha*, it is important to note that SL has a much smaller impact on thermal tolerance in this species than does prior temperature acclimation (McMahon *et al*. 1995; Rajagopal *et al.* 2005a; this study). The relatively small impact of SL on chronic thermal tolerance in *D. polymorpha* may account for the variation in this relationship reported among different studies.

It is unclear why there appears to be geographic variation in the effects of size on the chronic upper thermal tolerance of latitudinally separated *D. polymorpha* populations in the Mississippi River (Elderkin and Klerks 2005). Elderkin and Klerks (2005) suggest that the warm waters in the southern portions of the Mississippi River may be selecting for individuals with an increased thermal tolerance that allows them to survive through the summer and attain a large body size. Such selection could also explain the apparent positive relationship between SL and the chronic upper thermal tolerance limits in specimens of *D. polymorpha* from Lake Oologah (OK) which, like the southern portions of the Mississippi River (Elderkin and Klerks 2005), regularly experienced temperatures ≈30°C in the summer (Figure A29). However, a similar positive relationship between SL and chronic, upper thermal tolerance limit was also observed at exposure temperatures of 28°C and 29°C in specimens of *D. polymorpha*

from Hedges Lake (NY). In Hedges Lake (NY), ambient water temperatures never approach 30°C and, at best, may briefly attain a maximum summertime temperature of ≈25°C during extremely warm years, a temperature regime unlikely to exert thermal selection on its *D. polymorpha* population (Figure A30). In addition, there was no significant relationship between SL and chronic upper thermal tolerance limit in specimens of *D. polymorpha* from Winfield City Lake (KS) (Figure A21A and A21B), which was much warmer during summer months (maximum average daily water temperature = 29.4°C) than Hedges Lake (NY) (Figures A30 and A31). This result further suggests that selection for thermally tolerant individuals is unlikely to account for the reported latitudinal differences in correlation between SL and chronic thermal tolerance in *D. polymorpha* populations reported by Elderkin and Klerks (2005).

Nutritional condition (i.e., the relative amount of tissue, organic, energy stores) also appears to affect thermal tolerance. The results of this study indicated that large individuals of *D. polymorpha* from Winfield City Lake (KS) lost a higher relative proportion of their dry tissue biomass during warm summer months than did smaller mussels (see Chapter 3). Because chronic exposure to sublethal and lethal temperatures results in elevated catabolism of carbon energy stores (i.e., carbohydrates, lipids and proteins), the greater relative loss of biomass (i.e., energy stores) in larger relative to smaller individuals under warm ambient conditions may explain why some studies have indicated that larger, presumably less nutritionally competent, individuals of *D. polymorpha* appear less thermally tolerant than smaller, presumably more nutritionally competent, individuals (McMahon *et al*. 1995, Elderkin and Klerks 2005). In this

regard, it is interesting that specimens of *D. polymorpha* from Winfield City Lake (KS) had a significant positive correlation between SL and acute thermal tolerance (Figure A28). In the acute thermal tolerance testing methodology employed in this study, exposure duration to high temperatures was relatively short (i.e., temperature increasing at 0.2°C min^{-1} from 20°C) making the impacts of nutritional condition minimal. Similarly, McMahon and Ussery (1995) found no significant impact of SL on the acute upper thermal limit of specimens of *D. polymorpha* from the Niagara River (NY) but did find a negative correlation of SL with chronic thermal tolerance in mussels sampled from the same population.

2.4.2 Effects of Acclimation on Chronic Thermal Tolerance

The effects of acclimation temperature on the upper thermal tolerance limits of freshwater bivalves have been well documented with increased acclimation temperature conferring increased thermal tolerance (for reviews see McMahon 1996, McMahon and Bogan 1991). Similarly, the chronic or acute, upper thermal tolerance limits of *D. polymorpha* increase with increasing acclimation temperature (Iwansky and McCauley 1993, Hernandez 1995, McMahon *et al*. 1995, McMahon and Ussery 1995, Spidle *et al*. 1995, Rajagopal *et al*. 2005a). In this study, specimens of *D. polymorpha* collected from Lake Oologah (OK) in the summer of 2006 displayed an increase in upper thermal tolerance with increasing acclimation temperature (5, 10, 15, 20, 25 and 30°C) at all treatment temperatures (28–34°C) (Figure A12, Table B6). Similarly, *D. polymorpha* collected from Hedges Lake (NY) in 2006 displayed an increase in thermal tolerance with increasing acclimation temperature such that mussels acclimated to 30°C had

significantly longer median survival times on exposure to 33°C or 34°C than did individuals acclimated to lower temperatures (i.e., 5–25°C) (Figure A12, Table B5).

Specimens of *D. polymorpha* collected from Hedges Lake (NY) in 2006 displayed a similar increase in chronic, upper thermal tolerance with increasing acclimation temperature up to 25°C at treatment temperatures ≤32°C, with an apparent plateau in thermal tolerance between acclimation temperatures of 25°C and 30°C (Figure A12, Table B5). In contrast, the chronic thermal tolerance of specimens of *D. polymorpha* collected from Hedges Lake (NY) in 2007 was maximized among individuals acclimated to 30°C (Figure A17). The median chronic survival times of 30°C acclimated Hedges Lake (NY) and Lake Oologah (OK) specimens of *D. polymorpha* collected in 2007 were equal to or significantly greater than median survival times of 20°C and 25°C acclimated individuals (Table B7). Thus, the apparent plateau in thermal tolerance between 25°C and 30°C acclimated individuals from the 2006 Hedges Lake (NY) sample may have been of little biological significance.

2.4.3 Effects of Acclimatization on Thermal Tolerance

Acclimatization is a long-term process that induces changes in the physiological rate functions of organisms which cannot be negated by laboratory acclimation (McMahon 1996). Seasonal acclimatization is known to affect thermal tolerance of *D. polymorpha* with individuals collected from the lower Mississippi River (LA) and the Niagara River (NY) both displaying increased chronic thermal tolerance limits at 33°C during warmer months despite laboratory acclimation to the same temperatures (Hernandez 1995). In this study, samples of *D. polymorpha* from Lake Oologah (OK)

and Hedges Lake (NY) and *D. rostriformis bugensis* from Lake Mead (AZ/NV) were generally collected during early summer (see Section 2.2.2) such that specimens would have been acclimatized to warm natural waters prior to the attainment of peak summer temperatures that could have led to dry tissue mass loss and a decrease in physiologic condition (i.e., the capacity to tolerate environmental stress) (see Chapter 3). Collection at this time was anticipated to result in laboratory determinations of upper thermal tolerance limits that would be maximized among tested individuals.

In contrast, tests of chronic and acute thermal tolerance limits on *D. polymorpha* samples from Winfield City Lake (KS) were repeated through the summer and fall of 2008. Chronic thermal testing was performed for samples collected on 16 June 2008 and 31 August 2008 while acute thermal tolerance testing was performed for samples collected on June 29, July 19, August 8, August 31, and October 15, 2008. In chronic tests, after acclimation to 20°C, specimens of *D. polymorpha* collected in late summer (31 August 2008) had significantly longer median survival times than those collected in early summer (16 June 2008) at exposure temperatures of 31, 32 and 34°C while early-collected specimens had a significantly longer median survival time only at an exposure temperature of 33°C (Figure A19). These results were indicative of seasonal acclimatization with specimens collected in mid-summer after peak ambient water temperatures (Figure A31) having a generally elevated chronic thermal tolerance than individuals collected from cooler waters in early summer (Figure A31). Similarly, the acute thermal tolerance limits of *D. polymorpha* collected from Winfield City Lake (KS) during the month of August (August 8 and August 31, 2008) were almost 0.5°C

higher than recorded for individuals collected both before or after those dates (i.e., June 29, July 19 and October 15, 2008).

These results for both acute and chronic thermal tolerance suggest that specimens of *D. polymorpha* from Winfield City Lake (KS) were most tolerant of thermal exposure during and immediately after periods of peak summer ambient water temperature (Figure A31), despite a decline in physiologic condition associated with temperature-induced biomass loss (see Chapter 3).

Hernandez (1995) investigated the chronic thermal limits of *D. polymorpha* samples collected from the Niagara (NY) and Mississippi Rivers (LA). After acclimation to 5, 15 or 25°C, sampled individuals were chronically exposed to 33°C until 100% mortality was achieved. Even after acclimation, specimens of *D. polymorpha* from both collection sites displayed seasonal temperature tolerance acclimatization such that ambient water temperature at the time of collection significantly affected survival times at 33°C. Thus, individuals collected during periods of warmer ambient water temperature had increased survival times, regardless of laboratory acclimation (Hernandez, 1995). The results of Hernandez (1995) and this study indicate that seasonal temperature acclimatization is characteristic of *D. polymorpha*. It is presumably characteristic of *D. rostriformis bugensis*, but has yet to be confirmed by studies of seasonal variation in thermal tolerance in this species. Increased thermal tolerance in dreissenids during summer months due to acclimatization could potentially impact the application temperatures and/or times required for thermal treatment to mitigate mussel infestations in raw water using facilities (McMahon and

Ussery 1995) or affect 100% removal/mortality of mussels on boat hulls with thermal-spray wash systems (Morse 2009).

2.4.4 Effects of Feeding Regime on Chronic Thermal Tolerance

In this study, provision of food during chronic thermal exposure did not significantly impact the LT_{50} values of *D. polymorpha* or *D. rostriformis bugensis* at any treatment temperature (Figures A16 and A24). Similarly, Rajagopal *et al*. (2005a) found that there was no difference in chronic thermal tolerance between fed and unfed specimens of *D. polymorpha*, *Mytilus edulis*, or *Mytilopsis leucophaeata*. The availability of a nutritional food source should, in theory, have reduced the loss of nutritional condition that results from increasing metabolic rates at elevated treatment temperatures, thereby prolonging survival (See Section 2.4.1 above and Chapter 3). However, at temperatures above 20°C the ingestion rate of *D. polymorpha* decreases dramatically (Walz 1978a, Fanslow *et al*. 1995) such that at ≈30°C, consumption essentially ceases (Schneider 1992). In addition, scope for growth in adult mussels becomes negative (i.e., catabolism outstrips assimilation) above 20°C even under *ad libitum* feeding conditions (Walz 1978b), which suggests that feeding mussels during chronic exposures to lethal temperatures would have little or no impact on individual survival times relative to unfed individuals. However, my results did suggest that differences in the initial nutritional condition of individuals could have major impacts on chronic thermal tolerance limits. Thus, summer-collected individuals of *D. polymorpha* from Lake Oologah (OK) in 2006 and 2007 had unexpectedly lower thermal tolerance limits than individuals from the cooler Hedges Lake (NY). This

outcome presumably was a result of the high, summer, ambient, water temperatures experienced by the Lake Oologah (OK) population which resulted in individuals having reduced energy stores that lowered survival times under the increased catabolic rates induced by chronic exposure to elevated test temperatures (see Chapter 3 for details).

2.4.5 Extirpation of the Lake Oologah D. polymorpha population

Samples of *D. polymorpha* from Lake Oologah (OK) were less thermally tolerant than specimens of *D. polymorpha* from both Hedges Lake (NY) and Winfield City Lake (KS) despite having been collected from the southern-most, warmest, body of water sampled in this study. Thus, a standard 15-mm SL, 20°C acclimated specimen from these three lakes had median survival times of 19, 72 and 469 h, respectively, when chronically exposed to 31°C (Figure A25). At a depth of 2 m, the mean daily ambient water temperature at the Lake Oologah (OK) Redbud Bay Marina collection site over the warmest consecutive 28-d period during the summer of 2006 was 29.1°C (Figure A29). In the early summer of 2006, >95% mortality was achieved in a 20°C acclimated, standard 15-mm SL individual of *D. polymorpha* from Lake Oologah (OK) chronically exposed to 29°C over a 28-d period (Figure A10B). This result was attained just prior to a mid-summer mortality event that killed ≈90% of the Lake Oologah (OK) *D. polymorpha* population at the Redbud Bay Marina collection site. Late summer/fall reproduction in this population resulted in extensive settlement of juveniles in the fall of 2006. Juvenile growth was rapid, with individuals attaining an average SL of ≈1 cm by July 2007. A similar, but more extensive, mortality event occurred during the summer of 2007 during which the mean ambient water temperature was 29.1°C over the

warmest consecutive 28-d period (Figure A29). After this 2007 mortality event, living adult mussels could not be found at the Redbud Bay Marina despite repeated attempts to collect them. In the summer of 2007, laboratory testing indicated that a 20°C acclimated, 15-mm standard SL specimen of *D. polymorpha* collected from Lake Oologah (OK) were much less tolerant of chronic exposure to 29°C than in 2006 (see above) experiencing 100% mortality after only 10 d (Figure A14B). To date, the *D. polymorpha* population in Lake Oologah (OK) has not recovered from the summer 2007 mortality event, with no settled mussels found in fall 2007 through fall 2008 despite repeated collection attempts. Thus, the Lake Oologah (OK) *D. polymorpha* population appears to have been virtually extirpated, which would be the first recorded instance of a natural dreissenid population extirpation in North America, if confirmed.

The mortality events experienced by the *D. polymorpha* population in Lake Oologah (OK) appeared to result from a temperature-induced loss of nutritional condition during summer periods of elevated ambient water temperatures. As detailed in Section 2.4.1 and Chapter 3, at summer water temperatures >25°C specimens of *D. polymorpha* experience a reduction in organic energy due to the catabolic demands exceeding their energy assimilation rates. Thus, in Winfield City Lake (KS) this likely resulted in a major, but nonlethal, reduction of the tissue mass of adult mussels during the summer of 2008 (Chapter 3). In Lake Oologah (OK), where ambient water temperatures in 2007 remained above 25°C for approximately twice as long as they did at Winfield City Lake (KS) in 2008, adult specimens of *D. polymorpha* were recorded to have dry tissue masses only 32% that of similar-sized Winfield City Lake (KS)

individuals (Chapter 3). This result strongly suggested that through successive warm summers in Lake Oologah (OK), the energy stores of adult individuals of *D. polymorpha* eventually fell below that required for survival of prolonged summer water temperatures (>25°C) leading to the population's possible extirpation in 2007 (see Chapter 3 for details).

These results suggest that the chronic thermal tolerance of dreissenid mussel populations may be highly impacted by their nutritional condition (i.e., body mass per unit SL). Thus, individuals with reduced energy stores (i.e., reduced tissue mass) may be unable to tolerate prolonged exposure to even typically sublethal temperatures above 25°C as the resultant starvation reaches lethal levels. As appears to have occurred in Lake Oologah (OK), reduction of phytoplankton productivity, induced by the development of extensive dreissenid mussel densities after initial invasion may result in the nutritional condition of adult individuals eventually being reduced to levels insufficient for survival through extended periods of elevated, but normally sublethal, summer water temperatures inducing lethal levels of tissue biomass loss. This possibility warrants further study, particularly in southwestern water bodies recently invaded by *D. polymorpha* and/or *D. rostriformis bugensis* where the opportunity exists to monitor the long-term (i.e., >1 year), post-invasion, tissue biomass dynamics of adults in conjunction with population growth and reproduction.

2.4.6 Chronic Thermal Tolerance of D. rostriformis bugensis from Lake Mead

A standard 15-mm SL, 20°C acclimated individual of *D. rostriformis bugensis* from Lake Mead (NV/AZ) experienced negligible mortality when chronically exposed

for 28 d to temperatures ≤26°C and 100% mortality at temperatures ≥29°C (Figure A22). This result indicated that this population's incipient thermal tolerance limit was between 26°C and 29°C. When chronically exposed to 27°C and 28°C for 28 d, a standard 15-mm SL individual of *D. rostriformis bugensis* from Lake Mead (NV/AZ) experienced 24.7% and 75.0% mortality, respectively (Figure A22). Perhaps the best estimate of the incipient upper thermal limit is the LT_{50} value. The LT_{50} for a standard 15-mm SL, 20°C acclimated individual of *D. rostriformis bugensis* from Lake Mead (NV/AZ) was 27.2°C over a 28-d exposure (Figure A24).

It has been reported that North American specimens of *D. rostriformis bugensis* are somewhat less thermally tolerant than specimens of *D. polymorpha* (Domm *et al*. 1993, MacIsaac 1994, Mills *et al*. 1996, Spidle *et al*. 1995, Thorp *et al*. 1998). Similarly, this study found that 20°C acclimated specimens of *D. rostriformis bugensis* from Lake Mead (NV/AZ) (LT_{50} for a 20°C-acclimated, standard 15-mm SL individual after 12 d of exposure = 27.9°C) had a reduced chronic thermal tolerance relative to specimens of *D. polymorpha* from Winfield City Lake (KS) and Hedges Lake (NY) (LT_{50} for a 20°C acclimated, standard 15 mm SL individual after 12 d of exposure = 31.7 and 29.0°C, respectively) (Figure A26). In contrast, specimens of *D. rostriformis bugensis* from Lake Mead were more tolerant of elevated temperatures than specimens of *D. polymorpha* sampled from Lake Oologah (OK) in 2007 (LT_{50} for a 20°C acclimated, standard 15 mm SL individual after 12 d of exposure = 26.6°C) (Figure A26). However, this latter result may not represent the thermal tolerance of healthy specimens of *D. polymorpha* from Lake Oologah (OK) as the chronic thermal tolerance

of sampled individuals was likely confounded by extremely poor nutritional condition (see Sections 2.4.1, 2.4.5 and Chapter 3 for details).

Spidle *et al*. (1995) reported that, in two replicated chronic exposures to 30°C, 20°C acclimated specimens of *D. rostriformis bugensis* from Lakes Ontario and Erie (NY) achieved 96% and 52% mortality after 168 h, respectively. In this study, similarly sized and acclimated samples of *D. rostriformis bugensis* from Lake Mead (NV/AZ) chronically exposed to 30°C experienced 100% mortality within 120 h in the fed treatment and 108 h in the unfed treatment. Although specimens of *D. rostriformis bugensis* from Lake Mead (NV/AZ) appear slightly less tolerant than those from the Lakes Ontario and Erie (NY) (Spidle *et al*. 1995), no individuals from either population survived ≥30°C more than seven days. The inability to acclimate samples of *D. rostriformis bugensis* at 25°C occurred in both this study and that of Spidle *et al*. (1995). Based on this result, Spidle *et al*. (1995) suggested that the upper thermal limit of *D. rostriformis bugensis* must have been lower than this temperature, which he did not experimentally assess. In this study, the inability to acclimate specimens of *D. rostriformis bugensis* to 25°C appears to have been a result of something other than temperature stress as suggested by the negligible mortality that occurred in 20°C acclimated samples experimentally tested at ≤26°C for 28 d.

The reduced chronic thermal tolerance of Lake Mead (NV/AZ) specimens of *D. rostriformis bugensis* (this study) relative to that of specimens from populations experiencing cooler conditions in Lakes Ontario and Erie (NY) (Spidle *et al*. 1995) at first appears incongruous. The highest 2006, 28-d average, ambient water temperatures

in Lake Mead (NV/AZ) were 28.8, 28.5, 27.3 and 24.9°C at depths of 1, 6, 12 and 15 m, respectively (Figure A32). This study reports that a 20°C acclimated, standard 15-mm SL individual of *D. rostriformis bugensis* collected at a depth of 15 m from Lake Mead (NV/AZ) experienced mortalities of ≈0, 25, 75, and 100% when chronically exposed for 28 d to ≤26, 27, 28, and ≥29°C, respectively (Figure A22), thus indicating that *D. rostriformis bugensis* from Lake Mead (NV/AZ) may experience lethal temperature exposures at depths ≤12 m during summer months (Figure A32). However, at depths ≥12 m, moderate temperature regimes and an oxygenated hypolimnion should allow individuals to survive year round. The impacts of thermal tolerance on the distribution of *D. rostriformis bugensis* in Lake Mead (NV/AZ) suggested by this study's results are corroborated by the fact that in May 2007, National Park Service biologists dove to a depth of approximately 15 m before locating mussels in sufficient densities to be collected for the experiments conducted in this study (J. Rinella pers. comm.).

Interestingly, *D. rostriformis bugensis* have dispersed from Lake Mead into shallower reservoirs on the Lower Colorado and through water delivery systems to small reservoirs in southern California where they are likely to be subjected to summer water temperatures approaching or exceeding their incipient upper lethal limit of ≈27°C. They have also reached high densities in the surface waters of Lake Mead (NV/AZ) (i.e., depths of <1 m, David K. Britton, personal communication) where they are exposed to 28-d, average, daily, ambient, water temperatures ≥27.2°C which was determined in this study to be their 28-d incipient upper thermal limit. These observations, along with the elevated thermal tolerance recorded for the *D. polymorpha*

population in Winfield City Lake (KS) (see Section 2.4.7 below) suggest that selection for physiological lineages with elevated thermal tolerance limits may be occurring for both species in recently invaded water bodies in the southwestern United States. However, this supposition, even though it has major implications for the control and management of both species in southwestern U.S. water bodies, awaits further confirmation.

2.4.7 Thermal Tolerance of D. polymorpha from Winfield City Lake

Specimens of standard 15-mm SL, 20°C acclimated, *D. polymorpha* from Winfield City Lake (KS) were more thermally tolerant than similarly acclimated individuals of *D. polymorpha* from both Lake Oologah (OK) and Hedges Lake (NY) (Figure A25). Samples of *D. polymorpha* from Winfield City Lake (KS) experienced <25% and <80% mortality when exposed for 28 d to 30°C and 31°C, respectively (Figure A18). In contrast, 20°C-acclimated samples of *D. polymorpha* from Lake Oologah (OK) and Hedges Lake (NY) experienced 100% mortality within 28 d at both 30°C and 31°C in 2006 and 2007 with the exception of a Hedges Lake (NY) sample that experienced only ≈95% mortality when exposed to 30°C in 2006 (Figures A10 and A14). Samples of 20°C-acclimated *D. polymorpha* individuals collected in early summer from Winfield City Lake (KS), Hedges Lake (NY), and Lake Oologah (OK) chronically exposed to 31°C had median survival times of 469, 72, and 19 h, respectively. Similarly, samples of 20°C acclimated *D. polymorpha* collected in early summer from these three sites and chronically exposed to 32°C had median survival times of 168, 40.5, and 18.5 h, respectively, and when exposed to 33°C, had median

survival times of 30.5, 11, and 5.5 h, respectively (Figure A25). Generally, late-summer collected individuals of *D. polymorpha* from Winfield City Lake (KS), with median survival times of 580.5, 207.0, 24.0, and 9.25 h at chronic exposures to 31, 32, 33, and 34°C, respectively, were more tolerant than early-summer collected individuals with corresponding median survival times of 469.0, 168.0, 30.5 and 7.75 h (Figure A19). In contrast, times to 100% sample mortality were similar between early-summer and late-summer samples of *D. polymorpha* from Winfield City Lake (KS) where early-summer collected individuals experienced 100% mortality after 432, 84, and 18 h during chronic exposure to 32, 33 and 34°C, respectively. Corresponding 100% mortality times for late-summer collected individuals were only substantially greater at 33°C in which 100% mortality occurred after an exposure of 600 h.

The chronic, thermal-tolerance limits of the Winfield City Lake (OK) *D. polymorpha* population were elevated compared to other previously published values for this species. Elderkin and Klerks (2005) reported a mean time-to-death (TTD) of ≈80 h for chronically exposed 22°C-acclimated specimens of *D. polymorpha* collected from the Mississippi River near Baton Rouge (LA) and ranging in SL from 15–19.9 mm, which was considerably lower than the comparable value of 207 h recorded for late-collected individuals from Winfield City Lake (KS) acclimated to 20°C. However, the methodology of Elderkin and Klerks (2005) included a 1°C d^{-1} ramping period from 22°C which could have elongated the actual exposure time to elevated temperatures. Using a value of ≈28°C as the incipient upper lethal temperature for *D. polymorpha* based on this study, the ramping procedure would have included an additional 96-h

exposure to lethal temperatures yielding a 176 h mean time to death value which was still lower than the 207-h median survival time recorded in this study for late-summer collected individuals from Winfield City Lake (KS). As estimated from the linear regression models of Hernandez (1995), the median survival times for a 20°C acclimated standard 15-mm SL individual of *D. polymorpha* collected at an ambient water temperature of 25°C from the Mississippi River near Baton Rouge (LA) and chronically exposed to 33°C would be 20.1 h and from the Niagara River (NY), 16.8 h. Hernandez (1995) showed that the difference in mean survival times of mussels from the Mississippi (LA) and Niagara (NY) Rivers increased with decreasing acclimation temperature from 25–5°C which he attributed to selection for increased thermal tolerance in the warmer waters of the Lower Mississippi River (LA). Based on this study, the median survival time of a standard 15-mm SL individual from Winfield City Lake (KS) under similar conditions would be higher than that of specimens from either the Mississippi (LA) or Niagara (NY) Rivers at 30.5 h and 24.0 h for early- and late-summer collected individuals, respectively.

Similarly, McMahon *et al*. (1995) reported that 20°C acclimated samples of *D. polymorpha* collected from the Black Rock Navigation Lock on the Niagara River near Buffalo (NY) experienced 100% sample mortality within 472 h when chronically exposed to 31°C. By comparison, similarly treated late-summer collected samples of *D. polymorpha* from Winfield City Lake (KS) experienced only 25% mortality after 472 h and only 77% mortality by the end of the 672 h exposure period. In addition, using probit analysis, McMahon *et al*. (1995) developed models that estimated 50% sample

mortality times for 20°C acclimated individuals of *D. polymorpha* from the Niagara River (NY) to be 145.3, 52.5, 19.0 and 6.9 h on chronic exposure to 31, 32, 33, and 34°C, respectively, with corresponding 100% sample mortality values of 456.7, 148.1, 48.0, and 15.6 h. In this study, estimates of median survival times for a similarly treated, standard 15-mm SL, late-summer collected individual of *D. polymorpha* from Winfield City Lake (KS) were generally much greater at 580.5, 207, 24.0, and 9.25 h on chronic exposures to 31, 32, 33, and 34°C, respectively. Further, 20°C acclimated, late-summer collected standard 15-mm SL individuals of D. *polymorpha* from Winfield City Lake (KS) were estimated to experience only 77.5% sample mortality after 672-h at 31°C and a greatly elevated 100% sample mortality time of 600 h at 32°C compared to 148 h for mussels from the Niagara River (NY) (McMahon *et al.* 1995).

Other studies also suggest that populations of *D. polymorpha* at higher latitudes in North America have much lower chronic temperature tolerances than recorded in this study for the *D. polymorpha* population in Winfield City Lake (KS). For instance, based on the linear regression model of Iwansky and McCauley (1993), 20°C acclimated individuals of *D. polymorpha* from Lake Erie (NY) and Lake St. Clair (MI) ranging from 10–20 mm in SL had a mean survival time on chronic exposure to 31°C of just 37.5 h and experienced 100% sample mortality in 132.4 hours while similarly treated specimens from Winfield City Lake (KS) did not experience 100% mortality and had a median survival time of 580.5 h which was over 15 fold greater than that estimated by Iwansky and McCauley (1993). Similarly, samples of *D. polymorpha* from the Great Lakes region exposed to ambient temperatures of 20–25°C in flowing

river water experienced 95% mortality within 27 h when chronically exposed to 32°C (Harrington *et al*. 1997), while the same treatment required 22 times longer at 600 h to induce 100% sample mortality in 20°C-acclimated standard 15-mm SL individuals from Winfield City Lake (KS).

This study also indicated that the acute upper thermal tolerance limits of mussels from Winfield City Lake (KS) were higher than those of *D. polymorpha* populations at higher latitudes in North America. For instance, in this study, 20°C acclimated, standard 15-mm SL individuals of *D. polymorpha* collected on 8 August 2008 from Winfield City Lake (KS) and exposed to a rate of temperatures increase of 0.2°C min^{-1} had an estimated survival probability of ≈100% at 39°C, an LT_{50} value (i.e., estimated temperature for 50% sample mortality) of 40.1°C, and experienced 100% sample mortality at 41°C (Figures A27 and A28). Similarly treated, 20°C acclimated samples of *D. polymorpha* collected from the Lower Great Lakes had a median acute temperature tolerance of approximately 37°C (Spidle 1994) which was 3°C lower than that recorded for mussels (40.1°C) from Winfield City Lake (KS). Using the same acute thermal tolerance methodology as in this study, McMahon and Ussery (1995) reported that 20°C acclimated samples of *D. polymorpha* from the Niagara River (NY), had an LT_{50} value of 38.3°C and SM_{100} (i.e., actual temperature for 100% sample mortality) of 39°C compared to corresponding elevated values of 40.1°C and 41.0°C for specimens from Winfield City Lake (KS) experiencing the same experimental conditions. When exposed to a somewhat faster 0.3°C min^{-1} increase in temperature, 20°C acclimated specimens of *D. polymorpha* from Lake Erie (NY) had a mean critical

thermal maximum, defined as the temperature at which valve movement is lost in gaping individuals, of 37.0°C (Domm *et al*. 1993), 3°C lower than the LT_{50} value (40.1°C) recorded for mussels from Winfield City Lake (KS) experiencing the same experimental conditions except for a somewhat slower rate of temperature increase (i.e., 0.2°C min^{-1}). Interestingly, in the Domm *et al.* (1993) study, the faster rate of temperature increase utilized should have resulted in a higher critical thermal maximum value than would been attained at a slower rate of increase (McMahon and Ussery 1995), again indicative of the elevated acute thermal tolerance of *D. polymorpha* in Winfield City Lake (KS) relative to that reported for any other population in North America.

Winfield City Lake (KS) temperatures briefly peaked at 29.4°C in early August 2008 and averaged 27.4°C during the warmest 28-d summer period (Figure A31). Laboratory determinations of both chronic and acute thermal tolerance suggest that at these temperatures the Winfield City Lake (KS) *D. polymorpha* population would not experience mortality from thermal stress which was corroborated by concurrent field observations of behaviorally normal adult mussels, larval settlement and juvenile growth. The elevated acute and chronic thermal tolerance limits of the Winfield City Lake (KS) *D. polymorpha* population compared to North America populations at higher latitudes suggests that dreissenid populations in the southwestern United States may be rapidly evolving elevated thermal tolerance limits driven by the strong thermal selection pressures experienced in the warm isolated water bodies characteristic of this region. However, it is not known whether the relatively high thermal tolerance of the Winfield

City Lake (KS) *D. polymorpha* population resulted from adaptation or acclimatization in a warm water body. Nevertheless, a *D. polymorpha* population in the lower Mississippi River (LA) experiencing an annual thermal regime very similar to that of Winfield City Lake (KS) had a higher chronic thermal tolerance limit than did a more northern mussel population in the Niagara River (NY) with an annual thermal regime very similar to that of Hedges Lake (NY) (Hernandez 1995). This difference in thermal tolerance occurred despite extensive gene flow from more northern populations in the upper Mississippi River drainage and from Lake Michigan through the Chicago Sanitary Canal (i.e., Illinois River) via downstream dispersal of the mussel's planktonic veliger larva (Stoeckel *et al*. 1997, 2004). Hernandez (1995) suggested that the elevated thermal tolerance of adult *D. polymorpha* from the lower Mississippi River (LA) was most likely to result from extreme thermal selection associated with high mid-summer, thermally induced mortalities of juvenile mussels settled during the spring reproductive period. The capacity for such rapid temperature tolerance selection has been demonstrated for a *D. polymorpha* population in a Russian power station thermal discharge, in which allele frequencies at four of seven loci were significantly different from those of the source-water population from which extensive gene flow was received via veliger and suspended juvenile transport through the power station's raw water system (Fetisov *et al.* 1991). In contrast, lack of substantial gene flow from a cooler upstream water source via downstream veliger dispersal in Winfield City Lake (KS) may have allowed for more extensive selection of thermally tolerant individuals

resulting in its *D. polymorpha* population having the highest chronic and acute thermal tolerances recorded for any North American population of this species.

2.5 Conclusions

Reports of the effects of SL on the chronic upper thermal tolerance limits of dreissenid mussels have varied in the published literature including positive correlations between SL and chronic, thermal tolerance limit in *D. polymorpha* reported by Elderkin and Klerks (2005), a negative correlation by Elderkin and Klerks (2005) and McMahon *et al*. (1995) or no correlation (Rajagopal *et al*. 1997, Rajagopal *et al*. 2005a). Similarly, this study found contrasting results regarding the affect of SL on chronic upper thermal tolerance limits of dreissenid mussels from different populations. For instance, individuals of *D. polymorpha* from Lake Oologah (OK) and Hedges Lake (NY) displayed a positive correlation between SL and median chronic thermal tolerance times, while individuals of *D. polymorpha* from Winfield City Lake (KS) and of *D. rostriformis bugensis* from Lake Mead (NV/AZ) had no significant relationship between SL and chronic thermal tolerance. The basis for this apparent variation in the impact of SL on chronic thermal tolerance limits in dreissenid mussels remains unknown, however, the results of this study showed that exposure to ambient water temperatures ≥25°C had greater negative impacts on the nutritional/physiologic condition of larger versus smaller individuals of *D. polymorpha* in Winfield City Lake (KS) and were the apparent cause of an anomalously low chronic thermal tolerance limit of the Lake Oologah (OK) population, perhaps eventually leading to its extirpation. Because ambient temperature experience, and its resultant impacts on

physiologic condition, may disproportionately negatively impact the temperature resistance of larger adult individuals; the relation of thermal tolerance to SL in dreissenids may vary depending on the season and ambient water temperature at collection and subsequent holding conditions (i.e., holding temperature and feeding regimes), all of which could disproportionately impact physiologic condition of larger individuals prior to thermal tolerance testing.

In agreement with existing literature (Iwansky and McCauley 1993, Hernandez 1995, McMahon *et al*. 1995, McMahon and Ussery 1995, Spidle *et al*. 1995, Rajagopal *et al*. 2005a), this study suggested that there was a positive relationship between acclimation temperature and the upper thermal tolerance limits of dreissenid mussels. This study also demonstrated that food availability had negligible effect on the upper thermal tolerances of *D. polymorpha* as also reported by Rajagopal *et al*. (2005a). The demonstration of a positive effect of temperature acclimatization on the chronic upper thermal tolerance limits of *D. polymorpha* also concurred with that of Hernandez (1995). Thus, the positive relationship between temperature acclimation and acclimatization temperatures on chronic temperature tolerance appears well documented in *D. polymorpha* and may be characteristic of dreissenids in general (including *D. rostriformis bugensis*). This also appears to be case for the lack of impact of laboratory feeding regime on chronic thermal tolerance.

Laboratory-determined, chronic thermal tolerance limits of individuals of *D. polymorpha* from Lake Oologah (OK) indicated that summer water temperatures would induce extensive mortality events. Thus, the *D. polymorpha* population in Lake

Oologah (OK) suffered two successive mortality events in the summer of 2006 and 2007 possibly extirpating this population. If this population becomes extirpated it would be the first recorded instance of a natural dreissenid population extirpation in North America. However, two possibilities exist for the reestablishment of a *D. polymorpha* population in Lake Oologah (OK). First, the population could be reinitiated by additional invasion events, and second, reproduction by the few remaining, presumably more thermally tolerant individuals, could eventually repopulate the lake resulting in a genetically distinct, thermally tolerant lineage of *D. polymorpha* as appears to have been selected for in Winfield City Lake (KS). Such evolution of thermally tolerant dreissenid populations in southwestern water bodies could be the source for their further invasion into the warm waters of the southern and southwestern United States previously presumed to be thermally resistant to dreissenid mussel invasion. It also poses major implications for thermal mitigation techniques for the management of dreissenid mussel fouling in raw-water systems and for thermal-spray washing for mitigation of dreissenid mussel fouling on recreational boat hulls and on other mussel-infested equipment transported overland between infested and uninfested water bodies (Morse 2009).

The incipient, chronic (28 d), upper thermal tolerance limit of 27.2°C determined for *D. rostriformis bugensis* from Lake Mead (NV/AZ) in this study suggested that populations of this species could thrive in the lake at depths ≥12 m, but could be exposed to summer ambient water temperatures approaching or exceeding their incipient upper thermal limit at depths <12 m. The subsequent invasion of these

mussels into warm, shallow, southern-California and lower-Colorado River reservoirs may result in both acclimatization and genetic selection for more thermally tolerant lineages of *D. rostriformis bugensis*. Indeed, the recent development of dense adult populations of this species in the near-surface waters of Lake Mead (NV/AZ) may be a result of such thermal adaptation, threatening the further dispersal of *D. rostriformis bugensis* into warmer water bodies from which they were previously thought to be thermally excluded, particularly as this species was considered to have a incipient thermal tolerance limit that was ≈1–2°C lower than that of *D. polymorpha* (see Section 2.3.3 and literature cited in this chapter). Development of thermally tolerant lineages of *D. rostriformis bugensis* in isolated southwestern water bodies also has the same major implications for thermal mitigation and management of mussel fouling in raw-water systems and for thermal-spray washing for mitigation of quagga mussel fouling on recreational boat hulls and on other mussel-infested equipment described above for *D. polymorpha*.

The Winfield City Lake (KS) population of *D. polymorpha* appears to be the most thermally tolerant population of this species in North America. The highest, ambient, summer, water temperatures in Winfield City Lake (KS) are below the upper incipient (28 d) thermal tolerance limit of 30.7°C recorded for *D. polymorpha* from this water body. While it is possible that this result is due to acclimatization, this study, along with previously published data, presents strong evidence that it is more likely the result of thermal selection for a genetically based, thermally tolerant lineage. If its elevated incipient thermal tolerance limit is genetically based, the Winfield City Lake

(KS) *D. polymorpha* population and similar dreissenids presently infesting other warm southwestern water bodies could be carried over land by recreational boater traffic (Britton and McMahon 2005) and through interconnecting raw water pipelines and waterways to establish populations in other presently uninfested warm southwestern water bodies.

Raw-water users, such as electric power plants and water treatment plants, in the southeastern and southwestern United States are not well prepared to deal with dreissenid mussel fouling and are likely to incur extensive operating and mussel mitigation costs as populations of *D. rostriformis bugensis* and *D. polymorpha* expand their ranges in these regions. Indeed, this study suggests that adaptation of dreissenids to warmer, southwestern water bodies may reduce the effectiveness of present thermal mitigation treatments to control their macrofouling in raw water systems. In addition, this study presents evidence that the sensitive freshwater ecosystems of the southwestern and southeastern United States, from which dreissenids were once presumed to be thermally excluded, could support dense, thermally tolerant populations of both *D. polymorpha* and/or *D. rostriformis bugensis*. Unfortunately, most local, state and federal agencies, and their local water body managers, are not yet fully prepared or funded to implement the extensive and costly prevention and containment procedures required to limit the further range expansion of dreissenid mussels in southwestern water bodies and to deal with the resultant ecological and economic damage they entail (Western Regional Panel on Aquatic Nuisance Species 2009).

CHAPTER 3

IMPACT OF TEMPERATURE ON BODY TISSUE MASS IN ZEBRA MUSSELS, *DREISSENA POLYMORPHA*, FROM WINFIELD CITY LAKE, KANSAS

3.1 Introduction

3.1.1 Thermal Tolerance

The maximum, incipient (i.e., long-term), upper lethal temperature for samples of *Dreissena polymorpha* is approximately 30°C (McMahon 1996, McMahon and Ussery 1995, see Chapter 2). However, *D. polymorpha* populations inhabiting the lower Mississippi River (LA) appear to survive summer ambient water temperatures slightly above 30°C (Elderkin and Klerks 2005, Mihuc *et al*. 1999). Evidence of elevated thermal tolerance has been recorded in samples of *D. polymorpha* from the lower Mississippi River in Baton Rouge (LA), with specimens being able to tolerate 33°C longer than individuals from the more northern and much cooler Niagara River in Buffalo (NY) (Hernandez 1995). Similarly, individuals of *D. polymorpha* from Winfield City Lake (KS) tolerate elevated temperatures longer than specimens from the cooler Hedges Lake (NY) (See Chapter 2)

A seasonal pattern in upper thermal tolerance was recorded in *D. polymorpha* samples from the lower Mississippi River (Hernandez 1995, McMahon 1996). After acclimation to the same temperature (i.e., 5, 15 or 25°C), their upper thermal limit was still positively correlated to the ambient water temperature at the time of collection. Thus, even after acclimation for several weeks to the same temperature, specimens

sampled at high water temperatures during warm summer months had a higher upper thermal limit than specimens sampled at low ambient water temperatures during winter months (Hernandez 1995, McMahon 1996, this study–see Chapter 2). This suggests that there is a longer-term, seasonal pattern of acclimatization (i.e., seasonal changes in physiologic functions that cannot be removed by laboratory acclimation to constant conditions) impacting dreissenid mussel upper thermal limits in addition to short-term acclimation effects. These independently acting, long-term acclimatization and short-term acclimation impacts complicate the overall impact of ambient water temperature on the upper lethal temperature of dreissenid mussels (Hernandez 1995, McMahon 1996). Hernandez (1995) reported that, in addition to potential acclimatization effects, the lower Mississippi River (LA) population of *D. polymorpha* appeared to have undergone selection for elevated thermal tolerance compared to mussels from the Niagara River (NY). Thus, due to acclimatization to warmer, seasonal ambient water temperatures throughout the year and possible selection for thermally tolerant physiological lineages in warm water bodies, *D. polymorpha* sampled at the same time of the year from the southern and southwestern extremes of its North American range could potentially display a higher thermal tolerance than individuals sampled from cooler aquatic habitats in the northern regions of its North American range even after short-term acclimation of both groups to the same temperature.

3.1.2 Possibility of Selection for a Thermally Tolerant Physiological Lineage of D. polymorpha in Warm Freshwater Habitats in Oklahoma

Chapter two presented evidence supporting the possibility that populations of *D. polymorpha* or *D. rostriformis bugensis* in warm, southwestern water bodies such as

Winfield City Lake (KS), Lake Oologah (OK) and Lake Mead (NV/AZ) could be experiencing intense selection for development of a genetically based, thermally tolerant physiological lineages in water bodies where mean summer water temperatures approach or even exceed these species' upper thermal limits, relative to mussel populations in cooler waters at higher latitudes in North America where thermal selection is not experienced (for a review see Chapter 2). Summer water temperatures in Winfield City Lake (KS) and Lake Oologah (OK), which harbor populations of *D. polymorpha*, approach their incipient, upper lethal limit of ≈30°C (McMahon 1996). For instance, ambient, water temperatures recorded at a 2-m depth (the approximate depth of mussel collection) throughout 2006 and 2007 in Lake Oologah (OK) attained a brief August peak of 30.9° and 30.5°C, respectively. The mean daily ambient water temperatures in Lake Oologah (OK) at a 2-m depth during the warmest consecutive 28-d period in 2006 (4 August–31 August) and in 2007 (30 July–26 August) were both 29.1°C. Ambient water temperatures briefly (<1 d) reached 30.2°C at a 1-m depth in Winfield City Lake (KS) during August 2008 and the highest overall mean daily water temperatures at a 1-m depth during the warmest 28-d period in the summer of 2008 (17 July–13 August) was 27.4°C. Extremely high levels of *D. polymorpha* population mortality occurred in Lake Oologah (OK) during peak August temperatures in the summer of 2006 followed by population recovery in the spring of 2007. Elevated summer water temperatures in the summer of 2007 subsequently led to near extirpation of this population. Such apparent extensive annual thermal selection could lead to the development of populations with genetically elevated thermal tolerances in isolated,

southwestern, freshwater bodies recently invaded by dreissenid mussels. If thermally tolerant lineages of *D. polymorpha* and *D. rostriformis bugensis* are being selected for in southwestern United States water bodies, they could pose a potential threat as source populations for the eventual invasion into even warmer freshwater habitats in Texas and the southwestern United States from which they were once excluded by elevated summer water temperatures (i.e., >30°C). However, individuals of *D. polymorpha* were discovered in Lake Texoma (OK/TX) and in the Trinity River drainage above Lake Lavon (TX) in early 2009.

3.1.3 Anomalous Evidence for Negative Thermal Selection in Lake Oologah

Chronic thermal tolerance tests were performed on samples of *D. polymorpha* from Lake Oologah (OK) and from the cooler Hedges Lake (NY), where mussels presumably do not experience thermal stress (see Chapter 2). Since water temperatures in Lake Oologah (OK) were warmer, with a maximum ambient water temperature at a 2-m depth of 30.9°C, it was expected that mussels from that lake would have a relatively higher thermal tolerance, especially after several consecutive years of apparent, extreme thermal selection as maximum summer ambient water temperatures reached 30–31°C. However, this was not the case. Studies carried out in 2006 and 2007 revealed that samples of *D. polymorpha* from Lake Oologah (OK) did not tolerate chronic exposure to lethal temperatures as long as those from the cooler Hedges Lake (NY) (see Chapter 2). When held at a temperature of 29°C for 28 d, specimens of *D. polymorpha* from Lake Oologah (OK) had a higher mortality rate than did those from Hedges Lake (NY). The same result was recorded at exposure temperatures of 30–

34°C. In almost all cases, 100% mortality was recorded after 28 d at temperatures <30°C for samples from Lake Oologah (OK) while 100% mortality was not recorded over the same temperature range for specimens from Hedges Lake (NY) (see Chapter 2). Despite the fact that Lake Oologah (OK) was much warmer than Hedges Lake (NY), resulting in substantial summer population mortalities, samples of *D. polymorpha* from Lake Oologah (OK) had a lower, chronic thermal tolerance than did those from Hedges Lake (NY). This unexpected result suggested that, in August, *D. polymorpha* population mortality at water temperatures of 30–31°C in Lake Oologah (OK) may not have been the result of lethal thermal stress, but was influenced by another cause only indirectly associated with elevated ambient water temperatures.

3.1.4 Thermal Impacts on the Physiologic Condition of D. polymorpha

Individuals of *D. polymorpha* were sampled from Lake Oologah (OK) for thermal tolerance testing during the summers of 2006 and 2007 when surface ambient water temperatures were above 25°C (see Chapter 2). The study of Walz (1978a) indicated that at or above ≈20°C, specimens of *D. polymorpha* were no longer able to filter, ingest and assimilate food energy rapidly enough to support their temperature-induced elevated metabolic maintenance demands. Schneider (1992), using a bioenergetics model, suggested that above ≈24°C specimens of *D. polymorpha* (of 0.1 g wet tissue mass) would not be able to support their metabolic maintenance demands, resulting in negative growth. Thus, at ≥25°C, individuals of *D. polymorpha* appear to be subject to varying levels of negative growth (i.e., loss of tissue mass through time) depending on temperature and phytoplankton/nanoplankton food availability. Thus,

above an ambient, water temperature of 20–25°C, specimens of *D. polymorpha* could be expected to increasingly lose body mass in order to meet maintenance demands until mortality ensues. It is possible that such loss of nutritional condition (i.e., relative level of tissue, organic energy stores) would also result in the reduction of a mussel's incipient upper thermal limits. In other words, the thermal tolerance of individuals of *D. polymorpha* could be lowered due to progressive loss of physiologic function during prolonged body tissue loss, reducing the time over which they could tolerate exposure to lethal temperatures (i.e. ≥30°C), or even otherwise sublethal temperatures (28–29°C), by exacerbating tissue mass reduction to lethal levels within the relatively short exposure periods (i.e., 28 d) used to determined chronic thermal tolerance times (for details on chronic thermal tolerance determinations in dreissenid mussels see Chapter 2). This appeared to be the case among specimens of *D. polymorpha* sampled at water temperatures ≥25°C from Lake Oologah (OK) for thermal tolerance determinations during the summers of 2006 and 2007 (Chapter 2). Their poor nutritional condition during this period may have resulted in a concomitant loss of physiologic condition (i.e., reduction in capacity to tolerate environmental stress) resulting in an anomalously low thermal tolerance compared to specimens sampled from the cooler waters (<25°C) of Hedges Lake (NY) which had not experienced ambient water temperatures ≥25°C and thus, had presumably not undergone a loss of nutritional condition extensive enough to negatively impact their ability to survive 28-d exposures to lethal temperatures (Chapter 2).

The objective of this study was to determine the relationship between nutritional condition, defined as dry tissue mass to shell length (SL) ratio relative to ambient water temperature, in samples of *D. polymorpha* collected from Winfield City Lake (KS) from mid-June to mid-October, 2008. This information was then used to interpret the thermal tolerances and possible extirpation of *D. polymorpha* from Lake Oologah (OK).

The research presented in this chapter was a collaborative project with Esther Leung, a UT Arlington Honors College student, who used a portion of the information presented in this chapter for her Honors thesis. Esther's involvement primarily included determination of dry tissue weights for samples of *D. polymorpha* from Winfield City Lake (KS) while this author carried out all field collections, some of the laboratory procedures and the statistical analyses of the resulting data.

3.2 Methods

3.2.1 Sampling

Specimens of *D. polymorpha* were sampled periodically from Winfield City Lake (KS) during the summer of 2008. The lake is located 13 km northeast of the City of Winfield (KS) (Figure A2) and has a surface area at normal spillway levels of ≈5 km^2, a shore line of 33.8 km and a maximum depth of 15.25 m. The sampling site was within Winfield City Lake Park in a bay extending south from the southern edge of the main lake impoundment (37.3454°N, 96.8959°W) (Figure A5). Six mussel samples were collected throughout the summer until the beginning of fall by scraping specimens from mussel masses encrusting the surfaces of submerged plastic floats supporting the Park's utility barge dock. Collections of greater than 100 individuals were made on 16

June, 29 June, 19 July, 8 August, 31 August, and 15 October 2008. Individuals were randomly removed from their byssal attachments with a scalpel, immediately preserved in 95% alcohol and returned to the laboratory at The University of Texas at Arlington. Water temperatures were monitored at hourly intervals continually throughout the experimental period at the collection site with Onset Corporation® temperature data loggers (Part# UA-002-08). The data loggers were held at depths of 1, 2, and 4 m by suspending them from a weighted rope at the end of the Park's utility barge dock.

At each sampling period, a single 1-L water sample was taken from Winfield City Lake (KS) at a depth of 1 m with a van Dorn sampler. The water samples were kept on ice until they could be processed in the laboratory at UT Arlington (within 8 h). Water samples were divided into unfiltered and filtered subsamples, the latter created by pouring a portion of the sample through a 100-µm mesh filter. A single 30-ml subsample from both the filtered and unfiltered portion was vacuum-filtered through a 2.5-cm Whatman® 1-µm glass microfiber filter (GF/F) to remove all phytoplankton.

The filters, with phytoplankton, were placed (using forceps) into the bottom of a polypropylene scintillation vial containing 1 ml of saturated $MgCO_3$ and frozen at -12°C until chlorophyll *a* processing was performed utilizing the methodology of Welschmeyer (1994). To analyze the chlorophyll *a* content of the filtered material, 9 ml of acetone was added to each vial and the vials were incubated overnight in the dark at room temperature (20–23°C). Following incubation, the chlorophyll *a* content of each vials' solution was measured by lightly swirling each vial, pouring its contents into a 5-ml cuvette, and then measuring its fluorescence on a Turner Designs® 10-AU

fluorometer. The fluorometer was set to an excitation wavelength of 436 nm with a 680-nm emission filter.

Water temperature data was similarly monitored at Lake Oologah (OK) (36.4202°N, 95.6664°W) at depths of 1, 2, 4 and 8 m from 30 June 2006 through 31 August 2008 and chlorophyll *a* concentrations were similarly determined from water samples collected on 15 March, 05 April, 25 April, 17 May, 31 May, 29 June, 19 July, 08 August, 31 August, and 15 October 2008.

3.2.2 Measurements of D. polymorpha Sample Dry Tissue Weights

A randomly selected subsample of 100 individuals of *D. polymorpha* representative of the shell length range of the collected population was taken from each original sample. The shell length (i.e., the greatest linear distance from the anterior tip of the shell valve umbos to the posterior margin of the shell valves) was measured to the nearest 0.01 mm for each individual with digital calipers. The shell valves of each subsampled individual were then completely dissolved in a 15% nitric acid solution (by volume). After shell dissolution and removal of the shell periostracum, remaining soft tissues were carried through three distilled water rinses to remove any traces of nitric acid. The remaining soft tissue of each individual was blotted free of excess water on paper toweling. The tissue was then placed in an aluminum pan pre-weighed to the nearest 0.01 mg on an electric balance. The pan and soft tissue of each individual were dried to constant weight in a drying oven at a constant temperature of 60°C for >48 h. After drying to constant weight, the pan and dried tissue were reweighed to the nearest 0.01 mg and individual dry tissue weight (DTW) determined to the nearest 0.01 mg by

subtraction of the pan weight from the pan plus specimen dry tissue weight. A single 100-individual sample of *D. polymorpha* collected from the Redbud Bay Marina on Lake Oologah (OK) on 29 June 2007 was also similarly analyzed to determine individual SL and DTW.

3.2.3 Analysis of Data

The data was fitted to a least square linear regression of the logarithmic transformation of shell length (SL) in mm treated as a continuous independent variable and collection date as a categorical independent variable versus the logarithmic transformation of DTW in mg as the dependent variable using SAS's Proc GLM (SAS Institute, Inc.). Regression slopes from the saturated model were compared for significant differences ($P < 0.0024$) across sampling periods with pair-wise Bonferroni corrected contrasts of the logarithmic transformation of shell length by sampling date interaction estimates for each collection. Sample DTW by SL regressions with significantly lower slopes were assumed to imply significantly reduced DTW per unit increase in SL for individuals relative to individuals from samples with significantly higher SL versus DTW regression slope values. Pair-wise Tukey-Kramer comparisons were made between collection dates to determine significant ($P < 0.05$) differences in model-estimated DTW for individuals at 5, 10, 15, 20, 25, and 30 mm. The dry tissue weights of standard-sized individuals from each collection were then fitted to least square regressions versus chlorophyll *a* concentration concurrently estimated at the collection site (see section 2.1 above) as well as a least square regression of chlorophyll *a* concentration versus ambient water temperature at collection.

3.3 Results

3.3.1 Dry Tissue Biomass in Winfield City Lake

When data for the six Winfield City Lake (KS) collections and one Lake Oologah (OK) collection were each fitted to a least square linear regression with the logarithmic transformation of SL and dates of collection as continuous and categorical variables, respectively, and the logarithmic transformation of Dry Tissue Weight (DTW) as the dependent variable, DTW was found to increase exponentially with SL (Figure A33; Table B12). Collection date had a significant effect on the DTW of *D. polymorpha* (Table B12). This result was reflected by the slopes for DTW/SL regressions generally being significantly greater (Bonferroni $P < 0.0024$) for Winfield City Lake (KS) samples taken in the early summer (i.e., 16 June and 29 June 2008), and in the fall (15 October 2008) compared to regression slopes for the samples taken in mid- and late-summer (i.e., 19 July, 8 August, and 31 August 2008) (Figure A34, Table B13). The regression slopes for Winfield City Lake (KS) samples taken on 16 June, 29 June, and 15 October 2008 were not significantly different from each other (Bonferroni $P < 0.0025$) as were those taken on 19 July, 8 August, and 31 August 2008 (Figure A34, Table B13). This result suggested that samples of *D. polymorpha* from Winfield City Lake (KS) had a significantly lower DTW in mid- and late-summer than they did in early summer and early fall (Figure A34).

When estimated from $\log_{10}$ DTW/$\log_{10}$ SL regressions, DTW of Winfield City Lake (KS) individuals significantly declined ($P < 0.05$) to minimum values during 19 July, 8 August, and 31 August 2008 in standard individuals of 20, 25 and 30-mm SL

when mean daily ambient water temperatures were >25°C (Figure A35A, Table B14). This pattern of summer decline in DTW at ambient water temperatures >25°C was less pronounced in smaller standard individuals (i.e., 10 and 15-mm SL), but still apparent with maximal DTW occurring on 29 June 2008 and significantly ($P < 0.05$) declining thereafter (Figure A35B, Table B15). The least seasonal impact on variation in DTW occurred in the smallest standard 5 mm SL individual (Figure A35B, Table B15) while seasonal impact on DTW was most pronounced in the largest standard 30-mm SL individual. The 30-mm SL standard individual attained a maximum DTW of 105.9 mg on 29 June 2008 followed by a 53% decline to a minimal DTW of 49.9 mg on 8 August 2008. Subsequently, after mean daily water temperatures of Winfield City Lake (KS) fell below 25°C, the DTW of a 30 mm SL standard individual rose to 71.4 mg on 15 October 2008 (67% of the maximum DTW recorded on 29 June 2008) (Figure A35A).

3.3.2 Chlorophyll a Concentrations

Chlorophyll *a* concentrations in Winfield City Lake (KS) water samples (total and after sample filtration through a 100-µm filter) attained maximum values of 2.96 and 3.04 µg/L, respectively, on 29 June 2008. Subsequently, chlorophyll *a* concentrations after filtration through a 100-µm filter declined to relatively stable levels over the remainder of the sampling period, ranging from 1.40–2.12 µg/L (46.1–69.7% of the maximum 29 June 2008 value) (Figure A36A). Similarly, total chlorophyll *a* concentrations also declined after the 29 June 2008 maximum to relatively stable levels of 1.28–2.39 µg/L or 43.2-77.4% of maximal values (Figure A36A). Chlorophyll *a* concentrations (both total and after sample filtration through a 100-µm filter) at

Winfield City Lake (KS) did not display a significant ($P > 0.05$) relationship with mean, daily, ambient water temperature (Figure A36B, Table B16). Least square linear regression analysis also revealed no significant ($P > 0.05$) relationship between the DTW of any standard-sized specimens and chlorophyll *a* concentration (both total and after sample filtration through a 100-µm filter) (Tables B17 and B18, also see Figure A36C for a 25-mm SL example).

3.3.3 Dry Tissue Biomass in Lake Oologah

3.3.3.1 Comparison with Winfield City Lake Results

The DTW of *D. polymorpha* samples collected from Winfield City Lake (KS) on 29 June 2008 was compared to those collected exactly one year earlier from Lake Oologah (OK) on 29 June 2007. The slopes of the logarithmic transformation of DTW versus the logarithmic transformation of SL least square linear regressions of these two samples were significantly different ($P < 0.0001$), with that for Lake Oologah (OK) specimens being considerably lower than that for individuals from Winfield City Lake (KS) (Figure A37, Table B12). This result indicated that *D. polymorpha* samples from Lake Oologah (OK) had a much lower DTW across all size classes than did specimens from Winfield City Lake (KS) collected at the same time of year (Figure A38). For each standard-sized individual, the percentage of DTW of Lake Oologah (OK) individuals relative to that of Winfield City Lake (KS) was determined. The DTW of a standard 5-mm SL individual from Lake Oologah (OK) was 80.5% that of the DTW of a standard 5-mm SL individual from Winfield City Lake (KS). Similarly, the DTW values of 10, 15, 20, 25 and 30-mm SL standard sized individuals from Lake Oologah

(OK) were 56.6, 46.0, 39.7, 35.4 and 32.3% of similarly-sized standard specimens from Winfield City Lake (KS), respectively (Figure A38). These results indicated that reduction in the DTW of Lake Oologah (OK) individuals relative to those from Winfield City Lake (KS) became progressively more pronounced with increasing size, becoming maximal at 32.3% of the DTW in the largest standard individual of 30-mm SL (Figure A38).

3.3.3.2 Comparison of Ambient Water Temperatures

Temperatures in Lake Oologah (OK) varied with season reaching peak values of ≈30°C in mid-summer and minimum values ≈2°C in midwinter from 2006–2008 (Figure A39). A very similar pattern of seasonal temperature change was recorded in Winfield City Lake (KS) during the summer and fall of 2008 suggesting that both lakes likely had a similar pattern of seasonal temperature variation (Figure A39). However, when daily mean, ambient, water temperature records were compared for Lake Oologah (OK) and Winfield City Lake (KS), it was clear that ambient water temperatures at Lake Oologah (OK) reached higher levels and remained above 25°C longer than in Winfield City Lake (KS) (Figure A40). Where full summer water temperature data was available in 2007 at Lake Oologah (OK) and 2008 at Winfield City Lake (KS) (Figure A40), the number of degree-days during which ambient water temperature was above 25°C was calculated as the sum of the °C >25°C for each day that mean daily ambient water temperature was >25°C (Figure A40). Based on this calculation, the degree-days above 25°C at Lake Oologah (OK) during 2007 at 193 degree-days were essentially twice that of Winfield City Lake (KS) at 97 degree-days in 2008. This difference was a

result of both higher water temperatures and a longer duration of temperatures above 25°C at Lake Oologah (OK) during 2007 compared to Winfield City Lake (KS) during 2008. At Lake Oologah (OK), mean ambient water temperatures continuously remained above 25°C for 80 d in 2007 (Figure A40). In contrast, at Winfield City Lake (KS) in 2008 mean daily ambient water temperatures continuously remained above 25°C for only 63 d (Figure A40). Mean daily water temperatures also tended to be warmer at Lake Oologah (OK), averaging 27.4°C during the 80-d period when water temperatures continuously exceeded 25°C. This mean daily water temperature value was only 26.5°C during the 63 d that mean daily water temperatures continuously exceeded 25°C at Winfield City Lake (KS) (Figure A40).

3.3.3.3 Comparison of Chlorophyll *a* Concentrations

On dates when collections overlapped (i.e., 29 June, 19 July, 8 August, 31 August, and 15 October 2008), the average total and filtered chlorophyll *a* concentrations of Lake Oologah (OK) water were generally equal to or greater than that for Winfield City Lake (KS). The mean total chlorophyll *a* content of Lake Oologah (OK) for these sample dates was 3.12 ± 0.46 ug L^{-1} while that for Winfield City Lake (KS) was 2.10 ± 0.18 ug L^{-1}. Similarly, the mean 100-µm filtered chlorophyll *a* content of Lake Oologah (OK) for these sample dates was 3.00 ± 0.44 ug L^{-1} while that of Winfield City Lake (KS) was 1.98 ± 0.11 ug L^{-1}.

3.4 Discussion

3.4.1 Dry Tissue Weight Loss at Ambient Water Temperatures >25°C

The metabolic demand of specimens of *D. polymorpha*, as indicated by respiration rates, rises with increasing temperature and reaches a peak at 30–35°C (Schneider 1992, Aldridge *et al*. 1995, Alexander and McMahon 2004). In contrast, the filtration rate of specimens of *D. polymorpha* decreases with increasing temperature above 24°C such that at 28°C the assimilation rate for individuals of *D. polymorpha* is estimated to be below maintenance levels (Aldridge *et al*. 1995). Moreover, at 32°C the filtration rate of specimens of *D. polymorpha* falls 78% below maximal levels and metabolic demand far exceeds energy ingestion/assimilation (Aldridge *et al*. 1995). Samples of *D. polymorpha* from Lake Huron displayed maximum filtration rates at ≈15°C with filtration rate dropping quickly above that temperature such that filtration ceased at 30°C (Fanslow *et al*. 1995). Walz (1978b) recorded an optimal ingestion rate at a water temperature of ≈12.5°C for specimens of *D. polymorpha* collected from Lake Constance, Germany, with rates of ingestion declining rapidly with further temperature increase.

When ambient water temperatures were >25°C in Winfield City Lake (KS), adult individuals of *D. polymorpha* experienced a loss in DTW, which progressively increased with increasing SL (Figure A35). A similar, summer loss of DTW in specimens of *D. polymorpha,* after attaining a spring maximum, has been reported in European freshwaters (Doregelo and Kraak 1993, Jantz and Neumann 1998). A summer loss of DTW has also been reported for North American specimens of *D.*

polymorpha from Lake St. Clair (Nalepa *et al.* 1993), the western basin of Lake Erie (Stoeckmann and Garton 1997) and Lake Michigan (Nalepa *et al*. 1995). Walz (1978a) demonstrated that larger individuals of *D. polymorpha* had a progressively greater rate of tissue loss as a result of an increasingly negative metabolic energy balance than smaller individuals at 25°C. Jantz and Neumann (1998) observed a similarly more pronounced summer DTW loss in larger individuals of *D. polymorpha* over an SL range of 10–20 mm in the Rhine River, Germany. However, they attributed the dependence of DTW loss on SL to coincide with spawning events determined by microscopic examination of dissected individuals (Jantz and Neumann 1998).

In this study, weight loss began at ambient water temperatures of 25°C, which is well above the 12°C limit for spawning (Borcherding 1991). Although the threshold for spawning in specimens of *D. polymorpha* appears to be ≈12°C, maximal gonad volume and spawning occur at temperatures of 18–22°C (Haag and Garton 1992, Fong *et al.* 1995, McMahon 1996, Nichols 1996, Ram and McMahon 1996, Roe and MacIsaac 1998), still well below 25°C, the temperature at which individuals of *D. polymorpha* in Winfield City Lake (KS) began to lose DTW in this study. Furthermore, spawning was minimal among individuals of *D. polymorpha* from Lake Erie at ambient water temperatures ≥25°C and during the mid-summer spawning cessation period, tissue lipid contents continued to decline indicative that it resulted from a negative energy balance rather than gamete release (Roe and MacIsaac 1998). Therefore, samples of *D. polymorpha* utilized in this study were likely to have already spawned before field collections began, suggesting that the DTW loss observed at temperatures >25°C

resulted from temperature-induced metabolic demands exceeding energy assimilation rates rather than spawning.

Nalepa *et al.* (1993) observed that in mid-summer in Lake St. Clair (MI) when water temperatures approached 25°C, the body lipid contents of adult individuals of *D. polymorpha* declined from maximum spring values. As reported in this study, maximal DTW losses in the Lake St. Clair (MI) population of *D. polymorpha* occurred as peak summer ambient water temperatures approached or exceeded 25°C in late June and July (Jantz and Neumann 1998, Nalepa *et al.* 1993 Nalepa *et al*. 1995). Nalepa *et al.* (1993) also observed that from June through August the total tissue carbon contents of specimens of *D. polymorpha* at two stations in Lake St. Clair (MI) declined while tissue nitrogen contents remained constant, leading to a decline in tissue carbon:nitrogen (C:N) ratios. This loss of lipids and decline in C:N ratio during the period of maximal summer, ambient water temperatures suggested that mussels were metabolizing tissue lipid and carbohydrate energy stores, leading to a loss of tissue mass (marked by DTW loss) reflecting tissue contents being dominated by protein (Nalepa *et al.* 1993). Roe and MacIsaac (1998) also recorded a loss of tissue lipid content in specimens of *D. polymorpha* experiencing elevated ambient water temperatures (up to 30°C) in the western basin of Lake Erie. At high, summer, ambient, water temperatures, concurrent decreases in tissue carbohydrate and lipid contents, tissue C:N ratios, and DTW, are thus indicative of tissue biomass reduction as tissue lipid and carbohydrate (i.e., glycogen) energy stores are metabolized to generate the additional energy needed for

elevated maintenance energy demands that cannot be met by filtration and assimilation of enough phytoplankton, bacterioplankton and microdetritus.

Laboratory studies by Walz (1978a) have indicated that when fed phytoplankton *ad libitum*, individuals of *D. polymorpha* displayed optimal growth (i.e., positive energy balance where energy assimilation exceeds metabolic energy expenditure) at 10–15°C, and maintained a positive energy balance (i.e., sustained tissue growth) up to 20°C. However, mussels entered negative energy balance (i.e., energy assimilation < metabolic energy expenditure) above 20°C, leading to tissue mass loss (i.e., starvation). In contrast, Baldwin *et al*. (2002) reported that specimens of both *D. polymorpha* and *D. rostriformis bugensis* could maintain positive growth rates at temperatures of 23°C when food availability was high. Schneider (1992) estimated that negative growth rates for specimens of *D. polymorpha* would begin at 25°C regardless of food availability while Thorpe *et al*. (1998) estimated a growth threshold at 25–30°C.

In this study and those of Jantz and Neumann (1998), Nalepa *et al.* (1993, 1995) and Stoeckmann and Garton (1997), phytoplankton densities measured as chlorophyll *a* concentrations tended to decline during late summer months. Because the metabolic rates of specimens of *D. polymorpha* increase exponentially with temperature (Alexander and McMahon 2004), their maintenance metabolic rates are maximally elevated by high summer ambient water temperatures (Stoeckmann and Garton 1997). Maximal metabolic demands during a summer period of elevated temperatures followed by reduced summer phytoplankton densities are likely to place natural *D. polymorpha* populations in a negative energy balance as recorded by Stoeckmann and Garton

(1997), leading to tissue mass loss above ambient water temperatures of 25°C (Doregelo and Kraak 1993, Jantz and Neumann 1998, Nalepa *et al.* 1993, 1995, Stoeckmann and Garton 1997, this study).

3.4.2 Chlorophyll a Concentrations and Dry Tissue Weight Loss

Chlorophyll *a* concentrations were not correlated with DTW levels during the summer and fall at Winfield City Lake (KS) (Figure A36). Similarly, Dorgelo and Kraak (1993) found no correlation between algal cell densities and DTW during summer months in a *D. polymorpha* population from Lake Maarsseveen, the Netherlands. Hincks and Mackie (1997) also found no correlation between chlorophyll *a* concentration and juvenile growth rate of *D. polymorpha.* Walz (1978a) showed that at ≥20°C, specimens of *D. polymorpha* lost tissue mass regardless of algal food availability and that the rate of tissue mass loss was directly related to temperature. Thus, rather than *D. polymorpha* tissue growth being directly related to phytoplankton density as occurs <20°C, above 20°C mussel growth and tissue mass accumulation appears to be decoupled from algal food availability. Thus, as was observed at Winfield City Lake (KS), the DTW loss of specimens of *D. polymorpha* during summer months may have been more related to the degree of exposure to elevated water temperatures (i.e., >25°C) than to algal food availability (Walz 1978a). This may also explain the decline in growth rate recorded for a *D. polymorpha* population at ambient water temperatures above 20°C in the Rhine River, Germany (Jantz and Neumann 1998).

3.4.3 Impacts of Water Temperature on Dry Tissue Weights in D. polymorpha from Lake Oologah

Daily ambient water temperatures in Lake Oologah (OK) were generally higher and remained above 25°C for longer periods during the summer than in Winfield City Lake (KS) (Figure A40). During the summer of 2007, Lake Oologah (OK) had twice the number of degree-days above 25°C (192.6 °C·days) than did Winfield City Lake (KS) during the summer of 2008 (97.3 °C·days) (Figure A40). This difference in the degree of temperature stress may have resulted in twice the starvation pressure on the *D. polymorpha* population from Lake Oologah (OK) and their much reduced DTW on 29 June 2007 compared with that of the *D. polymorpha* population from Winfield City Lake (KS) sampled on 29 June 2008 (Figures A37 and A38). On 29 June 2008, mussel DTW was at its peak in the Winfield City Lake (KS) *D. polymorpha* population (Figure A35). During June 2007 and 2008 ambient water temperatures were essentially similar in Lake Oologah (OK) and Winfield City Lake (KS), respectively (Figure A40). The similar temperature regimes experienced by the two populations suggests that Lake Oologah (OK) individuals would have been near maximal DTW when collected on 29 June 2007 as were Winfield City Lake (KS) specimens collected on 29 June 2008 (Figure A35). Because specimens of *D. polymorpha* from Lake Oologah (OK) had a much reduced DTW [i.e., 35.4% that of Winfield City Lake (KS) mussels for a standard 25 mm SL individual (Figure A38)], they were unlikely to have been able to tolerate as great a level of high-temperature induced tissue mass loss as did the more nutritionally competent Winfield City Lake (KS) mussel population during the summer of 2008. In 2007 specimens of *D. polymorpha* in Lake Oologah (OK) entered the summer season in

much poorer nutritional condition and experienced twice the temperature stress than did the Winfield City Lake (KS) *D. polymorpha* population in 2008, suggesting that the Lake Oologah (OK) adult *D. polymorpha* population may have been subjected to a lethal level of tissue loss. Loss of tissue mass may also have prevented the Lake Oologah (OK) population from reproducing in 2007, accounting for their ongoing extirpation and lack of population recovery during the spring and summer of 2008.

The very low summer DTW levels in Lake Oologah (OK) specimens of *D. polymorpha* in 2007 may also have reduced their physiologic condition to levels that led to the reduced chronic thermal tolerance limits recorded for individuals from this population compared to specimens of *D. polymorpha* from Hedges Lake (NY) (see Chapter 2). In these chronic thermal tolerance tests, individuals were held at high, but sublethal, water temperatures for up to 28 d at 28–30°C under which they would have been subjected to maximal levels of negative energy balance (Walz 1978a, see above). Thus, rather than resulting directly from temperature stress, Lake Oologah (OK) specimens may have died from tissue mass loss exacerbated by prolonged exposure to high, otherwise sublethal temperatures, resulting in a lethal reduction in their organic energy stores making them less thermally tolerant, particularly at low test temperatures of 28–30°C, than specimens from Hedges Lake (NY) (see Chapter 2). In contrast, when subjected to the same thermal tolerance testing, Winfield City Lake (KS) specimens of *D. polymorpha*, which had much higher summer DTW levels than Lake Oologah (OK) specimens, proved to have higher, chronic thermal tolerance levels than mussels from Hedges Lake (NY) (see Chapter 2). This result suggested that the low, chronic thermal

tolerance limits recorded for specimens of *D. polymorpha* from Lake Oologah (OK) was due to their poor nutritional/physiologic condition induced by excessive tissue mass loss rather than a reflection of their healthy thermal tolerance limit which, like that of Winfield City Lake (KS) individuals, almost certainly would have been higher than that of Hedges Lake (NY) individuals in order for them to survive the extended exposures to near lethal ambient water temperatures that this population must have experienced during summer months before 2006 (Figures A39 and A40).

Lake Oologah (OK) once had a thriving *D. polymorpha* population that reached densities of >100,000 mussels m^{-2} (Boeckman 2006). After their invasion of Lake Oologah (OK), the *D. polymorpha* population remained dense and apparently healthy throughout the summer. In the summer of 2006, the first major mortality event in the Lake Oologah (OK) *D. polymorpha* population was observed, but the population recovered to previous density levels as a result of fall 2006/spring 2007 reproduction and juvenile mussel settlement. In summer 2007, the Lake Oologah (OK) *D. polymorpha* population was essentially extirpated and has not yet recovered. This sequence of events suggests that the Lake Oologah (OK) *D. polymorpha* population was experiencing progressively greater levels of temperature-induced tissue mass loss during summer months until they were no longer in sufficient physiologic condition to survive the temperature-induced tissue mass reduction experienced in the summer of 2007.

Although the algal abundance in Lake Oologah (OK) appeared to be 1.5 times greater than that of Winfield City Lake, based on Chlorophyll *a* concentrations during

the summer of 2008, it is worth noting that the *D. polymorpha* population in Lake Oologah (OK) had already been greatly reduced while that of Winfield City Lake (KS) was quite dense. Thus, the filter feeding of the Winfield City Lake (KS) *D. polymorpha* population may have accounted for this difference in phytoplankton densities. It is likely that filter feeding by the Lake Oologah (OK) *D. polymorpha* population during years prior to 2006 progressively reduced algal food availability by reducing phytoplankton densities. In doing so, they may have removed much of the nitrogen and phosphorous micronutrients from the water column required for phytoplankton growth by storing it in the tissues of living individuals. In addition, some of the removed phosphates and nitrates would have been sequestered in the sediments as feces and pseudofeces, and in the proteinaceous periostracum covering their shell valves, preventing its return to the overlying water column to support phytoplankton productivity. Previous studies have recorded that in waters infested with dreissenid mussels, phytoplankton productivity tends to substantially decline as nitrate and phosphates are removed from the water column (Arnott and Vanni 1996, Karatayev *et al*. 1997, Reed-Andersen *et al*. 2000, Stanczykowska 1984). Nalepa *et al*. (1995) reported a decrease in chlorophyll *a* concentration, suspended particulate organic matter and population density of *D. polymorpha* in the Saginaw Bay, Lake Huron across 1991–1993. *D. polymorpha* population density declined from a peak value of 33,200 individuals m^{-2} in 1992 to 4,100 individuals m^{-2} in 1993. During 1992, the estimated ash-free dry tissue (AFDTW) weight of a 15-mm SL individual of *D. polymorpha* declined 62% from May through October and remained at low values through the

winter followed by a further 48% loss in AFDTW through October 1993 (Nalepa *et al*. 1995). Such loss in AFDTW between 1992 and 1993 appeared to have been a result of a corresponding decline in the concentration of phytoplankton and particulate organic matter food resources mimicking the decline in DTW observed at high summer temperatures in Winfield City Lake (KS).

3.5 Conclusions

Specimens of *D. polymorpha* appear to undergo extensive tissue loss during summer periods of elevated ambient water temperatures ranging above 20–25°C (Doregelo and Kraak 1993, Jantz and Neumann 1998, Nalepa *et al.* 1993, 1995, Stoeckmann and Garton 1997, 25°C determined in this study), resulting from a negative energy balance in which metabolic energy demands exceed energy assimilation, regardless of phytoplankton abundance (Walz 1978a, this study). Extensive loss of tissue biomass induced by elevated temperatures, as documented for the Winfield City Lake (KS) *D. polymorpha* population in this study, appeared to have been the basis for the poor nutritional/physiologic condition of individuals from Lake Oologah (OK) in the summers of 2006 and 2007. Their greatly reduced tissue biomass and, presumably, organic energy stores, appeared to have resulted in the anomalously reduced, chronic thermal tolerance limits recorded for this population compared to that of specimens from the cooler Hedges Lake (NY) (see Chapter 2) and their possible extirpation from the lake during the summer of 2007.

D. polymorpha and *D. rostriformis bugensis* populations have recently invaded water bodies in the southwestern United States and California, causing much concern

over their potentially negative economic and ecological impacts. The potential for dreissenid mussel populations to experience high-temperature induced tissue biomass loss or even eventual extirpation in the warm water bodies of the southwestern and southern United States may have important implications for both the eventual invasion and distribution of dreissenid mussels in this region and for their management and control. As such, the impact of high, summer, water temperatures on the nutritional condition, growth, reproductive capacity, physiological limits and long-term survivorship of both *D. polymorpha* and *D. rostriformis bugensis* populations in the southwestern United States clearly warrants further study.

CHAPTER 4

ANALYSIS OF NORTH AMERICAN DREISSENID INVASION BIOLOGY WITH AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLP)

4.1 Introduction

4.1.1 Dreissenid Evolutionary History

North American dreissenids, the zebra mussel, *Dreissena polymorpha*, and the quagga mussel, *Dreissena rostriformis bugensis*, are among the many important invasive species indigenous to the Ponto-Caspian region that have been introduced to, and become established in, the Laurentian Great Lakes (Ricciardi and MacIsaac 2000). Because genetic variation and heterozygosity are correlated with the ability to survive stressful environments and invade new habitats (Garton and Haag 1991), the geological and climatological instability of the Ponto-Caspian region may have led to the evolution of successful invasive species by promoting the retention and maximization of genetic variation (Gelembiuk *et al.* 2006). Alternatively, the fluctuation of temperature, depth, salinity and chemistry of regional seas in the Ponto-Caspian could have generated selection events that created genetic bottlenecks reducing genetic variation. Multiple catastrophic population reduction events due to unpredictable, stressful fluctuations in local environments favor the selection of species with life history strategies of reduced lifespans, semelparity, early maturity, rapid growth and elevated fecundity that support rapid population growth as is characteristic of dreissenid bivalves (Stepien *et al.* 2001, McMahon 2002).

4.1.2 Dreissenid Genetics

D. polymorpha has a diploid chromosome number of 32. Twenty of these chromosomes are small metacentrics while the other twelve are acrocentrics (Marsden *et al.* 1996). The haploid genome size of *D. polymorpha* is approximately 1662.6 Mbp, a fairly typical, although slightly larger value than generally reported for heterodont bivalves (Gregory 2003). The relatively large size of the *D. polymorpha* genome compared to other bivalves suggests that multiple transposable elements may be present (Gregory 2003) and potentially associated with this species' invasiveness as suggested for *Drosophila* by Vieira *et al.* (2002).

Studies of genetic diversity in dreissenids have yielded differing results. Analysis of differences in the mitochondrial cytochrome oxidase I (COI) gene or 16S rDNA revealed that *D. polymorpha* and *D. rostriformis bugensis* had low levels of intraspecific genetic diversity potentially due to bottlenecks during their evolutionary history (Stepien *et al.* 2001, Gelembiuk *et al.* 2006, May *et al.* 2006). Microsatellite, randomly amplified polymorphic DNA (RAPD), and allozyme analyses have all indicated a high level of intraspecific genetic variation in populations of both species (Hebert *et al.* 1989, Garton and Haag 1991, Marsden *et al.* 1995, 1996, Stepien *et al.* 2002, Astanei *et al.* 2005). Despite high levels of intraspecific variation, Astanei *et al.* (2005) could not differentiate between two different North American Great Lake populations of *D. polymorpha* with microsatellite analysis. Similarly, allozyme analysis found only slight genetic differences among North American populations of *D. polymorpha* in the Great Lakes and between North American and European populations

(Marsden *et al.* 1995). These results and those of others suggest that early North American founder populations have involved introductions of large numbers individuals and/or that they have been subject to frequent genetic mixing (Stepien *et al.* 2002, reviewed by Marsden *et al.* 1996). In contrast, a more recent RAPD analysis of North American *D. polymorpha* and *D. rostriformis bugensis* populations was able to differentiate populations within both species, although the resulting tree had low support at many nodes (Stepien *et al.* 2002).

Theoretically, populations of invasive species should not experience further genetic mixing after their initial introduction to a hydrologically isolated water body, thereby increasing the likelihood of becoming genetically differentiated from other populations over time. However, due to the short period of dreissenid population dispersal into North American water bodies since their initial invasion in approximately 1986, the potentially high levels of dispersal among populations through interconnecting waterways (Johnson and Carlton 1996) and human-mediated overland transport of mussels and larval stages (Johnson *et al*. 2001, Britton and McMahon 2005), it is possible that many molecular techniques may not be capable of differentiating among North American dreissenid populations.

4.1.3 Amplified Fragment Length Polymorphism (AFLP)

AFLP was developed by Vos *et al.* (1995) as a novel DNA fingerprinting technique for use on genomes of any origin or complexity. The technique involves restriction of genomic DNA with two enzymes, one that cuts frequently (MseI) and one that cuts less frequently (EcoRI). The restricted DNA fragments are ligated to specific

adapters before undergoing a pre-selective PCR amplification. A second, selective PCR amplification step utilizes primers with varying numbers of extra base sequences. The restriction enzymes and additional nucleotide bases on the pre-selective and selective PCR primers can be manipulated such that the technique can be utilized for any organism. Historically, the resulting DNA fragments were analyzed by gel electrophoresis but use of fluorescent primers and capillary electrophoresis has reduced data gathering and analysis times and decreased experimental error (Papa *et al.* 2005).

AFLP requires no prior knowledge of the genome sequence, gene specific primers, or DNA probes making it ideal for organisms on which few genetic studies have been performed (Meudt and Clarke 2007). AFLP markers are useful in teasing apart closely related populations and lineages, such as those of North American dreissenids, because they may contain non-coding sequences, but are much less useful in providing inferences among distant taxa (Mueller and Wolfenbarger 1999). Sample sizes of 10 individuals per population resulted in similar patterns of differentiation (i.e., variation) using AFLP when compared to microsatellite and allozyme marker analyses, indicating its ability to assess genetic variation in small sample sizes (Paupy *et al.* 2004). Mueller and Wolfenbarger (1999) found that the error rate among laboratories was similar for microsatellite and AFLP data. Generally, AFLPs outperform other techniques such as RAPD, non-anchored Inter Simple Sequence Repeat (ISSR), and microsatellites in terms of repeatability, robustness and informativeness (Campbell *et al.* 2003, Meudt and Clarke 2007).

AFLP has been used to characterize the population genetics of a wide variety of organisms including species of fish, insects, plants, bacteria, amphibians and birds (Busch *et al.* 2000, Tatsuta and Butlin 2001, Terefework *et al.* 2001, Campbell *et al.* 2003, Richardson *et al.* 2003, Fang *et al.* 2005, Mock and Miller 2005, Makowsky *et al.* 2009). Similar to other genetic techniques (see above), AFLP analysis revealed that populations of *D. polymorpha* along the Mississippi River had a high degree of intrapopulation (83%) compared to interpopulation genetic variance (17%) (Elderkin *et al.* 2004). Unlike the results of RAPD analysis for Great Lakes *D. polymorpha* populations (Stepien *et al.* 2002), Elderkin *et al.* (2004), using AFLP, were able to detect minor genetic differences among Mississippi River *D. polymorpha* populations but those differences did not correlate with geographic distance. It is likely that extensive mixing of genetic material among populations exists along the Mississippi River due to mussel immigration from other infested drainage systems at several points along its longitudinal gradient via downstream transport of veliger larvae and bi-directional transport of adults attached to the hulls of commercial vessels and barges (Johnson and Carlton 1996, Elderkin *et al.* 2004). The studies of Stepien *et al.* (2002) and Elderkin *et al.* (2004) both involved analysis of relatively few North American dreissenid populations (≤10) over relatively restricted geographical ranges. Genetic analysis of a larger number of populations over a greater North American geographical range, and across a broad range of invasion dates might increase the probability of identifying genetically differentiated populations. This could be particularly effective for newly-founded populations not subject to extensive post-invasion gene flow.

Indeed, Pollux *et al.* (2003) were able to genetically differentiate and determine the probable source of *D. polymorpha* introduction to Ireland (i.e., Great Britain) using AFLP to analyze the genetic structures of populations in Irish water bodies invaded over a period of only 4-5 years compared to the ≈23 years that zebra mussels have been in North America.

4.1.4 Genetic Tracing to Improve Risk Assessment Modeling

Increased knowledge of dreissenid population genetics and genetic diversity could aid invasion modeling for this group as well as provide important information for developing effective strategies aimed at preventing their further invasion of North American water bodies. It is generally accepted that trailered boats are an important overland dispersal vector for dreissenid mussels among isolated drainage systems (Padilla *et al.* 1996, Buchan and Padilla 1999, Johnson *et al*. 2001, Britton and McMahon 2005). The ability to genetically analyze the likelihood and modalities by which the overland transport of dreissenid mussels occurs could support development and application of the most appropriate and cost-effective mussel containment and prevention measures. Use of molecular techniques to assess the pathways and modalities of dreissenid mussel invasions could supplement current information derived from boater surveys on dreissenid mussel invasion patterns and generate important new data on dreissenid invasion biology.

Thus, this study utilized AFLP analysis to examine genetic diversity among 16 populations of *D. polymorpha* and six populations of *D. rostriformis bugensis* and to examine these species' invasion biology in water bodies across a broad geographical

range in North America. The populations examined included those from water bodies first colonized during the initial invasions of both species, as well as those most recently invaded in the western United States.

4.2 Methods

4.2.1 Collection Sites and Technique

Specimens of *D. polymorpha* and *D. rostriformis bugensis* were collected from 16 and six United States water bodies, respectively, ranging from coast to coast (Table B19, Figure A41). Water bodies sampled for both species included some of the earliest invaded by both species in the Great Lakes, to Lake Mead (NV/AZ) and San Justo Reservoir (CA), recently infested by *D. rostriformis bugensis* and *D. polymorpha*, respectively (Table B19, Figure A41).

Samples of adult mussels were collected from various water bodies by collaborators (see Acknowledgements) and preserved in 95–100% ethanol prior to being shipped to UT Arlington. Live adult specimens collected by the author where taken at Lake Oologah (OK), Wolf Lake (IL), Mosquito Creek Reservoir (OH), Lake Erie (OH), Robert S. Kerr Reservoir (OK), and Lake Michigan (MI) (Table B19, Figure A41). Specimens collected by the author were mechanically removed from substrates in the field, pried open by hand and placed in 100% ethanol. Twenty-four hours after collection the ethanol was decanted and replaced with fresh 100% ethanol to ensure that the soft tissues were well preserved. After all samples arrived in the laboratory at UT Arlington they were kept frozen at -12°C to prevent DNA degradation.

4.2.2 DNA Extraction

Genomic DNA was isolated from preserved specimens by removal of a section of the shell adductor muscle and/or foot with a scalpel. DNA was extracted with a Qiagen DNeasy Blood and Tissue Kit. RNase A was used as an optional step during DNA extraction to remove RNA from the sample. Genomic DNA samples were spectrophotometrically analyzed (Nanodrop ND-1000®, Nanodrop Tech.) and run on an agarose gel to ensure the quality and quantity of DNA after extraction. Extracted DNA samples were frozen at -20°C until used for AFLP analysis modified from the methods of Vos *et al.* (1995), Papa *et al.* (2005), and Wooten and Tolley-Jordan (2009). A summary of the general AFLP procedure is presented in Figure A42.

4.2.3 DNA Restriction

Genomic restriction of DNA samples was performed by reaction with: 20 U of EcoRI [New England Biolabs® (NEB)], 2 U MseI (NEB®), 2 µl 10X EcoRI buffer (NEB®), 2 µl sample genomic DNA, in 14.8 µl of molecular-grade sterile water. The restriction reaction was held at 37°C for 6 h.

4.2.4 Ligation

Equal parts of two Mse adapters (5'-GACGATGAGTCCTGAG, 75 µM; 5'-TACTCAGGACTCAT, 75 µM) were combined to create an Mse adapter mix and equal parts of two EcoRI adapters (5'-CTCGTAGACTGCGTACC, 75 µM; 5'-AATTGGTACGCAGTCTAC, 75 µM) were combined to create an EcoRI adapter mix. The adapter mixes were separately heated to 95°C for 3 minutes and allowed to cool to room temperature for ≥10 min prior to utilization. The ligation reaction was prepared

by adding the following reagents to the DNA digestion reaction: 3.0 µl 10X T4 ligase buffer (NEB®), 0.1 µl T4 DNA ligase (NEB®), 1.125 µl Mse adapter mix, and 1.125 µl EcoRI adapter mix in 4.65 µl of sterile H_2O. The ligation reaction was incubated at 16°C for 12 h. Following DNA restriction/ligation, the reaction volume was diluted by the addition of 75 µl of molecular-grade sterile water.

4.2.5 Pre-selective PCR

The pre-selective PCR was prepared as follows: 0.8 µl 25 mM $MgCl_2$ (Promega®), 2.0 µl 10X PCR Buffer, 0.8 µl 1:1 formamide (Applied Biosystems®), 3.0 µl 2.5 mM dNTP mix (TaKaRa Inc.®), 0.5 U HS ExTaq (TaKaRa Inc.®), 0.45 µl 20 µM MseI primer (GATGAGTCCTGAGTAA[GC]), and 0.275 µl 10 µM EcoRI primer (GACTGCGTACCAATTC[G]) (selective extensions are in brackets) in 11.62 µl molecular-grade sterile water with 2 µl of the ligated DNA sample. PCR reactions were conducted in a thermocycler (Eppendorf® Mastercycler gradient) at 72°C for 1 min, at 94°C for 50 s, at 56°C for 1 min, and at 72°C for 1 min before being subjected to 25 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 minute and a 1 minute extension phase at 72°C. Once complete, the PCR was held at 4°C until diluted with 150 µl of molecular-grade sterile water.

4.2.6 Selective PCR

The selective PCR was prepared by placing 2.0 µl 10X PCR Buffer, 3.0 µl 2.5 mM dNTP (TaKaRa Inc.®), 0.4 µl 25 mM $MgCl_2$ (Promega®), 0.8 µl 1:1 formamide (Applied Biosystems®), 0.5 U HS ExTaq (TaKaRa Inc.®), 2.5 µl 10 µM MseI selective primer (GATGAGTCCTGAGTAA[GCC]), 2 µl 5µM EcoRI 5' FAM labeled primer

(GACTGCGTACCAATTC[GA]) (selective extensions are in brackets) and 4.0 µl of pre-selective PCR product in 7 µl of molecular-grade sterile water. PCR reactions were conducted in a thermocycler (Eppendorf® Mastercycler gradient) at 94°C for 50 s, 57°C for 1 minute, and 72°C for 2 minutes before being subjected to 20 cycles of 94°C for 15 s, 56°C for 1 minute and 72°C for 1 minute before being held at 4°C until removal.

4.2.7 AFLP Product Precipitation

AFLP product from the selective PCR was precipitated by adding 4 µl NaOAc, mixing well, and then adding 80 µl of 100% ethanol. This mixture was incubated at -20°C for 20 min and centrifuged at 1500*g* for 45 min at 15°C. The supernatant was decanted and the reaction plate centrifuged upside-down at 300*g* and 15°C for 1 min to further dry. The DNA was then dried by incubating the reaction plate, without a lid, at 50°C for 5 minutes in a thermocycler (Eppendorf® Mastercycler gradient). The DNA was then resuspended in 14 µl of pure formamide (Applied Biosystems®) and 0.7 µl Rox size standard (Applied Biosystems®, Genescan 400HD)

4.2.8 AFLP Band Analysis

Precipitated PCR product was loaded into a 96-well sequencer plate and amplified fragments were scanned on an Applied Biosystems® 3130XL/Genetic Analyzer. AFLP data were analyzed with GeneMarker® software (Softgenetics, LLC.®) with an amplitude threshold ≥50 rfu (1% max + local max), minimum fragment size of ≥100 bp and a 5% stutter peak filter. A standardizing panel was created from all individuals containing well defined peaks (i.e., AFLP markers or loci) representing DNA fragments in the 100 to 400 bp range. Following application of the standardizing

panel for all 178 individuals analyzed, AFLP markers present in fewer than 10 individuals were deleted from further analysis. In addition, to detect markers in low-quality profiles (i.e., with reduced peaks), the leading cause of genotyping errors in AFLP data (Bonin *et al.* 2007), AFLP markers were checked visually for every individual prior to statistical analysis. Peak data were exported as a binary matrix of marker presence (1) or absence (0) for all individuals analyzed.

4.2.9 Statistical Analysis

4.2.9.1 AFLP Repeatability and AMOVA Analyses

Repeatability of the AFLP procedure was assessed by evaluating discrepancies between presence/absence of fragment peaks from the genomic DNA of three *D. rostriformis bugensis* individuals and six *D. polymorpha* individuals from eight different populations subjected to the complete AFLP procedure twice on separate occasions. Repeatability was expressed as the percent difference between the two AFLP marker patterns from the same individual. An experiment-wide estimation of AFLP discrepancies was taken by averaging the percent difference between the two AFLP marker patterns for all nine individuals.

An analysis of molecular variance (AMOVA) was performed by GenAlEx v.6© (Peakal and Smouse 2006) on the species-specific datasets for all sampled individuals of *D. polymorpha* and *D. rostriformis bugensis* in order to determine partitioning of variance among the populations.

4.2.9.2 Population Assignment Analysis

Population assignment analysis was performed using Structure v2.2® (Pritchard *et al.* 2000). The AFLP marker data were split into three different datasets, the complete set, a set containing only *Dreissena polymorpha* individuals and a set containing only *D. rostriformis bugensis* individuals. For each dataset, a no-admixture model (Burnin: 50000 iterations followed by Markov Chain Monte Carlo (MCMC) runs: 50000 iterations) was run for each proposed number of groups (*K*) ranging from one to the number of sample collection sites in the analyzed dataset. Each run was repeated five times and the correct number of groups (i.e., genetically distinct populations) was determined by evaluating the mean ln P(X|K) [the log probability (P) of binary marker patterns (X) data given *K* number of groups ranging from one to the maximum number sample sites in the data set] (Pritchard *et al.* 2000) and by the methodology described in Evanno *et al.* (2005). The absolute mean difference of successive ln P(X|K) values was obtained by subtraction and then divided by the standard deviation of ln P(X|K) to yield ΔK. The distribution of ΔK is modal at the true number of genetically distinct groups (*K*) for the population (Evanno *et al.* 2005). No-admixture was assumed to be appropriate for the sampled populations because individual mussels are sessile and a majority of the collection sites represent isolated bodies of water where gene flow from other populations is likely to be low. Analysis of data with the no-admixture also increases the likelihood of discovering subtle population structure (Pritchard *et al.* 2000).

The species-specific datasets were also analyzed using collection site information to *a priori* assign each individual to a population based on their collection site. One individual was randomly chosen from each sampled species population and their population identifier was not used to assess marker frequencies of the populations. The remaining pre-defined individuals from each population were then used as a learning set to establish each population's genetic structure (Burnin: 10000 iterations, MCMC runs: 10000 iterations). The probability of the undefined (i.e., removed) individual originating from the correct collection site was then determined based solely on its marker frequencies. This process was repeated four times, using different undefined individuals from each population. This analysis was performed with a migration prior set to 0.001 (i.e. little gene flow among populations), each individual was assessed without migration (i.e. within 0 generations) and only genetic data from the learning set were used to update marker frequencies.

The dataset containing individuals of both *D. polymorpha* and *D. rostriformis bugensis* was also analyzed for the presence of hybrid individuals using Structure®. The program settings were adjusted such that an admixture model (Burnin: 10000 iterations, MCMC runs: 10000 iterations) was run assuming that there were two groups (K). The probabilities of group assignment were reviewed to determine if some individual mussels appear to have an equal probability of being placed into either *D. polymorpha* or *D. rostriformis bugensis*. Individuals with an assignment probability that fell within 40–60% were considered possible hybrids (Albaladejo and Aparicio 2007).

4.2.9.3 Distance Analyses

Genetic distances (Nei 1978) were determined between collection sites for the *D. polymorpha* and *D. rostriformis bugensis* datasets and the combined dataset using the program TFPGA© (Miller 1997). The genetic distance data were then used to develop a cluster analysis tree by an Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering analysis, also utilizing the program TFPGA© (Miller 1997). Bootstrapping (5000 iterations) was utilized to estimate node support reported as the percentage of replicated trees that contained the node inferred from the original analysis. Additionally, a Fisher's Exact Test (Raymond and Rousset 1995) was employed to compare marker frequency differences between populations.

4.2.9.4 Principal Coordinate Analysis

Principal coordinate analysis (PCoA) was performed on the species-specific and combined molecular data sets utilizing the GenAlEx v.6© program developed by Peakal and Smouse (2006). This analysis was utilized to detect AFLP marker separation for the combined and species-specific datasets. GenAlEx© was also used to test for significant genetic isolation by geographic distance with a Mantel Test (Mantel 1967), utilizing collection site coordinates and the AFLP marker information.

4.3 Results

4.3.1 AFLP Fragment Markers

Of the 161 DNA fragments created by the AFLP procedure, only one was present in all tested individuals, across both species. The remaining 160 fragments

were at least partially informative. On average, 52% of tested individuals shared any given AFLP fragment (range: 6–100%).

4.3.2 AFLP Repeatability

Differences in marker (DNA fragment) presence among the nine individuals of *D. polymorpha* and *D. rostriformis bugensis* for which AFLP analysis was duplicated revealed an average error rate (± SD) of 9.4 ± 5.4% with a range of 1.9–19.9%. The fragment chromatograms of the individual with the highest percent difference (19.9%) displayed low peak amplitude which can lead to peak detection errors. This sample was removed from further analyses because it was not possible to determine which, if any, of the fragment chromatograms were correct. The difference among the remaining eight individuals for which AFLP analysis was duplicated averaged (± SD) 8.1 ± 4.0% with a range of 1.9–13.0%.

4.3.3 AMOVA

AMOVA (Analysis of Molecular Variance) revealed significant variation (Φ_{st} = 0.055; $P < 0.001$) among the 16 sampled *Dreissena polymorpha* populations despite the great majority of variation occurring within populations (5.5% among population variation, 94.5% within population variation). AMOVA also revealed an almost identical pattern of variation (Φ_{st} = 0.052; $P < 0.001$) among the six sampled *D. rostriformis bugensis* populations with 5.2% among population variation and 94.8% within population variation.

4.3.4 Population Assignment Analysis

The entire AFLP dataset (n = 178) contained 52 specimens of *D. rostriformis bugensis* from six collection sites and 126 specimens of *D. polymorpha* from 16 collection sites. Population assignment analysis utilizing the Structure® program for the combined dataset assuming that collection sites were fully discrete (i.e. no admixture) indicated that the AFLP fragment patterns fell into two groups (K = 2), which coincided with the two species. All specimens of *D. rostriformis bugensis* grouped separately from all specimens of *D. polymorpha* with no individuals being incorrectly assigned to either species in any of the five program repetitions. Increasing the number of groups beyond two (K = 3–22) resulted in one group containing all individuals of *D. rostriformis bugensis*, while specimens of *D. polymorpha* had an equal probability of being assigned to 2–21 of the remaining groups, indicating a lack of within-species population structure.

To further explore population structure, the population assignment analysis was repeated with individuals from each of the two species independently. For both the *D. polymorpha* and *D. rostriformis bugensis* datasets, the analysis was incapable of distinguishing among different collection sites using population assignment analysis. Additionally, the Structure® program was unable to correctly assign selected undefined individuals into populations that were predefined by collection site. The average probability (±95% CI) that an undefined specimen of *D. rostriformis bugensis* (n = 24) was placed into its correct population was 18.3 ± 7.8%, while that for an undefined specimen of *D. polymorpha* (n = 64) was 12.4 ± 1.4%.

Based on an initial threshold probability of <0.60 for correct species assignment (see Methods section 4.2.9.2) as indicative of hybrids, analysis by the Structure® program suggested that it was unlikely that any of the sampled individuals were hybrids between *D. polymorpha* and *D. rostriformis bugensis*. Of the total, 178 specimen sample subjected to AFLP analysis, the analysis was able to assign 161 individuals to the correct species (previously determined by specifies-specific differences in shell morphology) with a probability ≥0.99. Of the remaining 17 individuals only two had a probability <0.90 of being assigned to the correct species. Of these two individuals, a specimen from Hedges Lake (NY) had a 0.855 probability of being correctly assigned to *D. polymorpha* and that from Lake Mead (NV/AZ), a 0.832 probability of being correctly assigned to *D. rostriformis bugensis*. These results indicated that AFLP was highly accurate in differentiating between *D. polymorpha* and *D. rostriformis bugensis.*

4.3.5 Genetic Distance Analyses

Fisher's Exact Tests for differentiation of AFLP marker frequencies indicated that there were no significant differences among the six tested populations of *Dreissena rostriformis bugensis* (Table B20). In contrast, each of the six *D. rostriformis bugensis* populations were significantly ($P < 0.0001$) differentiated from the *Dreissena polymorpha* population from Lake Oologah (OK) (Table B20). Fisher's Exact Tests for population differentiation also indicated no significant differences among the 16 tested populations of *D. polymorpha* although each population was significantly differentiated from the Lake Mead (NV/AZ) *D. rostriformis bugensis* population (Table B21).

Bootstrapping (to determine data consistency) of the *D. rostriformis bugensis* UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering analysis grouped the St. Lawrence River (NY) and Detroit River (MI) populations in 84% of the bootstrap pseudoreplicates (Figure A43). Bootstrapping also indicated that 100% of program iterations supported the grouping of the Lake Mead (NV/AZ) *D. rostriformis bugensis* sample outside of the other five quagga mussel populations (i.e., bootstrap value = 100%) (Figure A43). The Lake Oologah (OK) *D. polymorpha* population, included to provide a more distant member for comparison, also had 100% bootstrap support (Figure A43). All other tree nodes had low bootstrap support (<35%).

UPGMA analysis indicated that the San Justo (CA) sample of *D. polymorpha* grouped outside of the other 15 tested *D. polymorpha* populations with a bootstrap value of 82% (Figure A44). The UPGMA tree included the Lake Mead (NV/AZ) *D. rostriformis bugensis* population which had 100% bootstrap support (Figure A44). All other tree nodes had low bootstrap support (<46%).

Based on analysis of AFLP marker frequencies by TFPGA© (Miller 1997), the mean of Nei's (1978) unbiased genetic distances among populations of *D. rostriformis bugensis* was 0.044 ± 0.012 SD (range = 0.023–0.063) (Table B22) with an average marker heterozygosity of 0.2759 (Table B23). Similarly, mean unbiased genetic distances among all 16 populations of *D. polymorpha* was 0.053 ± 0.013 SD (range = 0.020–0.083) (Table B22) with an average marker heterozygosity of 0.2877 (Table B23). Among all individuals (n = 52) of *D. rostriformis bugensis*, 75.8% (122/161) of AFLP markers were polymorphic (i.e. they were not fixed for either presence or

absence) while for all 126 specimens of *D. polymorpha*, 78.3% of AFLP markers (126/161) were polymorphic (Table B23).

4.3.6 Principle Coordinate Analysis

For the combined datasets (n = 178) for the 16 population samples of *D. polymorpha* (n = 126) and six population samples of *D. rostriformis bugensis* (n = 52) Principle Coordinate Analysis estimated that the first two coordinates explained 79.17% of the variation in AFLP marker frequencies. AFLP marker data varied widely among individuals within populations displaying extensive within-species population overlap (Figure A45). In contrast, there was no overlap between species as clearly demarcated by the first coordinate.

Principle Coordinate Analysis of the data set containing the six populations of *D. rostriformis bugensis* (n = 52) indicated that only 39.98% of AFLP marker frequency variation was accounted for by the first two coordinates (Figure A46). AFLP marker data for individuals within populations varied widely with extensive interpopulation overlap with the single exception of individuals from Lake Mead (NV/AZ) which clustered to the right on coordinate one and displayed little overlap with the other five tested populations. A Mantel Test (Mantel 1967) revealed that the genetic distances among the six *D. rostriformis bugensis* population samples were correlated with geographic distance among the populations (Mantel r = 0.288, P = 0.001). Similarly, Principle Coordinate Analysis of the data set containing all individuals (n = 126) in the 16 *D. polymorpha* population samples estimated that only 38.69% of AFLP marker frequency variation was accounted for by the first two coordinates (Figure A47). As

occurred in the *D. rostriformis bugensis* analysis, *D. polymorpha* intrapopulation marker frequencies varied widely with extensive interpopulation overlap (Figure A47). In contrast to the *D. rostriformis bugensis* dataset, a Mantel Test (Mantle 1967) of genetic isolation by geographic distance found no significant correlation between geographic distance and genetic distance (Mantel $r = 0.015$, $P = 0.369$).

4.4 Discussion

4.4.1 AFLP Repeatability

The AFLP procedure's reproducibility had been questioned by Jones *et al.* (1997) and others and its reproducibility is not often reported in studies of population genetic structure. However, Holland *et al.* (2008) reported an AFLP replication error rate among *Ipomoea batatus* (sweet potato) and *Ourisia* sp. (creeping mountain fox glove from New Zealand) of 15% (range = 9–18%) and 10% (range = 6–13%), respectively. These values were somewhat higher than the 8.1% ($n = 8$, 5% of the total analyzed sample) reported in this study. However, their methodology did not include the visual assessment and rescoring of AFLP markers performed in this study. The authors estimated that their error rate was mostly a result of AFLP peak scoring error, such that the error rate associated with PCR artifacts was closer to 5% and 4% for *I. batatus* and *Ourisia* sp., respectively (Holland *et al.* 2008). An AFLP study of *Cannabis sativa* (hemp) reported an experiment-wide error rate of 10.8% among replicated samples (Datwyler and Weiblen 2006). Jones *et al.* (1997) studied the error rate in AFLP profiles of 172 markers for two *Beta vulgaris* (sugar beet) clones across nine different laboratories. Initially, unfamiliarity with the AFLP procedure resulted in

error rates >50%. However, with increased AFLP experience, the error rate declined to <0.01% with only a single mismatch among the laboratories. Bonin *et al.* (2007) suggested that an acceptable error rate for AFLP studies should not exceed 10% and should be based on ≥5% of all analyzed individuals. This study met the requirements of Bonin *et al.* (2007) suggesting that is was acceptable for analyzing the genetic structures of *D. rostriformis bugensis* and *D. polymorpha* populations in the United States.

4.4.2 Heterozygosity

Heterozygosity has been positively correlated with fitness in other bivalves (Garton *et al.* 1984, Koehn and Gaffney 1984). This study found high levels of heterozygosity among the AFLP markers for both North American *D. polymorpha* (28.77%) and *D. rostriformis bugensis* (27.59%) populations. Estimations of heterozygosity using AFLP markers assume Hardy-Weinberg Equilibrium and may estimate high levels of heterozygosity when compared to other techniques. However, previous work utilizing a variety of molecular techniques also recorded high levels of heterozygosity in *D. polymorpha* populations (Hebert *et al.* 1989, Garton and Haag 1991, Marsden *et al.* 1995, Lewis *et al.* 2000, Stepien *et al.* 2002, Astanei *et al.* 2005). As reported in this study, both Stepien *et al.* (2002) and Therrialt *et al.* (2005) found that populations of *D. rostriformis bugensis* in North America and Europe, respectively, had levels of heterozygosity similar to those reported for *D. polymorpha.* In contrast, based solely on allozyme analysis, May and Marsden (1992) reported that a single *D. rostriformis bugensis* population from Lake Ontario (NY) had approximately 67% less genetic variation than did six *D. polymorpha* populations from the Erie Canal (NY) and

five sites in the Great Lakes. The reduced genetic variation in *D. rostriformis bugensis* reported by May and Marsden (1992) may have resulted from restricting the analysis to a single population and from the fact that the analysis occurred in the very early stages of North American invasion by *D. rostriformis bugensis* which was first reported in the Erie Canal near Palmyra (NY) during August 1991 (May and Marsden 1992).

4.4.3 Intra- and Interpopulation Genetic Variation

In this study the vast majority of the genetic variation in *D. rostriformis bugensis* and *D. polymorpha* was within populations at 94.5% and 94.8% of total variation, respectively, indicative of relatively little interpopulation variation in either species. Previous genetic evaluations of North American *D. polymorpha* populations have similarly reported little interpopulation genetic variation. May *et al.* (2006) found no measurable interpopulation variation in sequences of the mitochondrial cytochrome oxidase I gene among either *D. rostriformis bugensis* or *D. polymorpha* populations in the Great Lakes. Amplified fragment length polymorphisms (AFLP), by their random nature, contain coding and non-coding regions that should better assess variation among populations of the same species or among populations of congeneric species (Mueller and Wolfenbarger 1999). Utilizing AFLP, Elderkin *et al.* (2004) found significant interpopulation variation (17%) among six populations of *D. polymorpha* along a latitudinal gradient in the Mississippi River, although the majority (83%) was intrapopulation variation. The analyses of molecular variance in this and previous studies, using AFLP and other techniques, suggested that high levels of variation are present in North American *D. rostriformis bugensis* and *D. polymorpha* populations;

however the vast majority of that variation occurred within populations. The limited interpopulation genetic variation observed among both *D. rostriformis bugensis* and *D. polymorpha* populations in this and other studies may make the identification of origin in newly founded populations and the patterns of invasion for both North American dreissenid species difficult to determine.

Population assignment and Principle Coordinate Analyses of AFLP data indicated that the AFLP markers were capable of discriminating between *D. polymorpha* and *D. rostriformis bugensis* with 100% efficiency, with all tested 178 individuals being correctly assigned to species (Figure A45). In contrast, population assignment and Principle Coordinate Analyses of AFLP marker frequency data could not discriminate among the different populations within each species, even when species-specific datasets were individually analyzed (Figure A46 and A47). Thus, in this study, individuals with an undefined population source had a probability of <0.2 of being correctly assigned by AFLP analysis to their population of origin. Inability to correctly assign individuals to their population of origin and to discriminate among populations within species (based on the protocol used in this study) indicated that AFLP analysis is not likely to be useful in determining the origin of newly founded populations or patterns of invasion for both dreissenid species in North America. It is possible that the inclusion of a greater number of AFLP markers and increased sample size per population, or greater number of tested populations could provide enough information to achieve these goals, but this possibility requires further study.

4.4.4 D. polymorpha X D. rostriformis bugensis Hybridization

In this study, AFLP analysis revealed no *D. polymorpha* X *D. rostriformis bugensis* hybrids among 178 tested individuals even though samples were taken from six water bodies where both species are known to co-occur [i.e., Mohawk River (NY), Lake Erie (OH), St. Lawrence River (NY), Lake Ontario (NY), Lake Michigan (IL), Millbrook Quarry (VA), and Detroit River (MI)]. *D. polymorpha* X *D. rostriformis bugensis* hybrids have been artificially created under laboratory conditions indicating a potential for inter-specific hybridization in mixed-species natural populations (Nichols and Black 1993). However, there are no published records of hybrid larvae being raised to maturity suggesting that settled hybrid juvenile mussels are unlikely to occur in nature. There are species-specific sperm attractants in dreissenids that may further reduce the likelihood of naturally occurring hybrids (Miller *et al.* 1994). In addition, a genetic evaluation of dreissenid mussels using species-specific alleles of two protein coding regions did not reveal any *D. polymorpha* X *D. rostriformis bugensis* hybrids in water bodies where the two species co-occurred (Spidle 1994). While this and Spidle's (1994) study did not reveal the presence of adult *D. polymorpha* X *D. rostriformis bugensis* hybrids in a number of natural populations, the data presented should not be interpreted as indicating that such hybridization does not occur in nature. It may be possible that hybrids could appear rarely in co-occurring populations of *D. polymorpha* and *D. rostriformis bugensis* requiring the testing of a much larger number of individuals than used in either this or Spidle's (1994) study in order to detect them.

4.4.5 Taxonomic and Systematic Implications

In this study, the mean unbiased genetic distances (Nei 1978) among the 16 tested populations of *D. polymorpha* was 0.053 (range = 0.020–0.083). This was similar to that for the six tested populations of *D. rostriformis bugensis* which was 0.044 (range = 0.023–0.063). Spidle (1994), using allozyme markers, found a genetic distance of 0.02 among two North American and two European *D. polymorpha* populations and 0.068 among three North American and one European *D. rostriformis bugensis* population, respectively. Spidle's (1994) analysis also indicated a genetic distance of 1.69 between the two species and he suggested their separation into different subgenera (*Dreissena* and *Pontodreissena*). In this study of 16 populations of *D. polymorpha* and six populations of *D. rostriformis bugensis*, the maximum genetic distance observed between the two species (D = 0.446) occurred between the San Justo Reservoir (CA) *D. polymorpha* and Millbrook Quarry (VA) *D. rostriformis bugensis* populations (Table B22), which were both sampled within a year of their first discovery, indicating that *D. polymorpha* and *D. rostriformis bugensis* should not be placed in different subgenera as suggested by Spidle (1994).

4.4.6 Genetic Differentiation among Geographically Isolated Populations

This study revealed little genetic differentiation among either the 16 sampled populations of *D. polymorpha* or the six sampled populations of *D. rostriformis bugensis* throughout their North American ranges. Previous allozyme analysis among 18 collection sites for *D. polymorpha* in the Great Lakes indicated that the genetic distances among sites was ≤0.028 (Marsden *et al.* 1995), marginally less than the 0.053

recorded for 16 *D. polymorpha* populations in this study, although genetic distances estimated from the two different techniques may not be directly comparable. Marsden *et al.* (1995) suggested that the low level of interpopulation genetic differentiation among the 18 collection sites was due to high levels of gene flow among them and predicted that populations in isolated water bodies would have higher genetic distances. However, although the genetic distances among the 16 *D. polymorpha* populations sampled in this study were somewhat higher (Table B22) than those reported by Marsden *et al.* (1995), Mantel Analysis revealed no significant genetic isolation by geographic distance among them, including a number of populations restricted to water bodies in different drainage systems. In addition, Fisher's Exact Testing revealed no significant genetic differentiation among the 16 *D. polymorpha* or six *D. rostriformis bugensis* populations sampled (Tables B20 and B21). Furthermore, the genetic distance between closely adjacent *D. rostriformis bugensis* populations sampled in Lake Ontario (NY) and the lake's outflow into the Thousand Islands region of the St. Lawrence River (NY) had a genetic distance of 0.044 which was not the lowest recorded among the six tested populations including two more widely distant sites in the Great Lakes/St. Lawrence River drainage and two totally isolated populations in Lake Mead (NV/AZ) and Millbrook Quarry (VA) (Table B22). In contrast, utilizing 32 AFLP markers, Pollux *et al.* (2003) were able to differentiate among nine European and one North American population of *D. polymorpha* by principle component analysis despite low sample sizes ($n \leq 7$ per population). Although the variation explained by their principle axes is not reported, the differentiation of populations under these circumstances seems

surprising given the inability of this study to differentiate among either populations of *D. polymorpha* or *D. rostriformis bugensis* using 161 AFLP markers and larger sample sizes ($n \geq 6$ per population). However, mainland European and British populations of *D. polymorpha* have existed much longer (>150 yr) than North American populations (<25 yr) and may have undergone significant differentiation during this time. In this study, Genetic Distance and Principle Coordinate Analyses revealed little evidence for clustering of *D. polymorpha* populations based on habitat drainage interconnectivity (Figures A44 and A47). Similarly, an AFLP study examining the population genetics of *D. polymorpha* populations in the Mississippi River recorded no population genetic differentiation with downstream distance which was attributed to extensive gene flow both downstream by hydrologic transport of the planktonic veliger larva and upstream by transport of adults attached to barge hulls (Elderkin *et al*. 2004). Therrialt *et al.* (2005) also found little genetic differentiation by geographic distance using analysis of six microsatellite genetic markers to differentiate 13 *D. rostriformis bugensis* populations in the Black Sea, Caspian Sea, Volga River and Lake Erie. This result indicated a high level of gene flow among these populations. In contrast, Astanei *et al.* (2005) found significant genetic variation correlated with geographic distance when analyzing five recently established Irish *D. polymorpha* populations using microsatellite analysis. However, as recorded in this study for the *D. rostriformis bugensis* population in Lake Mead (NV/AZ), the reported geographically correlated genetic variation for *D. polymorpha* depended on a single, distantly located sample site. As reported for both *D. polymorpha* and *D. rostriformis bugensis* populations within single drainage systems

in this study, there was no significant genetic differentiation among geographically separated *D. polymorpha* populations within the Irish Shannon-Erne River drainage system (Astanei *et al*. 2005). This and the above mentioned studies all strongly suggest that this lack of genetic differentiation among geographically separated North American populations of *D. polymorpha* and *D. rostriformis bugensis* is a result of either high levels of gene flow either among populations within drainage systems due to this species high natural capacity for dispersal and across continental drainage systems via high levels of human-mediated transport or that founding populations consist of a large number of individuals whose gene pool harbors a majority of the dreissenid species' overall genetic diversity.

In this study, UPGMA analysis of genetic distances among populations of *D. rostriformis bugensis* suggested that this species' population in the Detroit River was more similar to a population in the St. Lawrence River than to any other tested population and that AFLP marker data grouped those two populations together in 84% of bootstrap replicates (Figure A43). The *D. rostriformis bugensis* population in Lake Ontario (NY) grouped with these two populations (although with very low bootstrapping values of <35%) and was not more genetically similar to the closely adjacent St. Lawrence River (NY) populations. UPGMA analysis of specimens of *D. rostriformis bugensis* from Lake Mead (NV/AZ), the most geographically isolated site sampled, grouped them outside all other sampled *D. rostriformis bugensis* populations with a bootstrap value of 100% indicative of genetic differentiation between the Lake Mead (NV/AZ) population and the other five sampled populations (Figure A43). In

contrast, genetic differentiation of the Lake Mead (NV/AZ) *D. rostriformis bugensis* population was not supported by population assignment analysis nor Fisher's Exact Tests (Table B20), although Principle Coordinate Analysis provided some support for this result as evidenced by the comparatively limited genetic overlap of individuals sampled from Lake Mead (NV/AZ) with those from the five other *D. rostriformis bugensis* populations (Figure A46). Similarly, UPGMA analysis suggested that specimens of *D. polymorpha* sampled from San Justo Reservoir (CA), the most geographically isolated *D. polymorpha* population, grouped outside all 15 other sampled *D. polymorpha* populations with a bootstrap value of 82%, again indicative of some differentiation relative to the other sampled populations (Figure A44). In contrast to the Lake Mead (NV/AZ) *D. rostriformis bugensis* population, population assignment analysis, Principle Coordinate Analysis (Figure A45) and Fisher's Exact Tests (Table B21) could not differentiate the San Justo Reservoir (CA) *D. polymorpha* population from the other sampled *D. polymorpha* populations.

4.4.7 Dreissenid Invasion Biology

Stepien *et al.* (2002), using random amplified polymorphic DNA (RAPD) analysis found bootstrap values supported genetic differentiation among Eurasian and North American *D. polymorpha* populations although bootstrapping support values were generally lower than the threshold 70% value used in this study for recognizing valid population genetic differentiation (Hillis and Bull 1993). The authors used these data to suggest that North American *D. polymorpha* populations were founded by multiple invasion events from several locations within Eurasia (Stepien *et al*. 2002).

While multiple *D. polymorpha* invasion events in North America are possible, it is difficult to explain why *D. polymorpha* populations in hydrologically connected North American water bodies would cluster separately from each other in the presence of extensive upstream and downstream gene flow and cluster intermittently with seemingly random sites from Eurasia as reported by Stepien *et al.* (2002). In contrast, using allozyme analysis, Marsden *et al.* (1995) found no significant genetic differentiation among North American *D. polymorpha* populations and, unlike Stepien *et al.* (2002), reported that North American populations clustered separately from European populations suggestive of a single North American *D. polymorpha* introduction event. Similarly, utilizing cytochrome oxidase I (COI) gene sequence analysis, May *et al.* (2006) found no genetic differentiation among Great Lakes *D. polymorpha* populations, indicative of a single North American invasion by this species.

This study also found little genetic differentiation among isolated populations in recently invaded North American water bodies of either *D. polymorpha* [Offutt Air Force Base Lake (NE), San Justo Reservoir (CA)] or *D. rostriformis bugensis* [Lake Mead (NV/AZ), Millbrook Quarry (VA)]. The broad genetic diversity characteristic of these newly founded populations suggests that successful dreissenid invasions must result from either multiple overland introductions into an isolated water body or a successful single invasion by a large number of genetically diverse individuals. It is unlikely that the dreissenid populations in at least three sites included in this study, San Justo Reservoir (CA) (*D. polymorpha*); Offutt Air Force Base Lake (NE) (*D. polymorpha*); and especially Millbrook Quarry Lake (VA), which did not receive

recreational boat traffic and whose *D. rostriformis bugensis* population was known to have been established from a single, purposeful, human-mediated introduction (Virginia Department of Game and Inland Fisheries 2005), were a result of multiple introductions. This outcome strongly suggests that the high levels of genetic variability recorded for these populations were the result of single introductions of a large number of individuals with a diverse gene pool. Therefore, the results of this study support the hypothesis that both *D. polymorpha* and *D. rostriformis bugensis* invasions of isolated water bodies, and the initial invasion of North America, were primarily the result of a single introduction of a large number of genetically diverse individuals.

For two recently invaded sites, UPGMA analysis separated individuals of *D. polymorpha* from San Justo Reservoir (CA) from 15 other sampled *D. polymorpha* populations (bootstrap value = 82%, Figure A44) and individuals of *D. rostriformis bugensis* from Lake Mead (NV/AZ) from the five other sampled *D. rostriformis bugensis* populations (bootstrap value = 100%, Figure A43). That both of these recently established populations, while displaying extensive genetic diversity, were somewhat genetically different from the other North American populations sampled, supports their establishment from single introductions. Presumably, populations resulting from multiple introductions, originating from different infested water bodies, would likely prevent development of genetically differentiated populations.

This study recorded high levels of intrapopulation genetic variation in all tested dreissenid populations indicating a lack of genetic bottlenecks. It is unlikely that considerable gene flow has occurred among hydrologically isolated water bodies

because successful long-distance, overland transport of aquatic nuisance species appears to be a relatively rare event (Buchan and Padilla 1999). Therefore, lack of genetic evidence for bottlenecks suggests that initial dreissenid invasions by overland dispersal on trailered boats and other equipment requires introductions of a large number of individuals with high levels of fecundity as is characteristic of both *D. polymorpha* and *D. rostriformis bugensis* (Puurtinen *et al*. 2004) Certainly, trailered boats carrying extensive infestations of *D. polymorpha* and/or *D. rostriformis bugensis* attached to their hulls have been intercepted by law enforcement, wildlife, or marina personnel (L. Dalton, personal communication, 18 May 2009) suggesting that single introductions of large numbers of dreissenids are possible, if not relatively common.

While all current evidence suggests that populations of *D. polymorpha* and *D. rostriformis bugensis* are both highly genetically uniform across North America (Marsden *et al.* 1995, Elderkin *et al.* 2004, May *et al.* 2006, this study), this study presents evidence that at least some populations, particularly in the warm water bodies of the southwestern United States, may be undergoing intense thermal selection for increased thermal tolerance (Chapter 2) and the capacity for increased energetic efficiency at temperatures >25°C (Chapter 3). Ultimately, such thermal selection could eventually lead to the development of genetically distinct, physiological lineages of *D. polymorpha* and *D. rostriformis bugensis* better adapted to, and more likely to further invade, warm southwestern U.S. water bodies from which these two species had been previously hypothesized to be thermally excluded (McMahon 1996).

4.4.8 Implications for Prevention and Containment of Dreissenids Infestations

The suggestion that populations of both *D. polymorpha* and *D. rostriformis bugensis* appear to be founded in isolated, uninfested water bodies by an initial overland transport and introduction of a large number of individuals has important implications for the future containment and prevention of spread for both species in North America and particularly in the western United States. Why would an initial introduction of a large number of individuals be required to establish a dreissenid population in a previously uninfested water body? Temporally separated multiple introductions of small numbers of individuals may not be capable of establishing viable dreissenid populations. Introduction of a small number of individuals to a previously uninfested water body may result in mussels settling on substrates that are distant from one another. Even under ideal laboratory conditions only 41.0–51.8% of *D. polymorpha* eggs are successfully fertilized (Ciereszko *et al.* 2001), but in natural populations of externally fertilizing species, fertilization success decreases linearly with decreasing adult density falling below 10% at 0.5 individuals m^{-1} (Claereboudt 1999). Further, <2% of *D. polymorpha* veliger larvae develop to settled juveniles even under ideal laboratory conditions (Nichols 1993). Thus, based on these estimates, only 0.82–1.03% of spawned eggs are likely to be fertilized and survive to the settled juvenile stage under ideal conditions in dense populations. However, survivorship of spawned eggs to settled juveniles would be likely to greatly decline to <0.003% (0.17% x 2%) in newly introduced populations of very low density (<0.1 m^{-1}) based on the data of Nichols

(1993), Ciereszko *et al.* (2001) and Claereboudt (1999). Thus, based on his model for fertilization success in externally spawning species, Clareboult (1999) states that

> ---for free-spawning, colonizing species (such as the zebra mussel *Dreissena polymorpha*), the probability of these recruits to start a new population remains low unless sufficiently high numbers of new recruits settle simultaneously at the same location. This implies that either several successful settlement episodes or a massive immigration would be required before a self sustainable density of colonizers is reached.

The necessity for a large number of founding individuals to form a sustainably reproducing *D. polymorpha* or *D. rostriformis bugensis* population suggests that prevention and containment measures that eliminate adult mussels from recreational boats and other submerged equipment may not require 100% efficacy to be effective. Thus, preventative measures such as removal of mussels from boats prior to launching in an uninfested water body and containment measures such as disinfection of attached juvenile and adult mussels on boats leaving an infested water body, although unlikely to eliminate 100% of attached mussels [as recently reported for hot-water spray washing by Morse (2009)], may still reduce the number of transported mussels to levels that prevent successful colonization of an uninfested water body. Therefore, diligent application and improvement of prevention and containment measures, effective monitoring of water bodies for the presence of dreissenid mussels allowing early detection of invasion, analysis of boater movements among water bodies, increased public awareness campaigns, the closing of unmanned, unmonitored boat launch sites, and the provision of efficient mussel disinfecting facilities appear to be potentially successful strategies for the prevention of further *D. polymorpha* or *D. rostriformis bugensis* dispersal to, and colonization of, isolated, uninfested water bodies in North

America, particularly in the western United States where many water bodies presently do not yet harbor populations of either of these species.

4.5 Conclusions

The AFLP protocol utilized in this study, as well as other molecular techniques in previous studies, indicated a high level of genetic variation within North American populations of both *D. rostriformis bugensis* and *D. polymorpha*. High levels of intrapopulation genetic variation relative to low levels of interpopulation variation make genetic differentiation of North American dreissenid populations difficult. Thus, this study was unable to differentiate among 16 populations of *D. polymorpha* or six populations of D. *rostriformis bugensis* using AFLP markers. The inability of AFLP markers to differentiate among North American intraspecific populations of either *D. polymorpha* or *D. rostriformis bugensis* suggested that the technique presented in this study may not be suitable for identifying the source populations for recent invasions or for use in water body invasion risk assessment models for either species. Therefore, other potentially more sensitive molecular techniques such as microsatellite or SNP arrays should be investigated for elucidating the invasion biology of dreissenid mussels in North America. However, the extensive intrapopulation genetic variation and relatively limited interpopulation variation recorded in this and other studies for both species may make genetic analysis of the invasion dynamics of either North American *D. polymorpha* or *D. rostriformis bugensis* populations unfeasible. While unable to distinguish among intraspecific populations of either North American *D. polymorpha* or *D. rostriformis bugensis*, this study did demonstrate that AFLP analysis was capable of

correctly identifying the species of each of the tested 178 specimens with 100% accuracy. Additionally, AFLP population assignment analysis detected no naturally occurring *D. polymorpha* x *D. rostriformis bugensis* hybrids among 178 tested individuals. The ability of AFLP analysis to accurately differentiate adults of *D. polymorpha* and *D. rostriformis bugensis* suggests that it should be investigated as a means of species identification of dreissenid veliger samples as presently carried out by PCR techniques (Claxton and Boulding 1998).

The general inability to differentiate among intraspecific populations of *D. polymorpha* and *D. rostriformis bugensis*, and the high intrapopulation variability of AFLP marker data suggested that recently established populations of dreissenid mussels via the overland transport of mussels attached to boat hulls have not been subjected to genetic bottlenecks. Furthermore, given the recent invasion and low probability of multiple invasions for several of the sampled populations in this study, the lack of genetic bottlenecks likely resulted from the introduction of a large number of initial colonizers to uninfested water bodies. The implied necessity for a large number of founding individuals to form a sustainably reproducing *D. polymorpha* or *D. rostriformis bugensis* population suggests that prevention and containment measures that eliminate adult mussels from recreational boats and other submerged equipment may not require absolute 100% efficacy to be effective in reducing the spread of dreissenid mussels in North America.

CHAPTER 5

CONCLUSIONS

Recent invasion of warm, often isolated, water bodies in the southwestern United States by *Dreissena polymorpha* and *D. rostriformis bugensis* has created a growing concern that it could lead to selection for thermally tolerant lineages in both species, resulting in the further expansion of their North American range. The incipient upper thermal tolerance limit of 27.2°C determined for *D. rostriformis bugensis* from Lake Mead (NV/AZ) in this study suggested that populations of this species could thrive in the lake at cooler depths ≥12 m in which water temperatures did not approach their incipient upper thermal limit. In contrast, Lake Mead (NV/AZ) populations of this species could be exposed to summer ambient water temperatures approaching or exceeding their incipient upper thermal limit of 27.2°C at depths <12 m. The subsequent invasion of *D. rostriformis bugensis* into warm, shallow southern California reservoirs and the lower Colorado River where summer ambient water temperatures exceed its known incipient upper thermal limit appears to be the result of genetic selection in populations isolated in upstream drainages. Indeed, the recent development of dense adult populations of this species in the near-surface waters of Lake Mead (NV/AZ) may be a result of such thermal adaptation, increasing the potential for future dispersal of *D. rostriformis bugensis* into other southwestern United States water bodies from which this species was previously thought to be thermally excluded, particularly

as it was considered to have an incipient thermal tolerance limit that was ≈ 1-2°C lower than that of *D. polymorpha* (see Section 2.3.3 and literature cited in this section).

Chronic thermal tolerance limits determined for individuals of *D. polymorpha* from Lake Oologah (OK) indicated that ambient summer lake water temperatures would induce extensive mortality events. Thus, the *D. polymorpha* population in Lake Oologah (OK) suffered two successive mortality events in the summers of 2006 and 2007 essentially extirpating this population. If this population has been extirpated it would be the first recorded instance of a natural dreissenid population extirpation in North America. However, two possibilities exist for the reestablishment of a *D. polymorpha* population in Lake Oologah (OK). First, the population could be reinitiated by additional invasion events, and second, the few remaining, presumably more thermally tolerant individuals, could repopulate the lake resulting in a genetically-distinct, thermally tolerant lineage of *D. polymorpha*.

This study has revealed that a thermally-tolerant lineage of *D. polymorpha* may already exist in Winfield City Lake (KS). With an incipient, upper lethal temperature of 30.7°C, the Winfield City Lake *D. polymorpha* population is the most thermally tolerant population of this species ever recorded in North America or Europe. The highest ambient summer water temperatures in Winfield City Lake (KS) were below the upper incipient thermal tolerance limit of 30.7°C recorded for specimens of *D. polymorpha* in that water body. While it is possible that this result was due to acclimatization and phenotypic plasticity it is not supported in the published dreissenid literature. In contrast, this study, along with previously published data, presents strong evidence that

it is very likely to be the result of thermal selection for a genetically based, thermally tolerant lineage.

Specimens of *D. polymorpha* from Winfield City Lake (KS) appear to undergo a major reduction in body tissue mass at water temperatures above 25°C which was most pronounced in larger individuals. This loss of body tissue mass at elevated ambient water temperatures suggested that individuals were in negative energy balance and could explain the progressively increasing extensive summer mortalities and possible extirpation of dreissenid mussels following extended thermal stress in the warmer waters of Lake Oologah (OK) in 2006 and 2007 (Figure A40). Indeed, in early summer (29 June 2007) a standard individual of 25-mm shell length from Lake Oologah (OK) had only 35.4% of the dry tissue body mass of a similar-sized specimen from Winfield City Lake. Considering that Winfield City Lake (KS) mussels had achieved maximum tissue mass on 29 June 2008, and that 25-mm SL specimens had lost 49% of that mass by early August (Figure A35), it is quite possible that already emaciated mussels from Lake Oologah (OK) may have reached lethal levels of tissue loss by that time. Poor physiologic condition due to extreme tissue mass loss in the Lake Oologah (OK) *D. polymorpha* population during the summers of 2006 and 2007 appeared to result in the anomalously reduced chronic thermal tolerance limits recorded for this population during the summers of 2006 and 2007 compared to that of specimens from the cooler Hedges Lake (NY). Additionally, because the negative impact of elevated ambient water temperatures on nutritional/physiologic condition is disproportionately expressed in larger individuals, differences in the size of individuals tested may partially account

for the variation in thermal tolerance reported for this species in North America and Europe.

The recent invasion of southwestern United States water bodies by *D. polymorpha* and *D. rostriformis bugensis* has caused much concern over their likely negative economic and ecological impacts in this region. As such, the potential for dreissenid mussel populations to experience high-temperature density reductions or even eventual extirpation in the warm water bodies of the southwestern and southern United States may have important implications for both the eventual invasion and distribution of dreissenid mussels in the southwestern United States and their management and control. As such, the impact of high summer water temperatures on nutritional condition, growth, reproductive capacity, physiological limits and long-term survivorship in both *D. polymorpha* and *D. rostriformis bugensis* populations in the southwestern United States clearly warrants further experimental attention.

Evolution of thermally tolerant dreissenid populations in southwestern water bodies could be a source for their further invasion into the warm waters of the southern and southwestern United States previously presumed to be resistant to dreissenid mussel invasion. It also poses major implications for thermal techniques utilized to mitigate and manage dreissenid mussel fouling in raw-water systems and for thermal spray washing mitigation of dreissenid mussel fouling on recreational boat hulls and on other mussel-infested equipment transported overland. Indeed, this study suggests that adaptation of dreissenids to warmer southwestern water bodies may reduce the effectiveness of present thermal mitigation treatments to control their macrofouling in

raw-water systems. In addition, the demonstration of a positive relationship between both short-term temperature acclimation and longer-term temperature acclimatization on the chronic upper thermal tolerance limits of *D. polymorpha* appears to be well documented and may be characteristic of dreissenids in general (including *D. rostriformis bugensis*). These positive relationships may also reduce the efficacy of thermal mitigation treatments utilized in the warm southwestern regions of the United States.

The genetic evaluation of North American dreissenid invasion presented in this study also has implications for mitigation techniques. The AFLP protocol utilized in this study, as well as other molecular techniques by other authors indicated a high level of genetic variation within North American populations of both *D. rostriformis bugensis* and *D. polymorpha*. The high levels of intrapopulation genetic variation relative to the low levels of interpopulation variation make genetic differentiation of North American dreissenid populations difficult to assess. The general inability to differentiate among intraspecific populations of *D. polymorpha* and *D. rostriformis bugensis*, and the high intrapopulation variability of AFLP marker data suggested that populations of dreissenid mussels recently established via the overland transport of individuals attached to boat hulls have not been subjected to genetic bottlenecks. Furthermore, given the recent invasion and low probability of multiple invasions for several of the sampled populations in this study, the lack of bottlenecks likely resulted from the introduction of a large number of initial colonizers to uninfested water bodies. The implied necessity for a large number of founding individuals to form a sustainably

reproducing *D. polymorpha* or *D. rostriformis bugensis* population suggests that prevention and containment measures that eliminate adult mussels from recreational boats and other submerged equipment may not require absolute 100% efficacy to be effective in reducing the spread of dreissenid mussels in North America.

Raw-water users such as electric power plants and water treatment plants in the southeastern and southwestern United States are presently not well prepared to deal with dreissenid mussel fouling and are likely to incur huge operating and mussel mitigation costs as populations of *D. rostriformis bugensis* and *D. polymorpha* expand their ranges into these regions. This study presents evidence that the sensitive freshwater ecosystems of the southwestern and southeastern United States, from which dreissenids were once presumed to be thermally excluded, could support dense thermally tolerant populations of both *D. polymorpha* and/or *D. rostriformis bugensis*. Unfortunately, most local, state and federal agencies and their local water body managers do not appear to be fully prepared or funded to implement the extensive and costly prevention and containment procedures required to limit the further range expansion of dreissenid mussels in southwestern water bodies and to deal with the resultant ecological and economic damage they entail. However, the results of this study also suggest that western U.S. water bodies may remain free of dreissenid mussels if a coordinated, integrated and region-wide prevention, containment and management plan is developed and adopted throughout the western states as detailed in the *Quagga-Zebra Mussel Action Plan for Western US Waters* by the Western Region Panel on Aquatic Nuisance Species (2009).

APPENDIX A

FIGURES

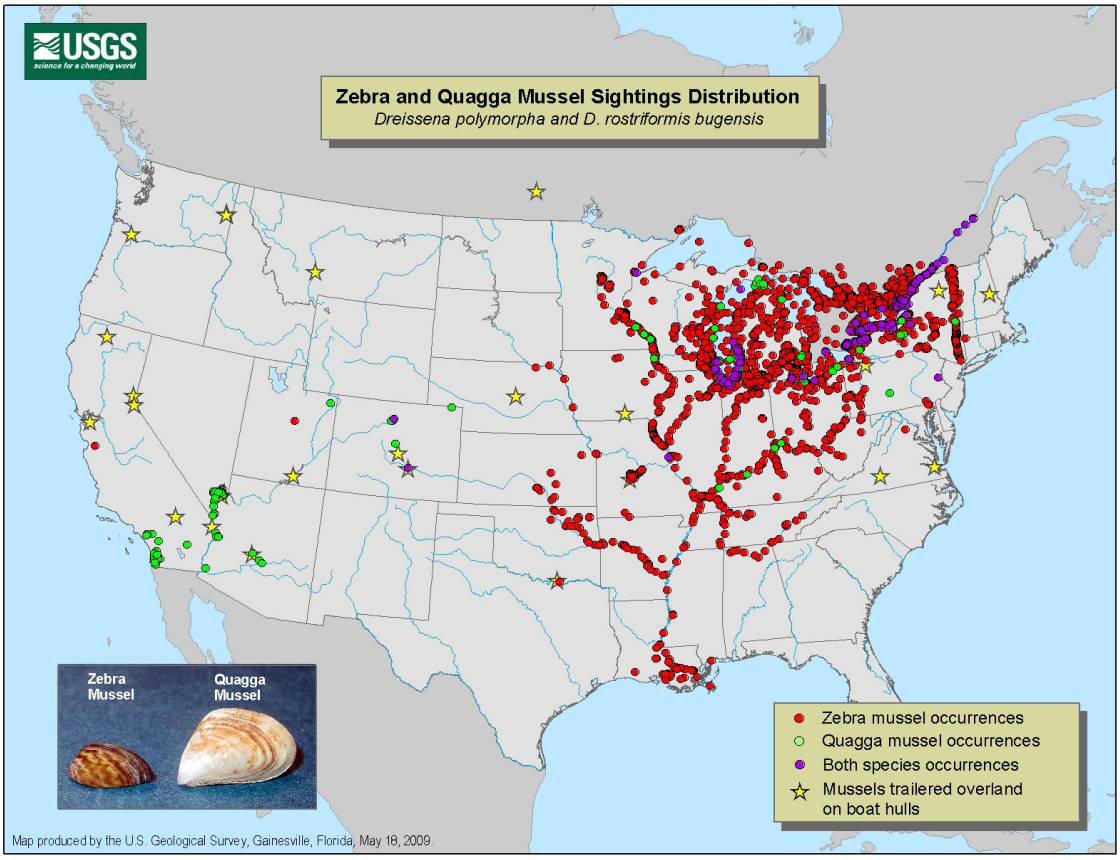

Figure A1. North American distribution of *Dreissena polymorpha* (red and purple circles) and *D. rostriformis bugensis* (green and purple circles) as of May 2009. Stars are locations where trailered boats with dreissenid mussel hull fouling have been intercepted. Reproduced from USGS (2009).

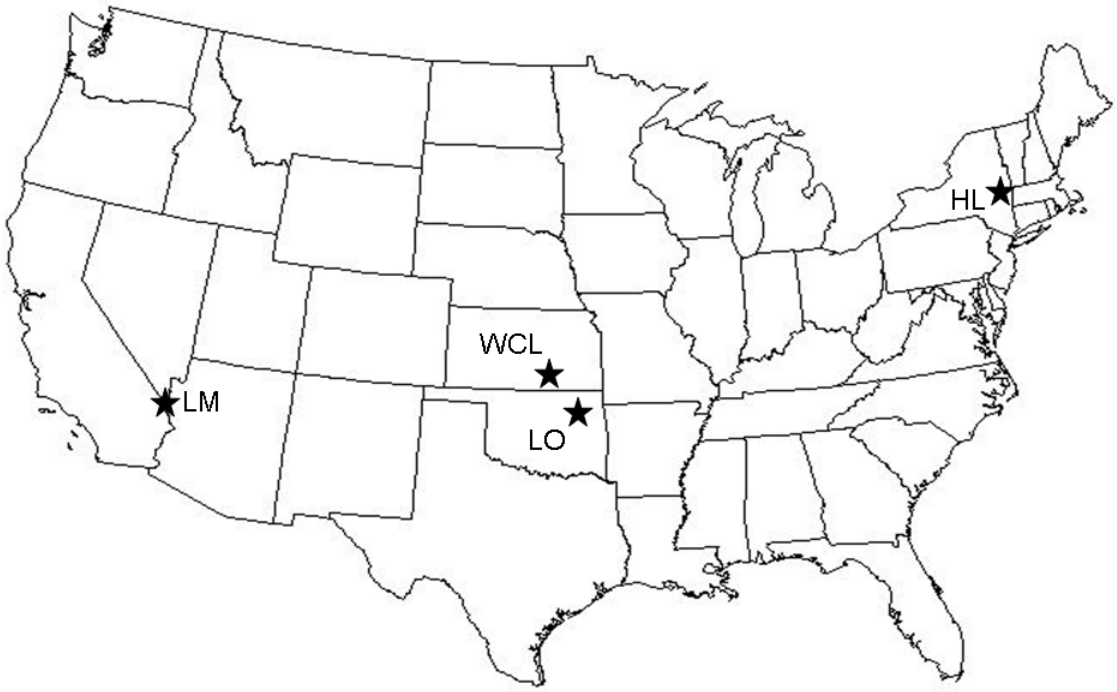

Figure A2. Specimens of *Dreissena polymorpha* were collected from Winfield City Lake, KS (WCL), Lake Oologah, OK (LO), and Hedges Lake, NY (HL) while specimens of *D. rostriformis bugensis* were collected from Lake Mead, NV/AZ (LM) for upper thermal tolerance limit testing.

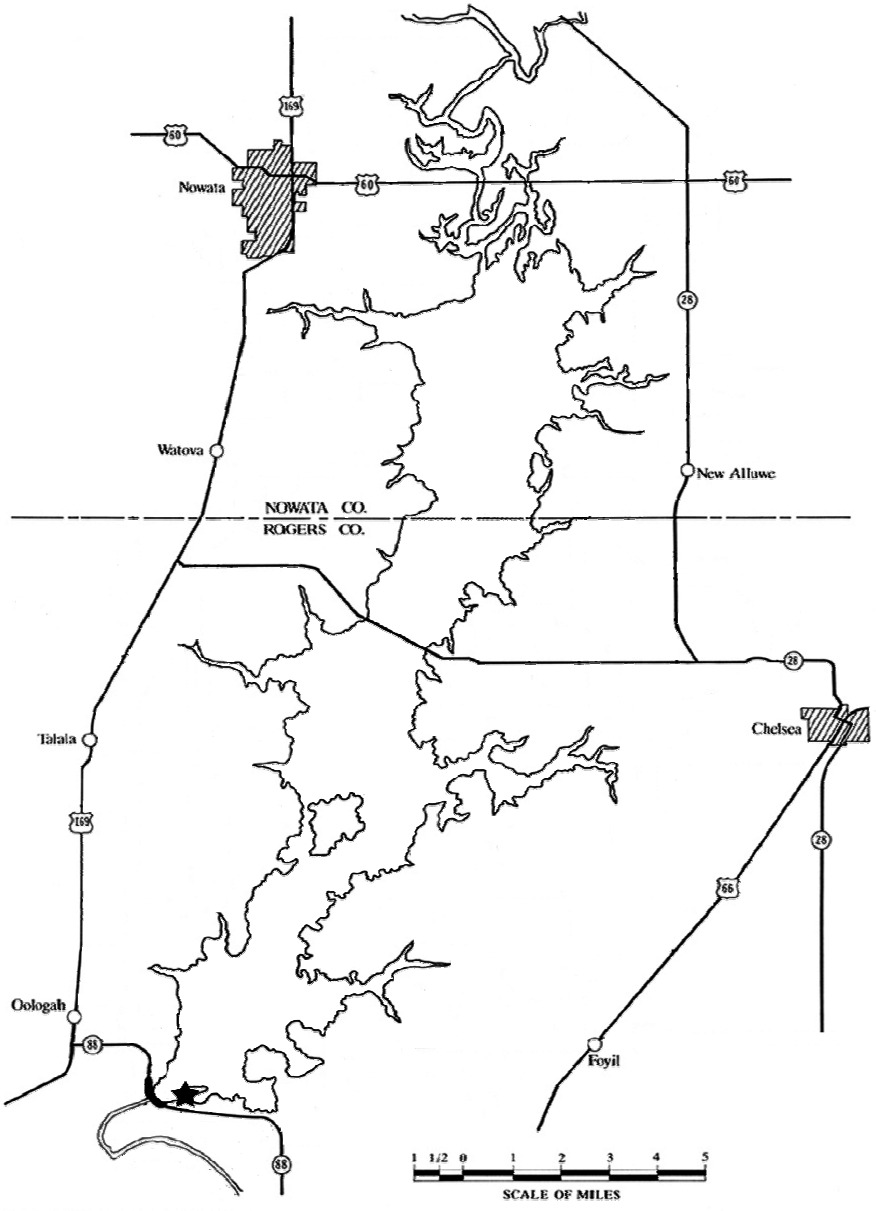

Figure A3. Map of Lake Oologah (OK) showing the location of the Redbud Bay Marina (black star) where specimens of *Dreissena polymorpha* were collected. Scale is in miles.

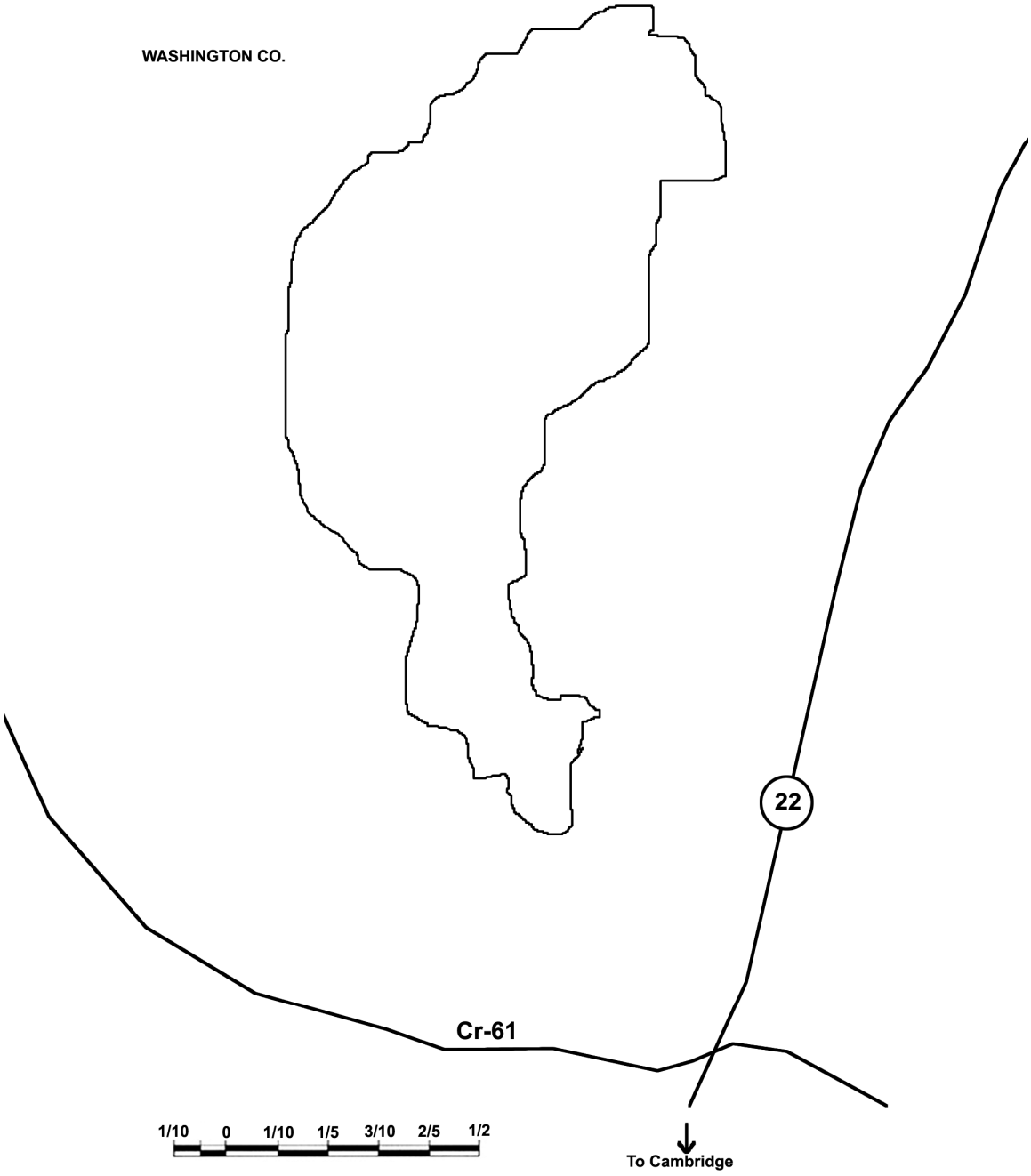

Figure A4. Map of Hedges Lake (NY) from which *Dreissena polymorpha* were collected for determination of their chronic upper thermal limit. Scale is in miles.

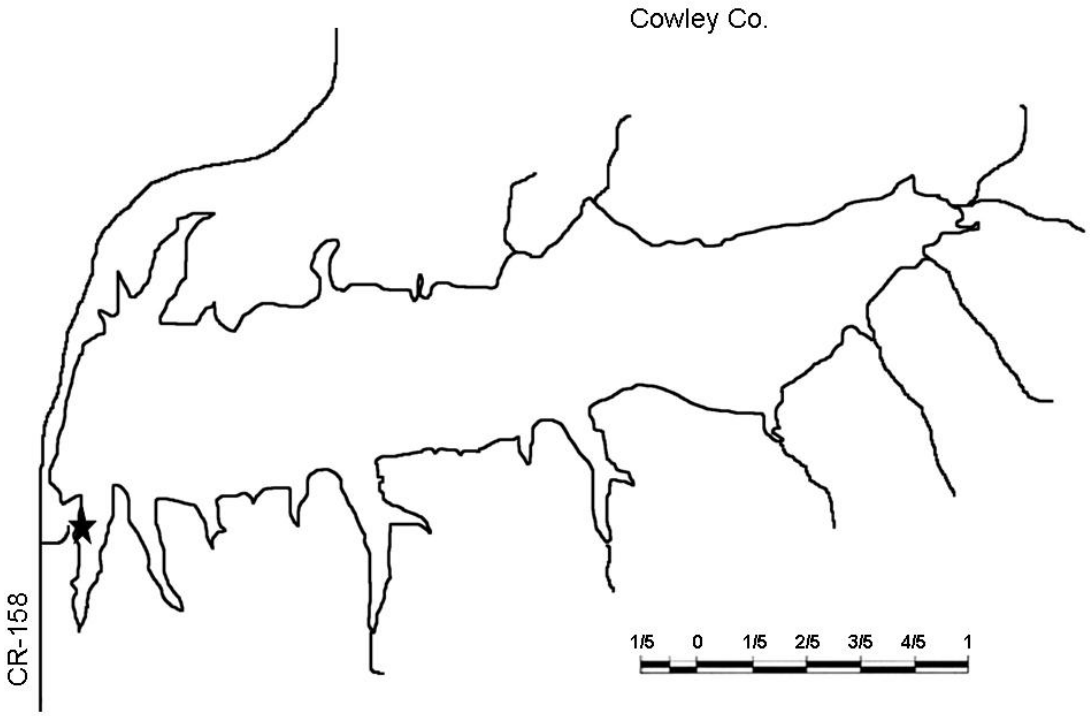

Figure A5. Map of Winfield City Lake (KS) showing where specimens of *Dreissena polymorpha* were collected (black star). Scale is in miles.

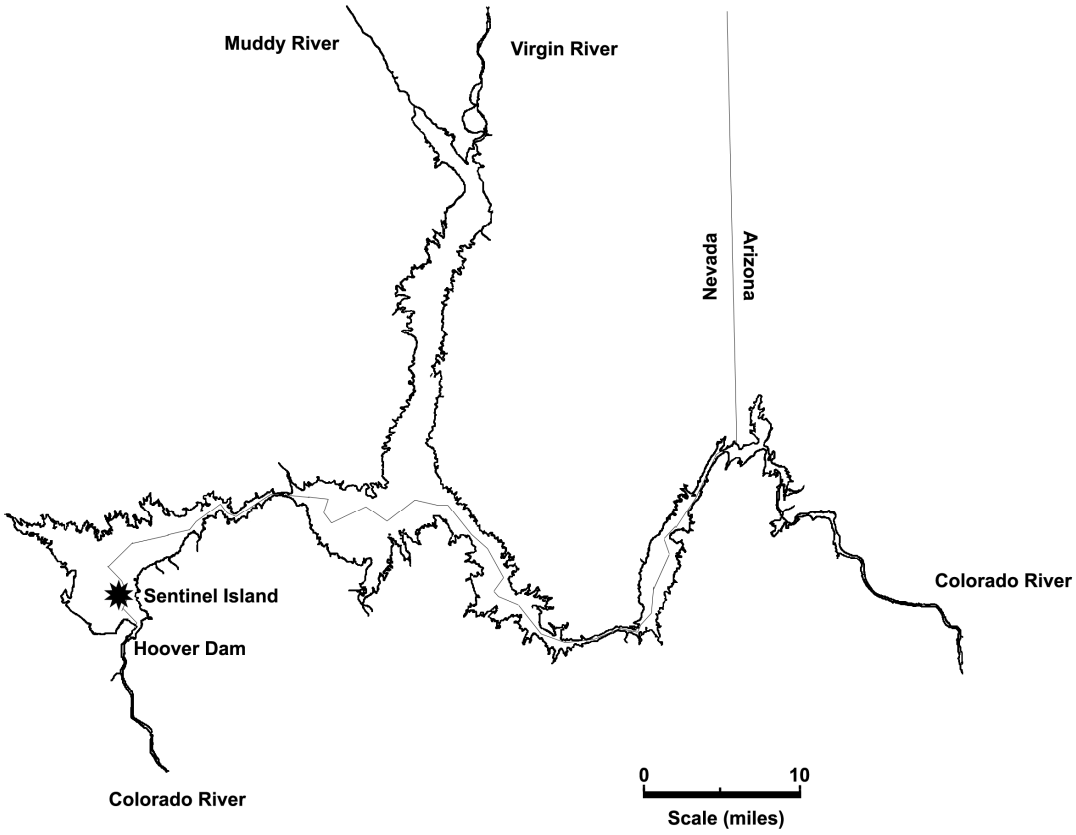

Figure A6. Map of Lake Mead (NV/AZ) showing the location of Sentinel Island (black star) where specimens of *Dreissena rostriformis bugensis* were collected. Scale is in miles.

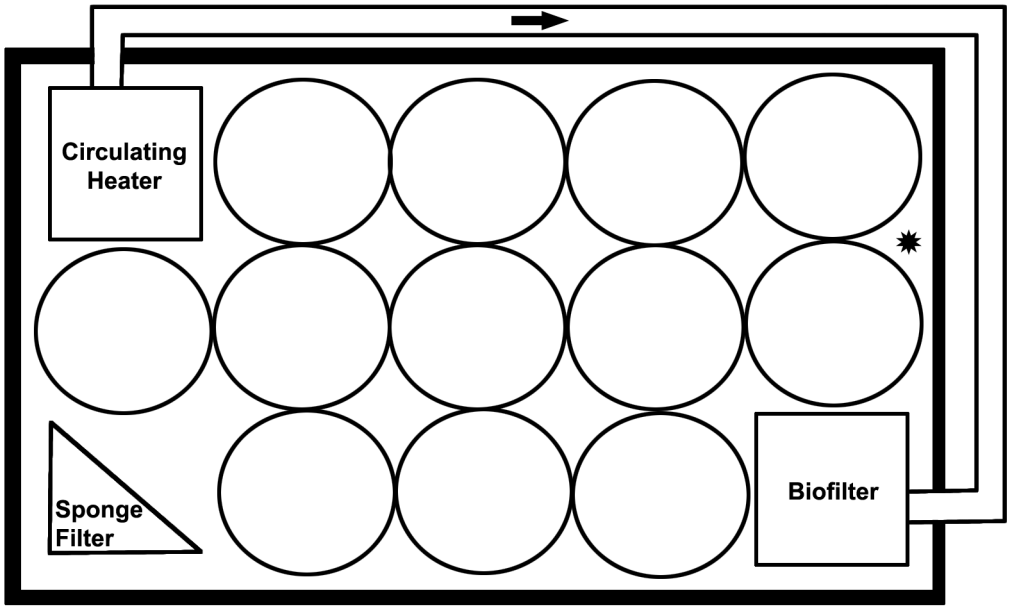

Figure A7. Experimental tank setup for dreissenid mussel, chronic thermal tolerance testing. The star represents the location of the air stone.

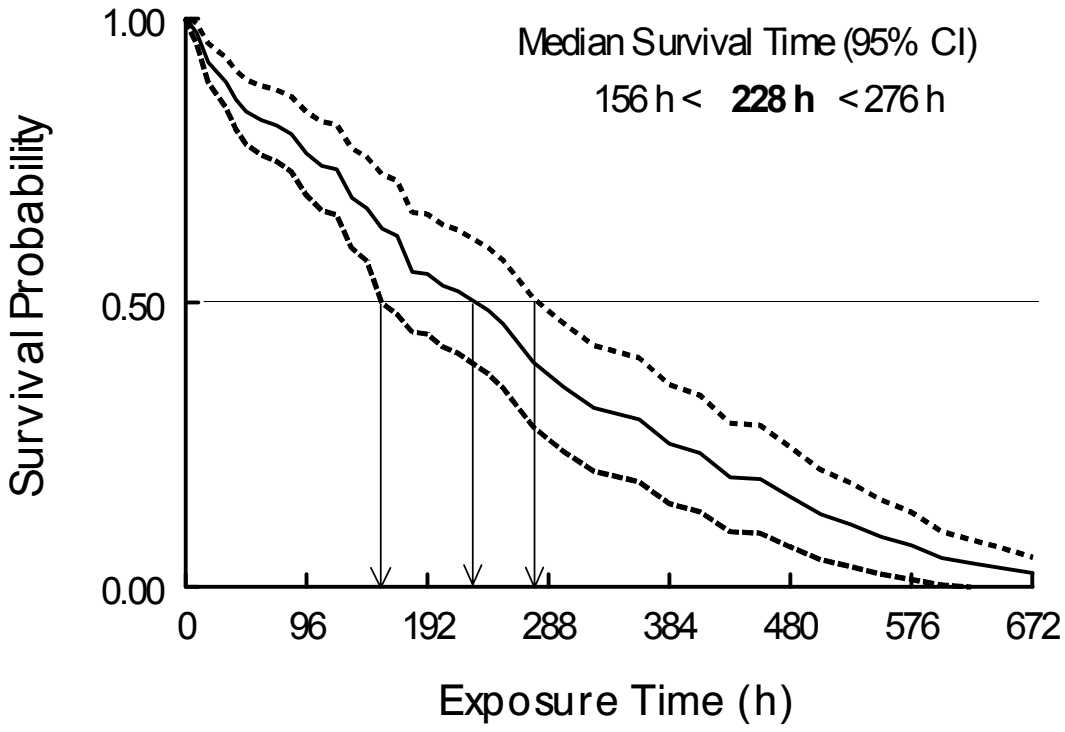

Figure A8. Depiction of the methodology used to determine the median survival time and its 95% confidence interval (CI) from a plot of the model estimated survival function and the function's 95% CI. The median survival time from this survival function would be 228 h with a 95% CI range of 156 h to 276 h.

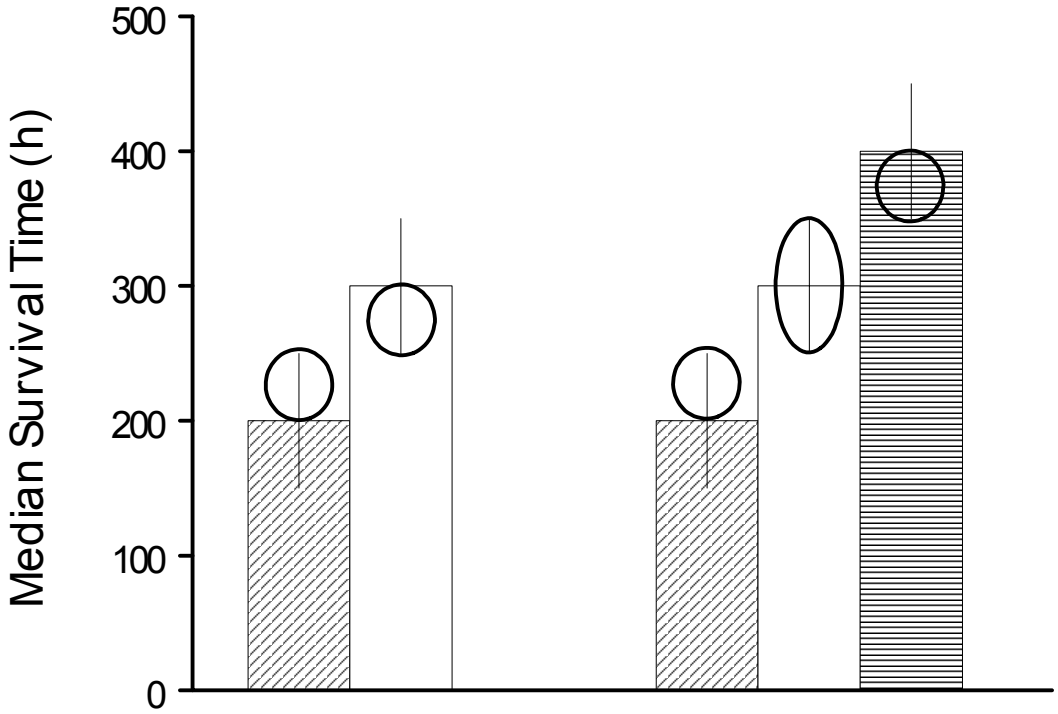

Figure A9. Determination of standard errors for Wald Chi-square comparisons of median survival time in the presence of non-symmetrical standard errors. The coupled bars on the left show which standard errors would be used to compare the two median survival times. The trio of bars on the right show which standard errors would be used to calculate the Wald statistic if more than two groups were compared. Note that the middle bar's standard error is actually a mean of its upper and lower standard errors.

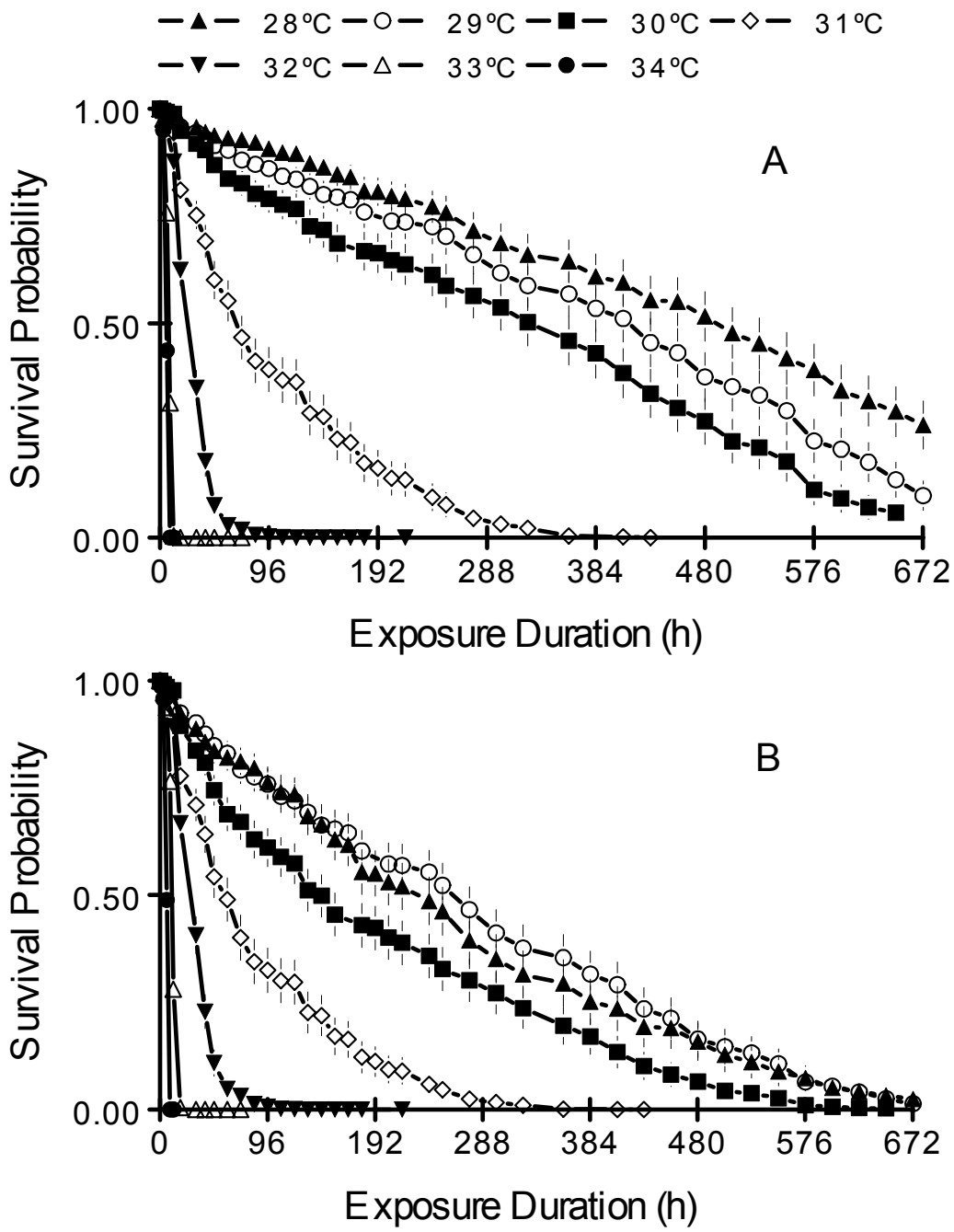

Figure A10. Survival curves for a standard 15-mm SL specimen of *Dreissena polymorpha* collected in 2006, acclimated to 20°C, and exposed to 28°C (black triangles), 29°C (open circles), 30°C (black squares), 31°C (open diamonds), 32°C (black upside down triangles), 33°C (open triangles), and 34°C (black circles) from (A) Hedges Lake (NY) and (B) Lake Oologah (OK). Bars represent standard errors.

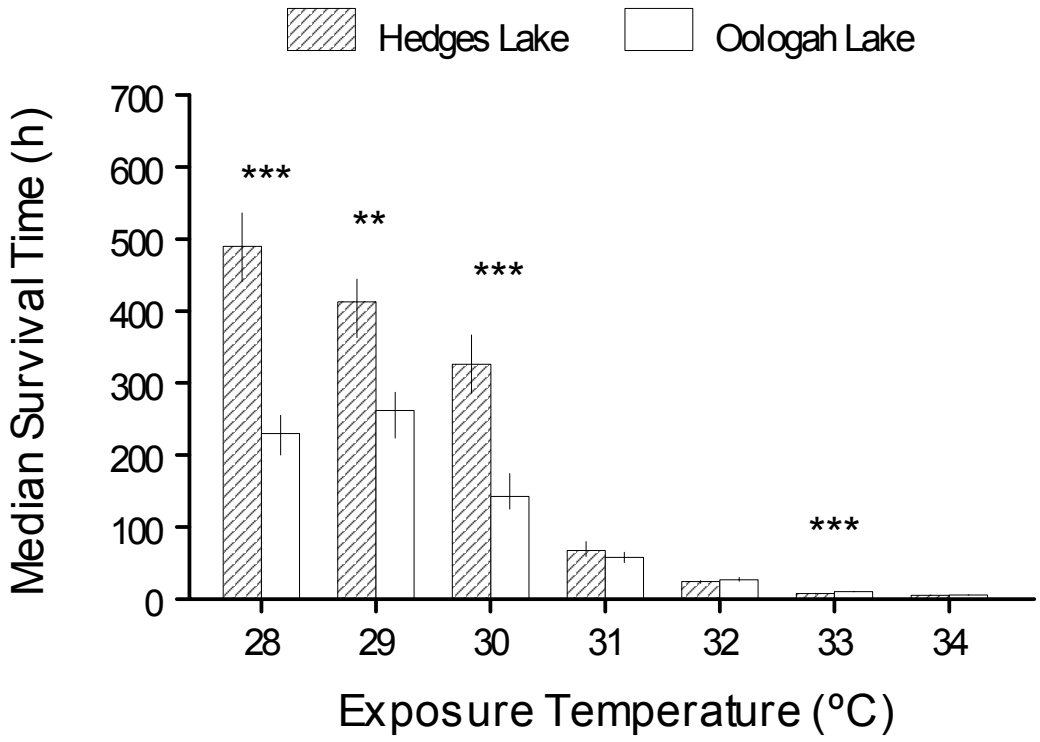

Figure A11. Median survival time (in hours) with standard error bars for standard 15-mm SL Lake Oologah (OK; open bars) and Hedges Lake (NY; striped bars) specimens of *Dreissena polymorpha*, collected in 2006, acclimated to 20°C, and exposed to a range of temperatures (x-axis) for 28 d. Asterisks represent significant differences between the two populations (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

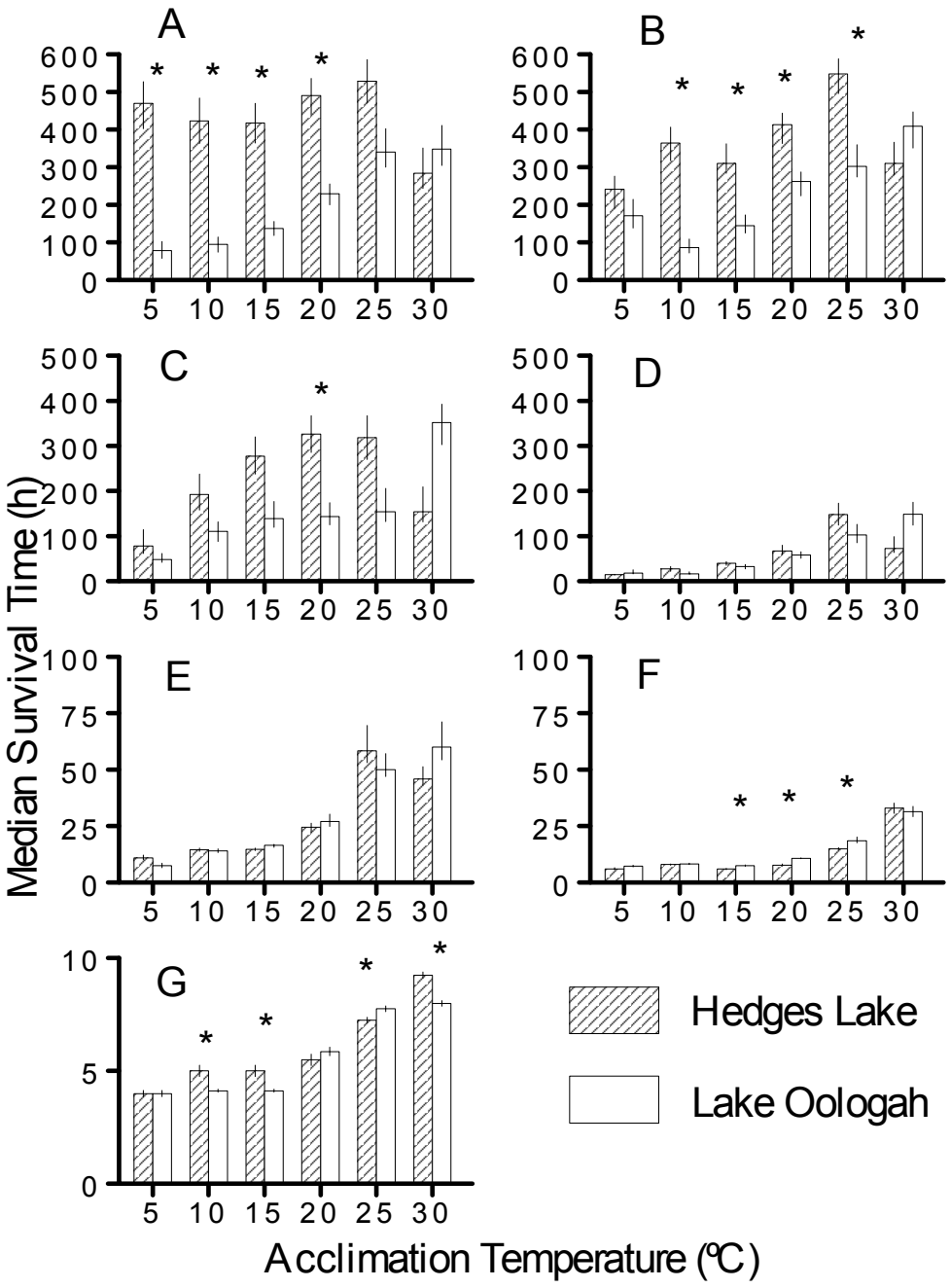

Figure A12. Median survival times with standard errors of 15-mm standard SL specimens of *Dreissena polymorpha* collected in 2006 from Hedges Lake (NY; striped bars) and Lake Oologah (OK; open bars), acclimated to different temperatures (x-axis) and exposed to 28°C (A), 29°C (B), 30°C (C), 31°C (D), 32°C (E), 33°C (F), and 34°C (G). Asterisks represent significant differences between the two populations after Bonferroni correction ($P < 0.008$).

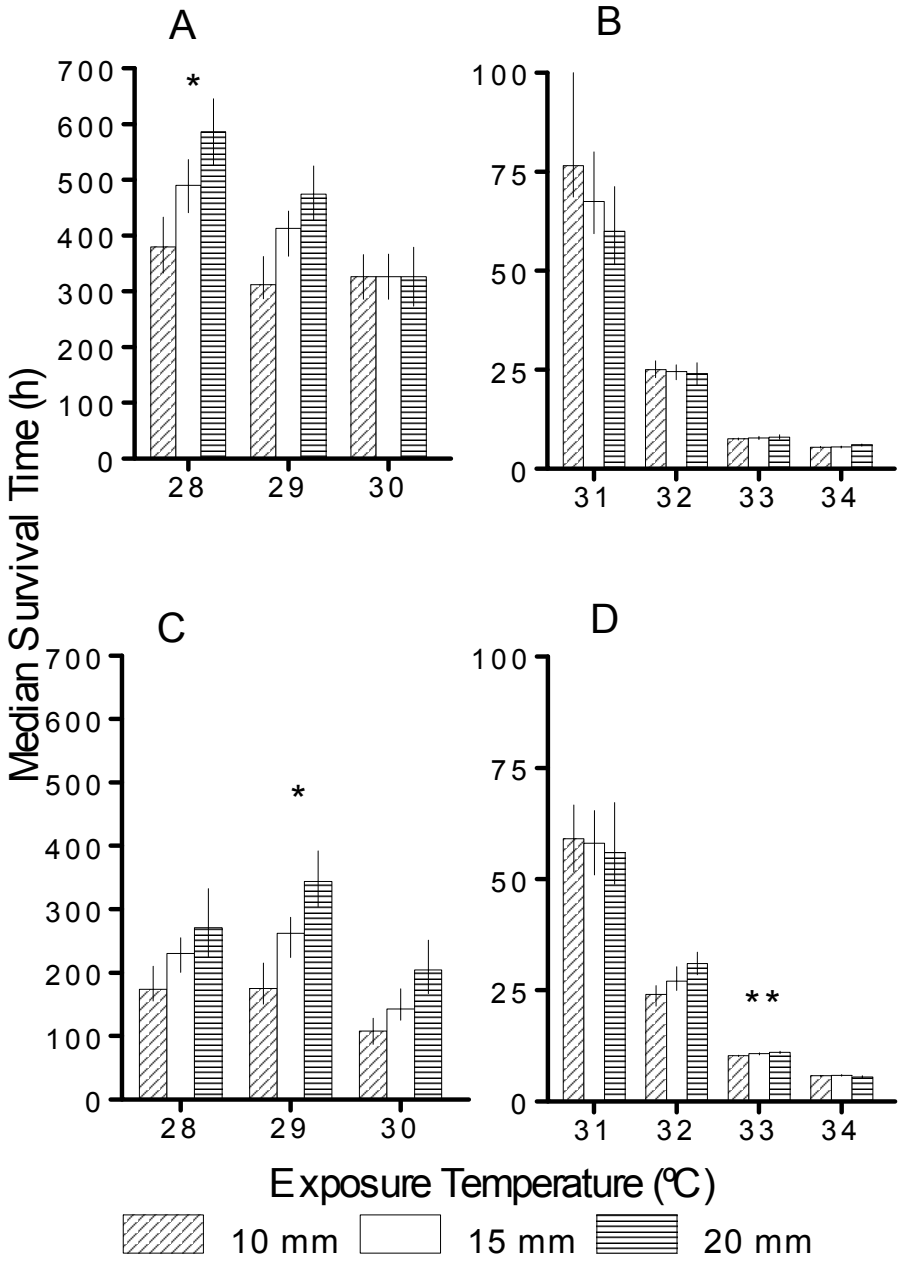

Figure A13. Median survival times (y-axis) with standard errors for 20°C acclimated, standard SL specimens of *Dreissena polymorpha* collected in 2006 from Hedges Lake (NY) (A and B) and Lake Oologah (OK) (C and D) and treated at various temperatures (x-axis). Diagonally striped bars, open bars, and horizontally striped bars represent standard 10, 15, and 20-mm SL individuals, respectively. Asterisks represent significance differences among all three size cohorts as determined by a Wald Chi-square statistic (* $P < 0.05$, ** $P < 0.01$).

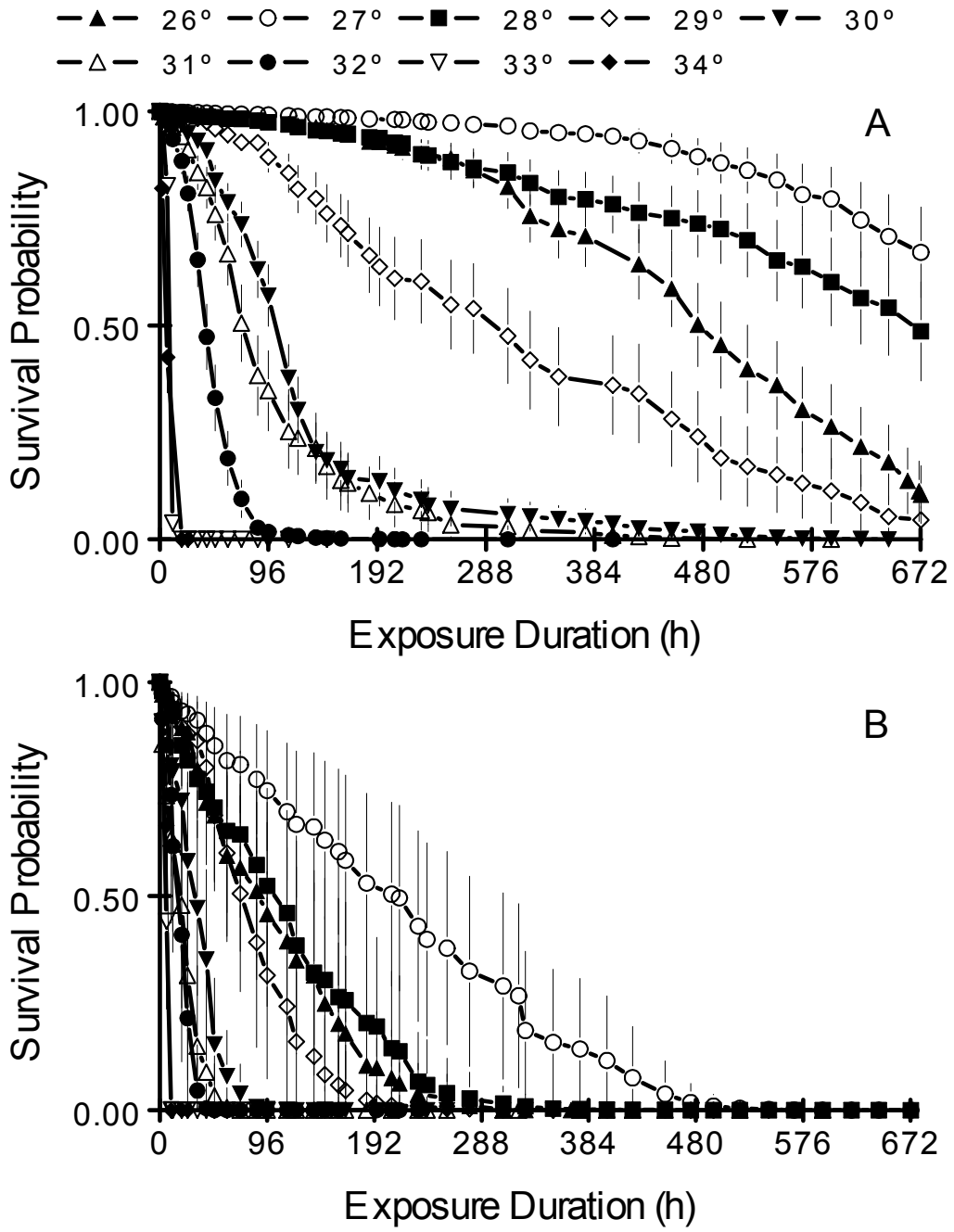

Figure A14. Survival curves for a standard 15-mm SL specimen of *Dreissena polymorpha* collected in 2007, acclimated to 20°C, and exposed to 26°C (black triangles), 27°C (open circles), 28°C (black squares), 29°C (open diamonds), 30°C (black upside-down triangles), 31°C (open triangles), 32°C (black circles), 33°C (open upside-down triangles), and 34°C (black diamonds) from (A) Hedges Lake (NY) and (B) Lake Oologah (OK). Bars represent standard errors.

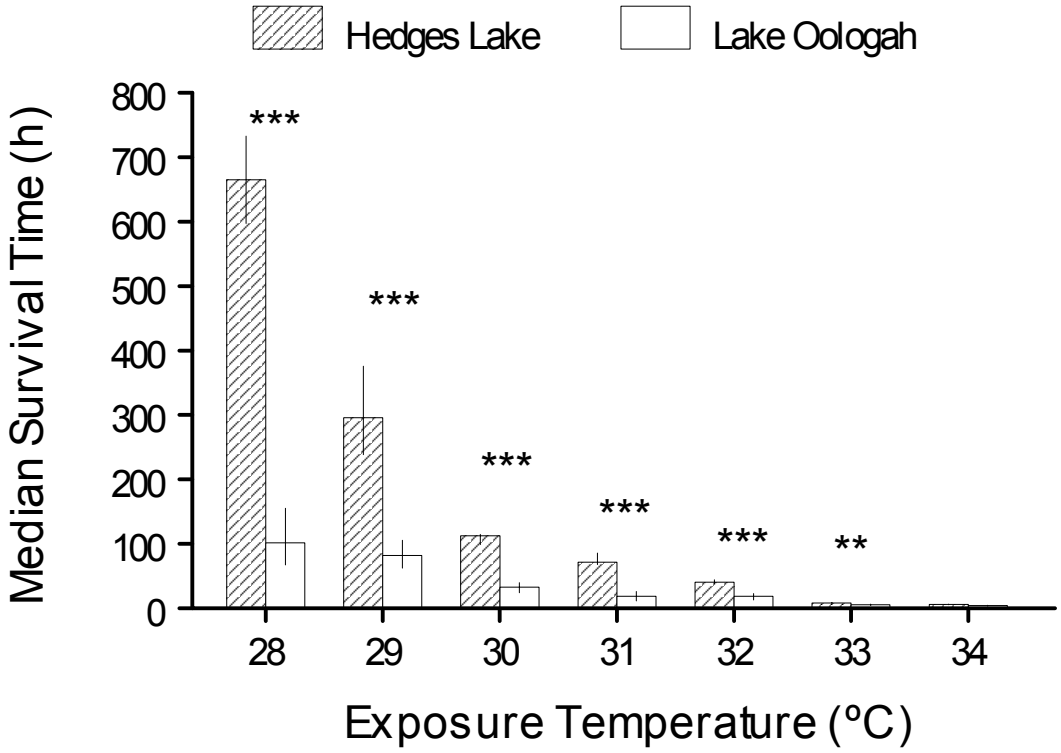

Figure A15. Median survival time (in hours; y-axis) with standard errors for unfed, 20°C acclimated, standard 15-mm SL specimens of *Dreissena polymorpha* collected in 2007 from Lake Oologah (OK; open bars) and Hedges Lake (NY; striped bars), and treated at a range of temperatures (x-axis) for 28 d. Asterisks represent significant differences between the two populations (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

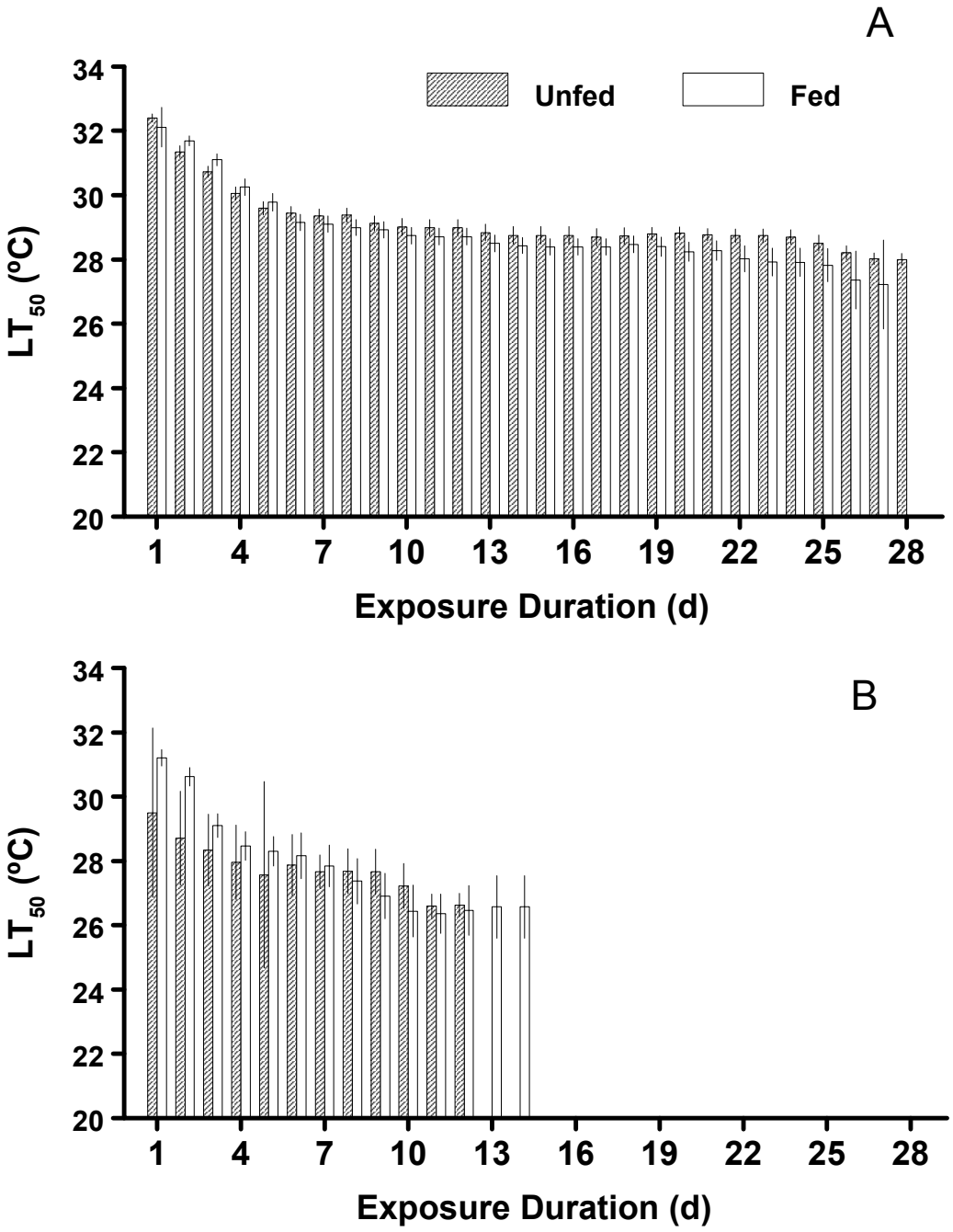

Figure A16. LT_{50} values with standard errors for standard 15-mm SL, 20°C acclimated, fed (open bars) and unfed (striped bars) specimens of *Dreissena polymorpha* collected in 2007 from (A) Hedges Lake (NY) and (B) Lake Oologah (OK). Missing values indicate that mortality was too rapid at all exposure temperatures to calculate an LT_{50} value.

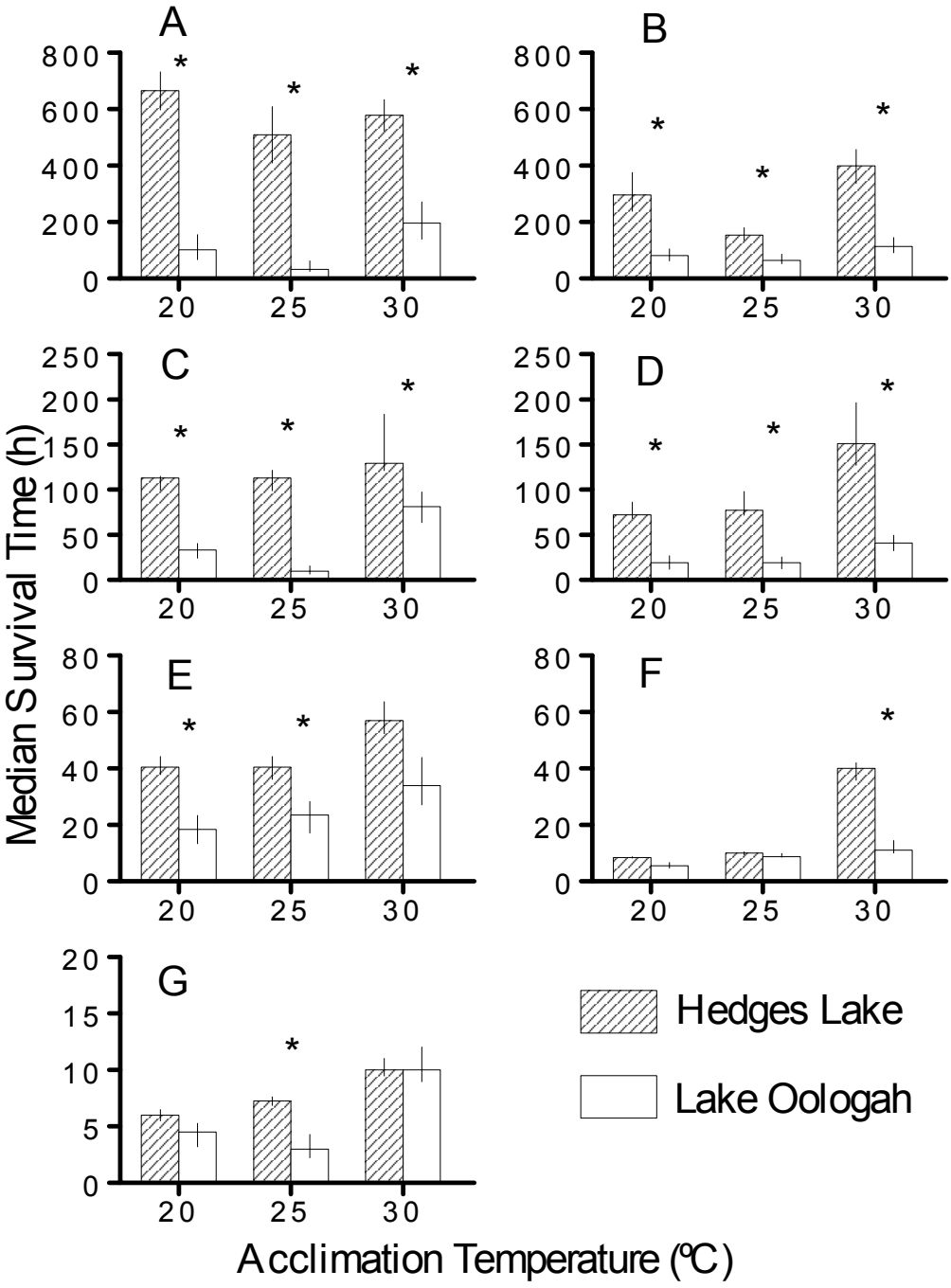

Figure A17. Median survival times with standard errors for a standard 15-mm SL specimen of *Dreissena polymorpha* collected in 2007 from Hedges Lake (NY; striped bars) and Lake Oologah (OK; open bars) and exposed to (A) 28°C, (B) 29°C, (C) 30°C, (D) 31°C, (E) 32°C, (F) 33°C, and (G) 34°C. Asterisks represent significant differences between the two populations after Bonferroni correction ($P < 0.017$).

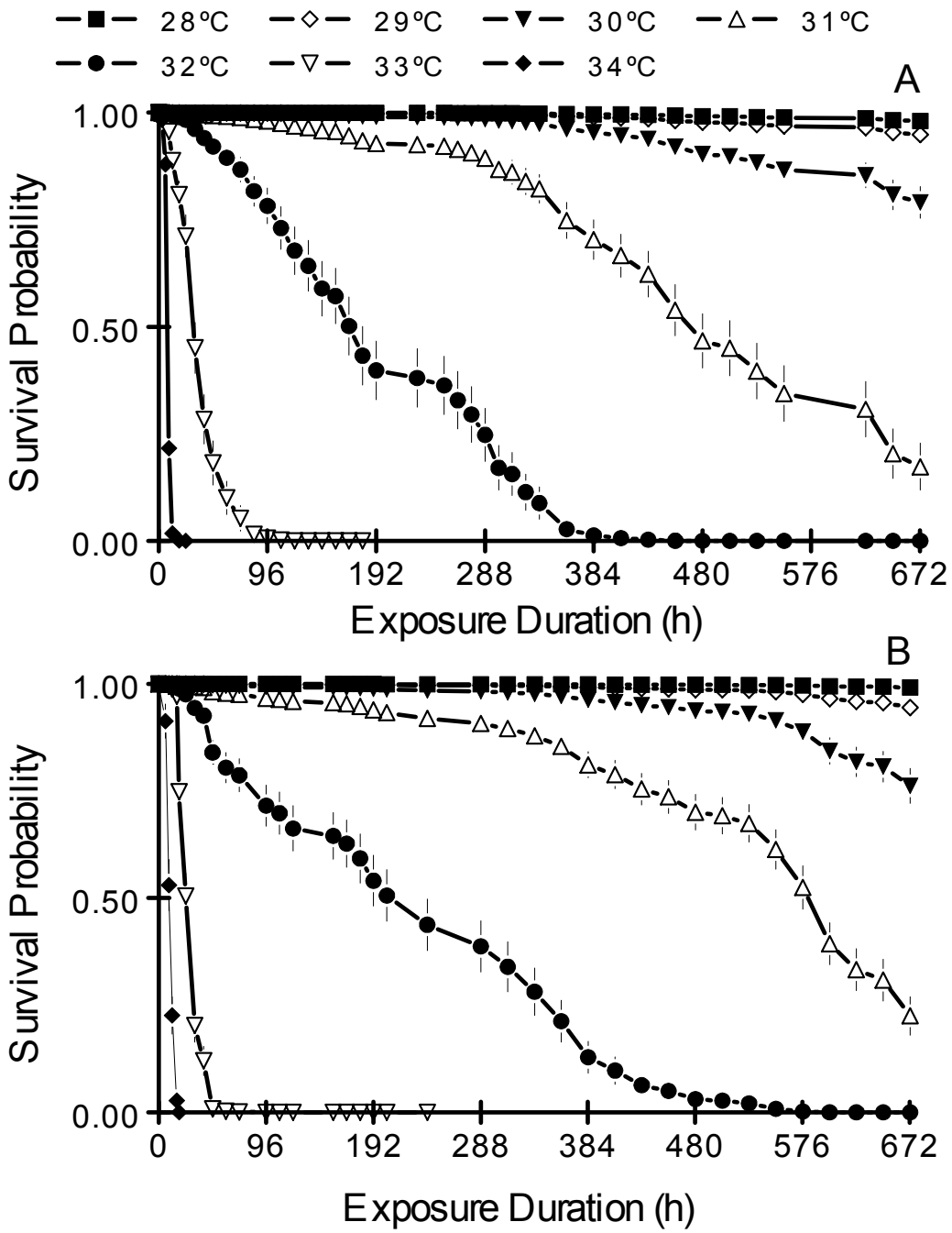

Figure A18. Survival curves for a standard 15-mm SL, 20°C acclimated specimen of *Dreissena polymorpha* from Winfield City Lake (KS) exposed to 28°C (black squares), 29°C (open diamonds), 30°C (black upside-down triangles), 31°C (open triangles), 32°C (black circles), 33°C (open upside-down triangles), and 34°C (black diamonds) collected from (A) early summer and (B) late summer. Bars represent standard errors.

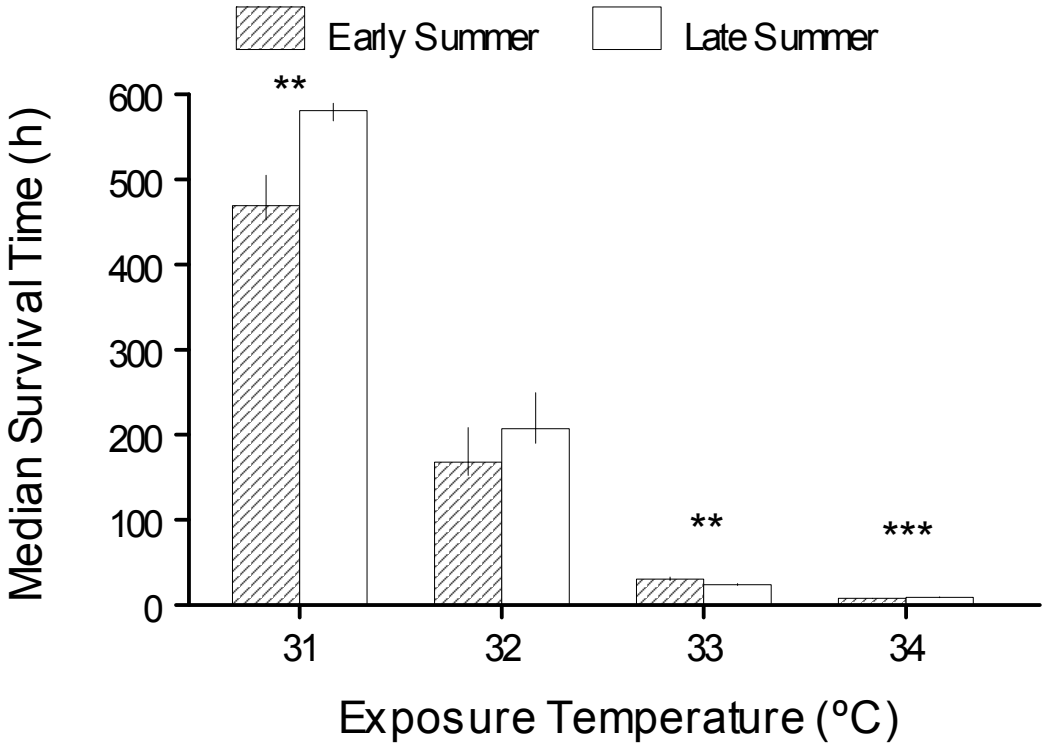

Figure A19. Median survival times with standard errors for early (striped bars) and late summer (open bars) collected, 20°C acclimated, standard 15-mm SL specimens of *Dreissena polymorpha* from Winfield City Lake (KS) and exposed to various temperatures (x-axis). Asterisks represent significant differences between the two collection periods (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

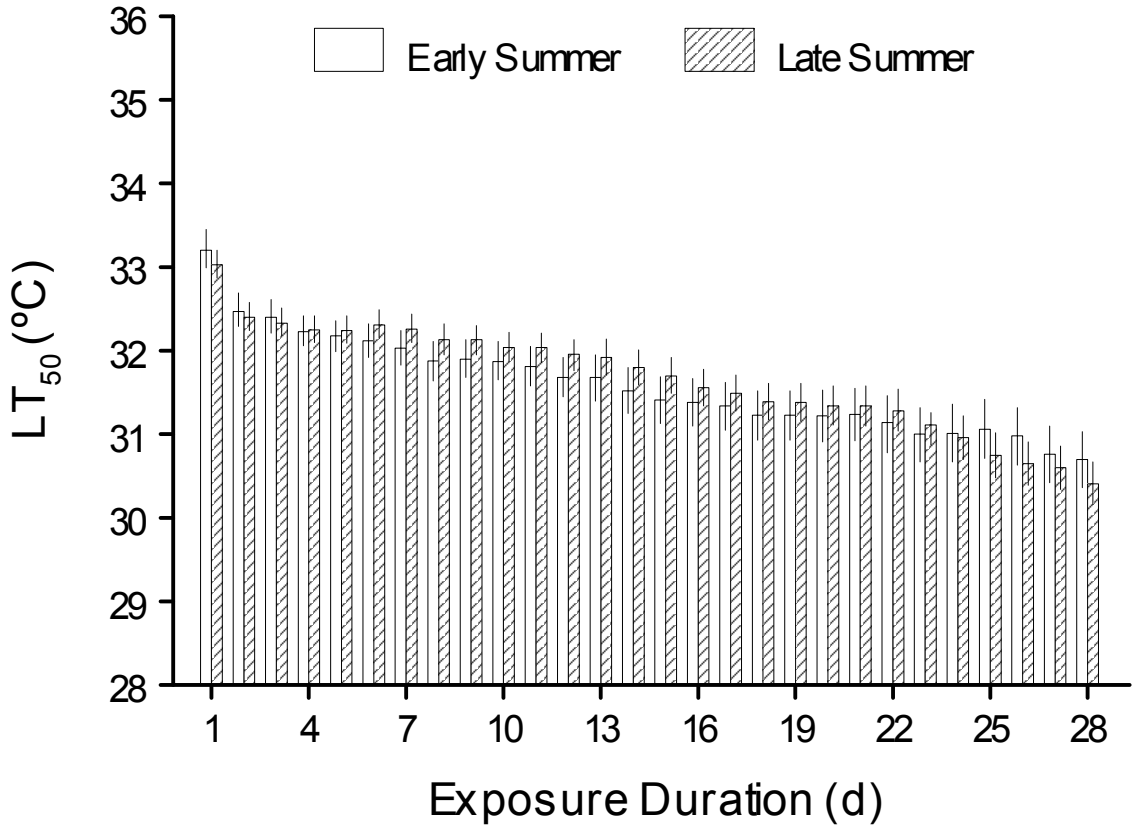

Figure A20. LT_{50} values (in °C; y-axis) with standard errors for standard 15-mm SL, 20°C acclimated specimens of *Dreissena polymorpha* collected in early (open bars) and late summer (striped bars) from Winfield City Lake (KS).

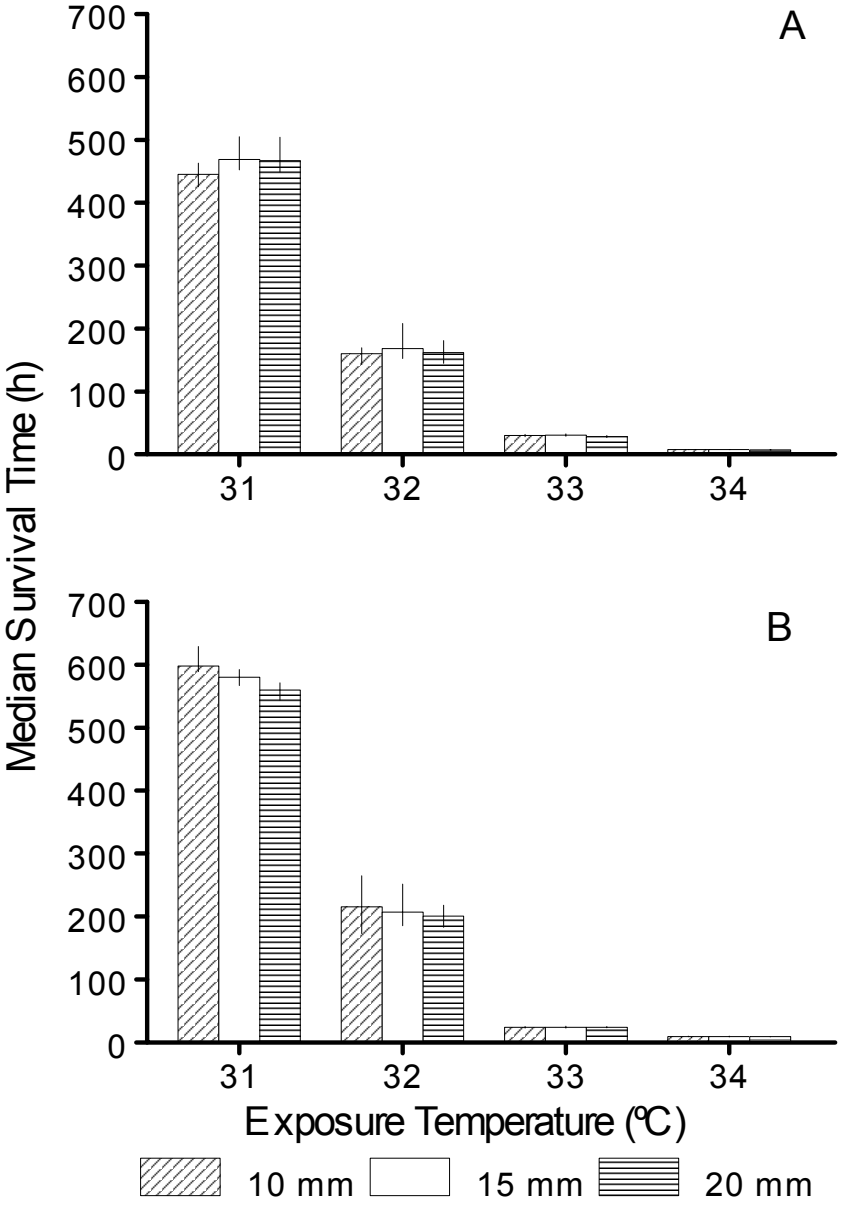

Figure A21. Median survival times (in h; y-axis) with standard errors for standard length, 20°C acclimated, early- (A) and late-summer (B) collected specimens of *Dreissena polymorpha* from Winfield City Lake and treated at various temperatures (x-axis). Diagonally striped bars, open bars, and horizontally striped bars represent standard 10, 15, and 20-mm SL individuals, respectively. There were no significance differences among the median survival times for all three size cohorts as determined by a Wald Chi-square statistic at each treatment temperature.

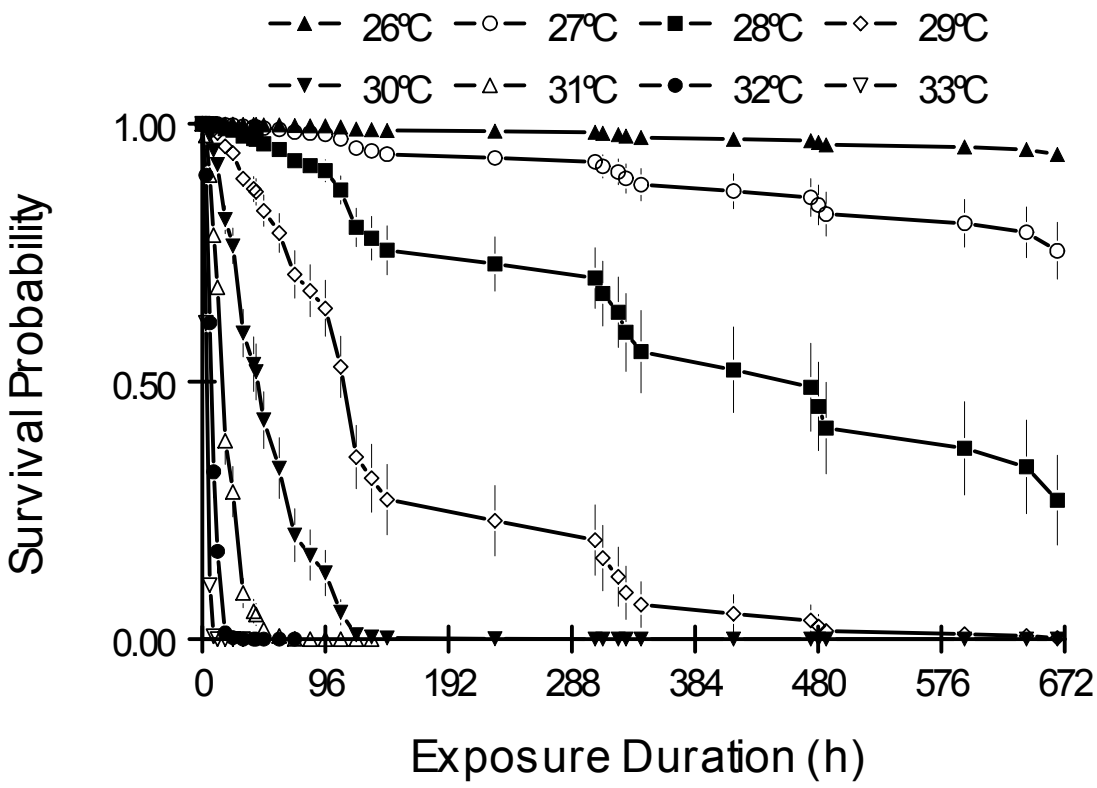

Figure A22. Survival curves for a standard 15-mm SL, 20°C acclimated specimen of *Dreissena rostriformis bugensis* collected from Lake Mead (NV/AZ) and exposed to 26°C (black triangles), 27°C (open circles), 28°C (black squares), 29°C (open diamonds), 30°C (black upside-down triangles), 31°C (open triangles), 32°C (black circles), and 33°C (open upside-down triangles). Bars represent standard errors.

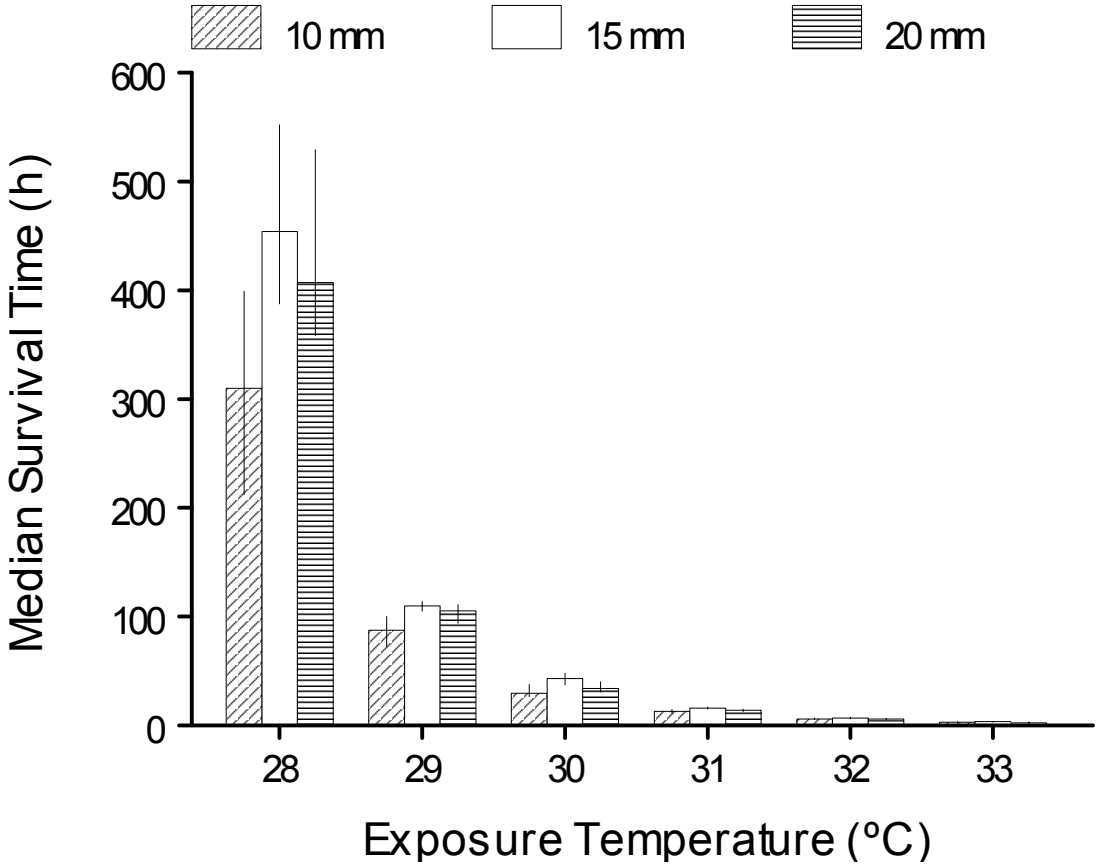

Figure A23. Median survival times (in h; y-axis) with standard errors for standard length, 20°C acclimated specimens of *Dreissena rostriformis bugensis* collected from Lake Mead (NV/AZ) exposed to various temperatures (x-axis). Diagonally striped bars, open bars, and horizontally striped bars represent standard 10, 15, and 20-mm SL individuals, respectively. There were no significance differences among the median survival times for all three size cohorts as determined by a Wald Chi-square statistic at each treatment temperature.

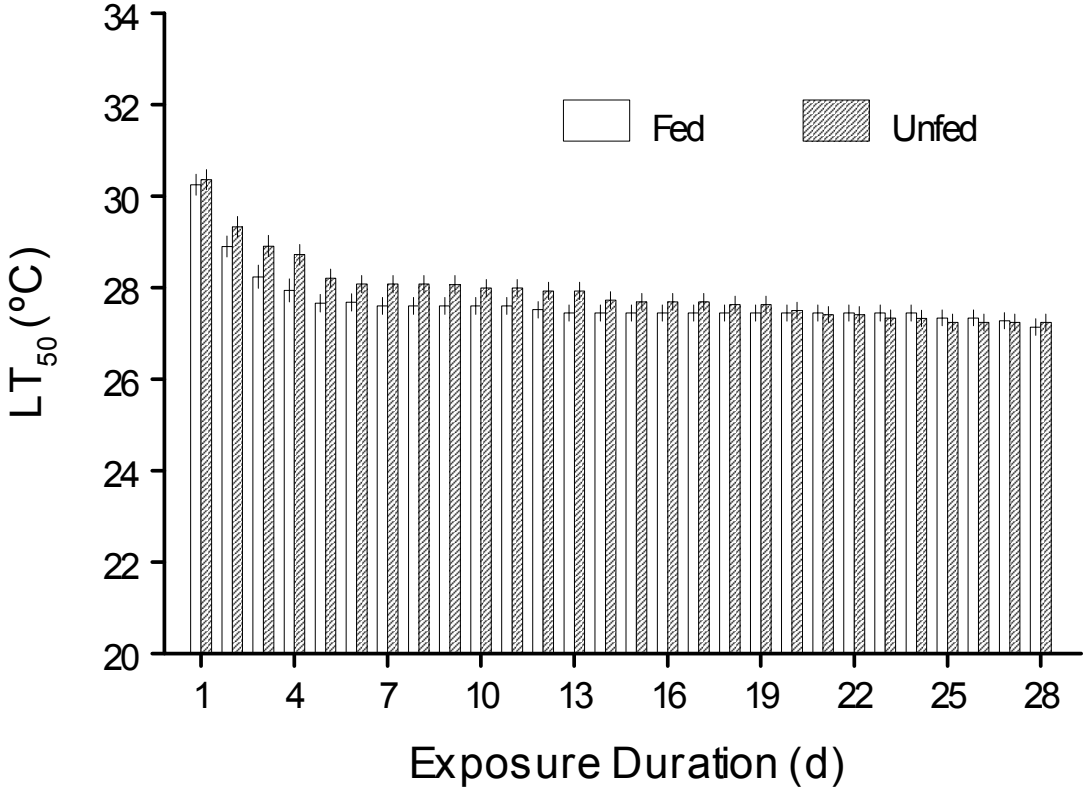

Figure A24. LT_{50} values (in °C; y-axis) with standard errors for standard 15-mm SL, 20°C acclimated, fed (open bars) and unfed (striped bars) specimens of *Dreissena rostriformis bugensis* collected from Lake Mead (NV/AZ).

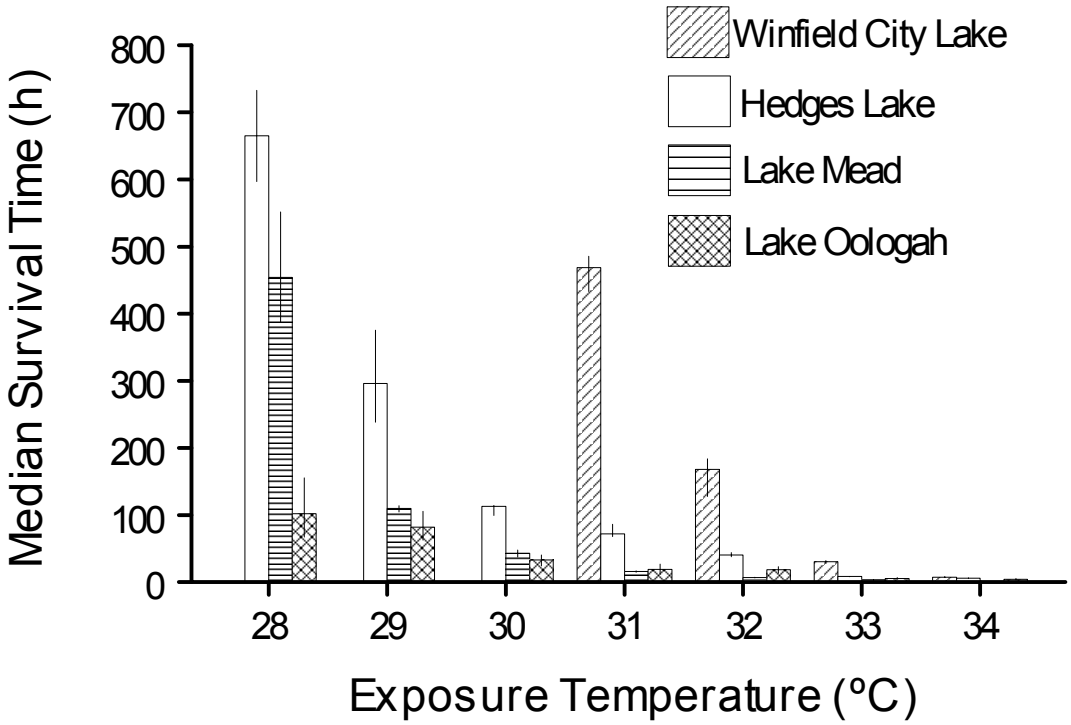

Figure A25. Median survival times (in h; y-axis) with standard errors for 20°C acclimated, standard 15-mm SL, unfed specimens of *Dreissena polymorpha* from Hedges Lake (NY; open bars), Lake Oologah (OK; hatched bars), and Winfield City Lake (KS; diagonally striped bars) and for similar specimens of *D. rostriformis bugensis* from Lake Mead (NV/AZ; horizontally striped bars).

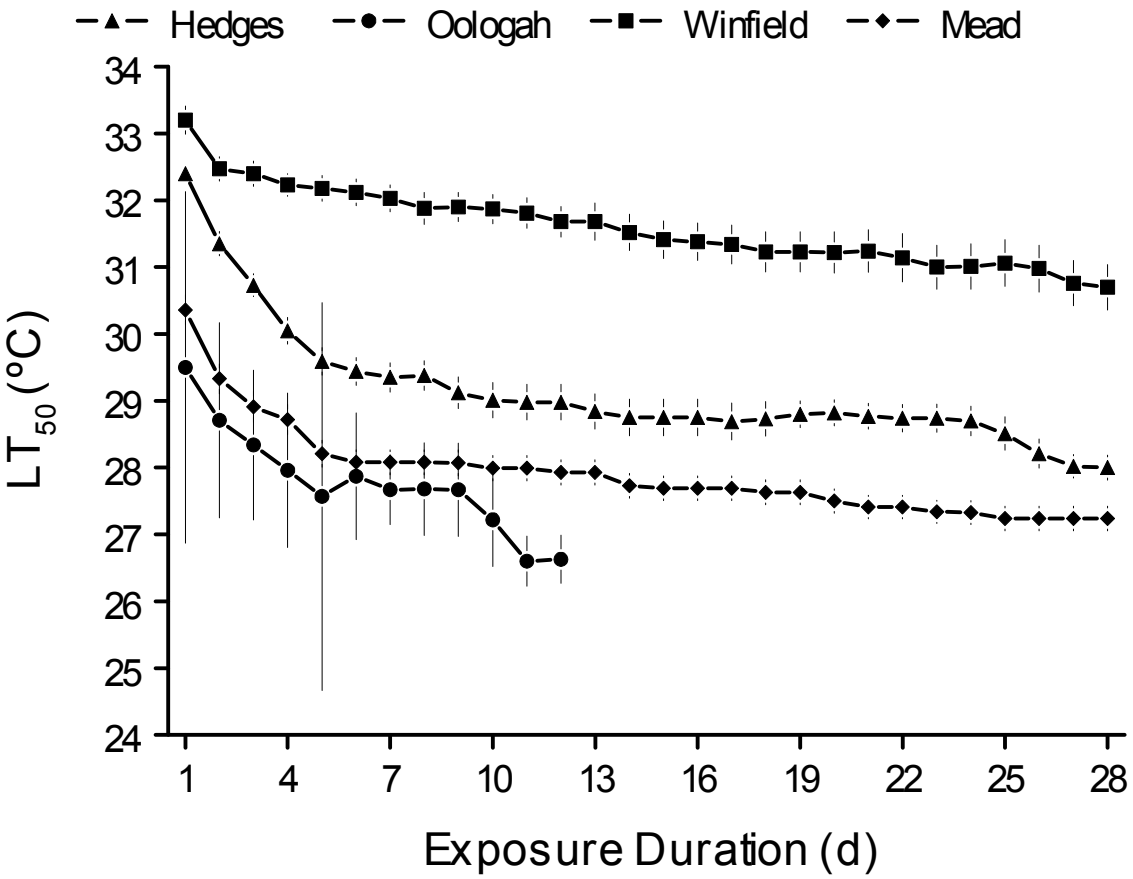

Figure A26. LT_{50} values (in °C; y-axis) with standard errors for 20°C acclimated, standard 15-mm SL individuals of *Dreissena polymorpha* from Hedges Lake (NY; triangles), Lake Oologah (OK; circles) and Winfield City Lake (KS; squares) and D. *rostriformis bugensis* from Lake Mead (NV/AZ; diamonds).

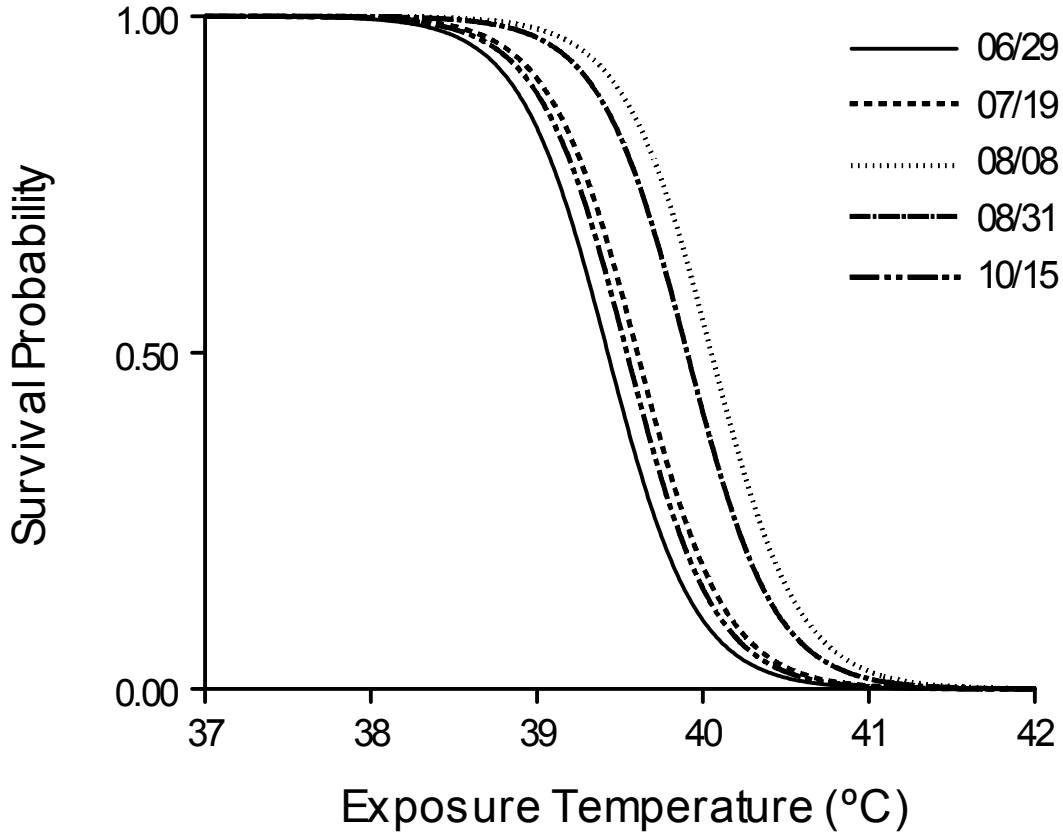

Figure A27. Acute thermal survival probability curves for standard length 15-mm specimens of *Dreissena polymorpha* from Winfield City Lake (KS) collected on 29 June (thin solid line), 19 July (dashed line), 8 August (dotted line), 31 August (single dot-dashed line) and 15 October 2008 (double dot-dashed line) experiencing a 0.2°C min^{-1} increase in temperature from 20°C to 50°C.

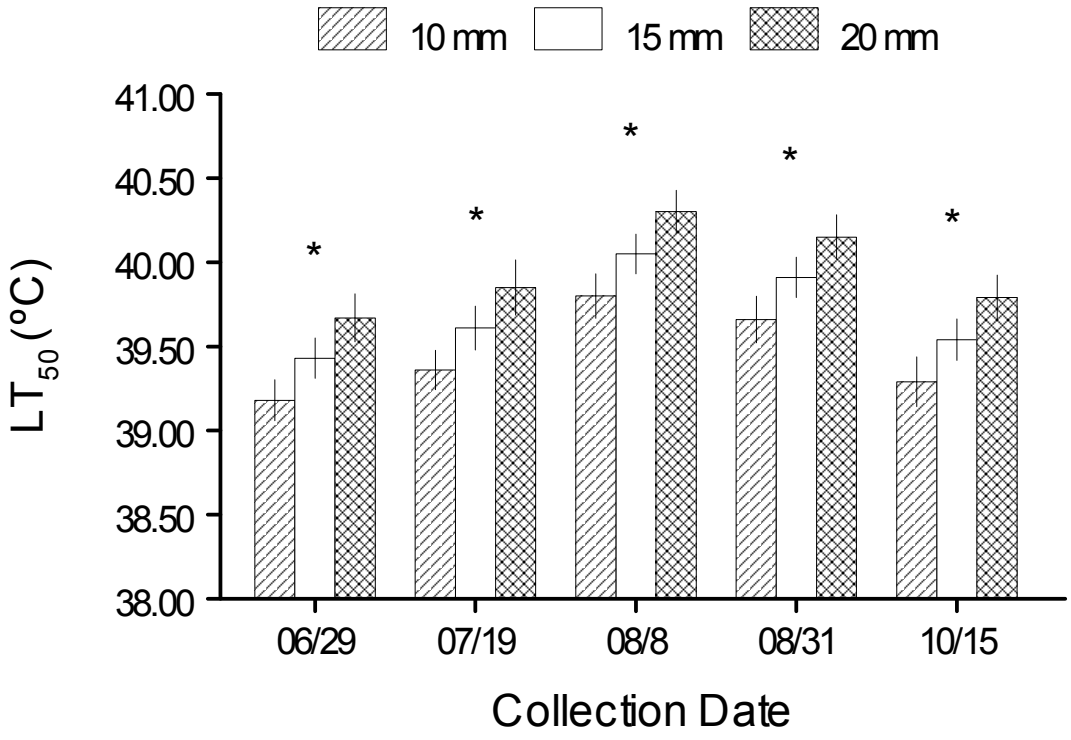

Figure A28. LT_{50} values (in °C; y-axis) for standard length specimens of *Dreissena polymorpha* collected from Winfield City Lake (KS) throughout the summer and exposed to acute thermal stress. Diagonally striped bars, open bars, and hatched bars represent standard 10, 15, and 20-mm SL individuals, respectively. Error bars represent 95% confidence intervals. Asterisks represent significance of the hypothesis that the LT_{50} values are equal for all three size cohorts as determined by a Wald statistic (* $P <$ 0.05).

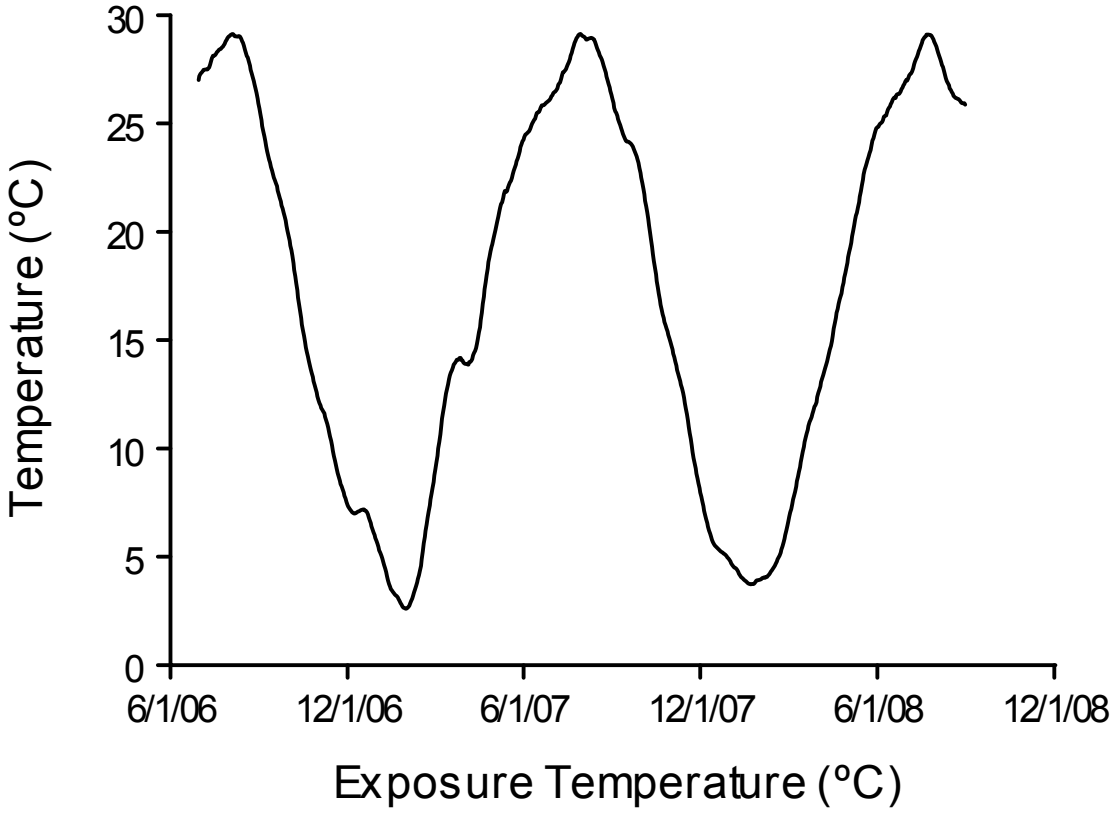

Figure A29. Average daily ambient water temperatures of Lake Oologah (OK) from summer 2006 through summer 2008 at a depth of 2 m.

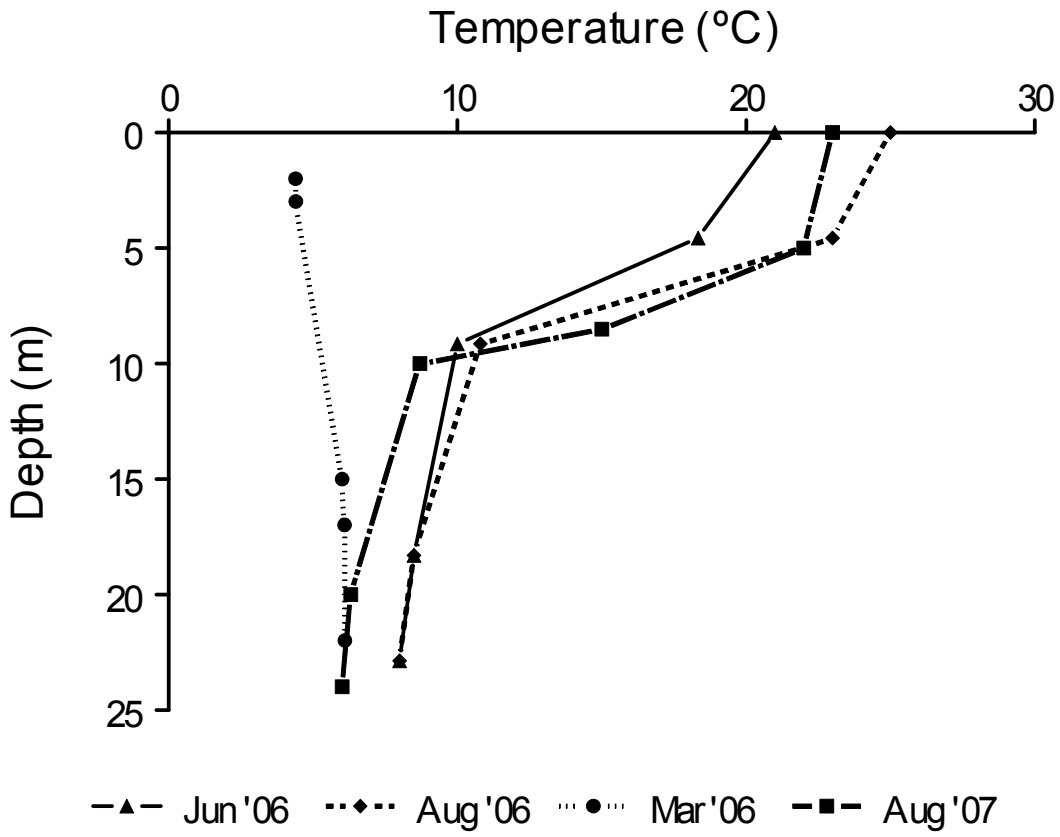

Figure A30. Ambient water temperature (in °C; x-axis) profiles at various depths (in m; y-axis) for Hedges Lake (NY) taken during mid-June 2006 (triangles, solid line), mid-August 2006 (diamonds, dashed line), mid-March 2006 (circles, dotted line) and mid-August 2007 (squares, single dot-dashed line). Data courtesy of Steve Butz of the Cambridge (NY) School District.

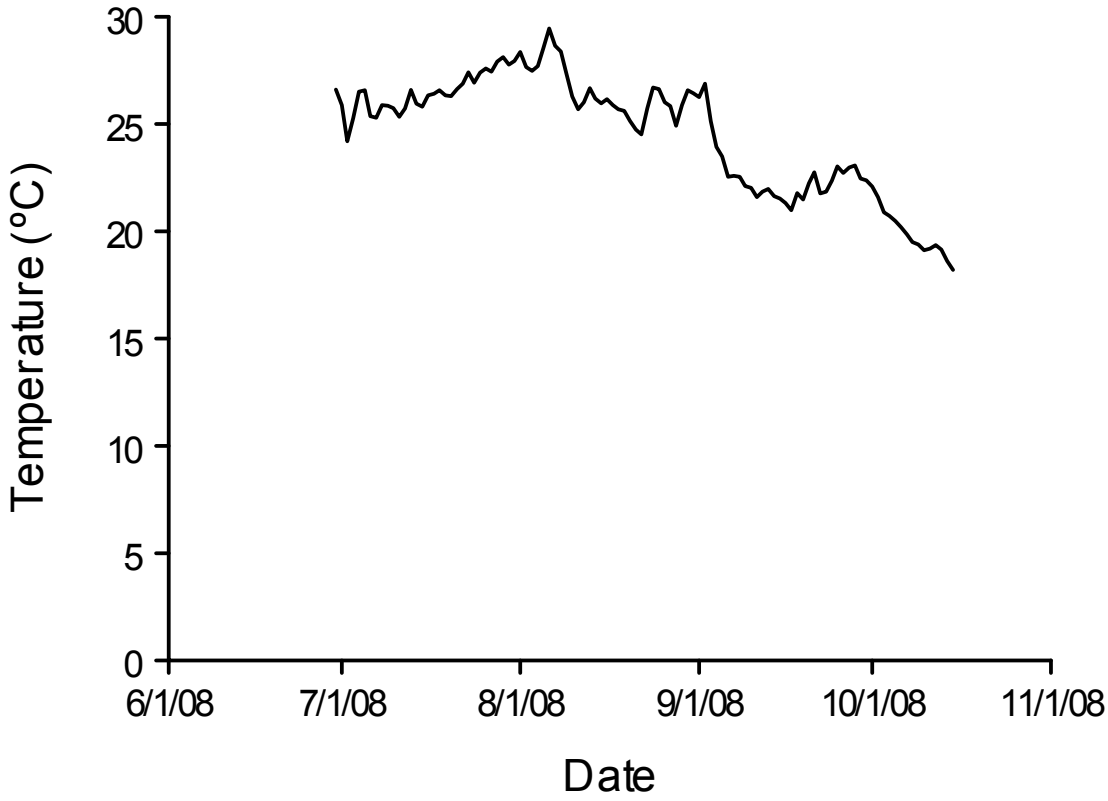

Figure A31. Average daily ambient water temperatures (in °C; y-axis) for Winfield City Lake (KS) through the summer of 2008 at a depth of 1 m.

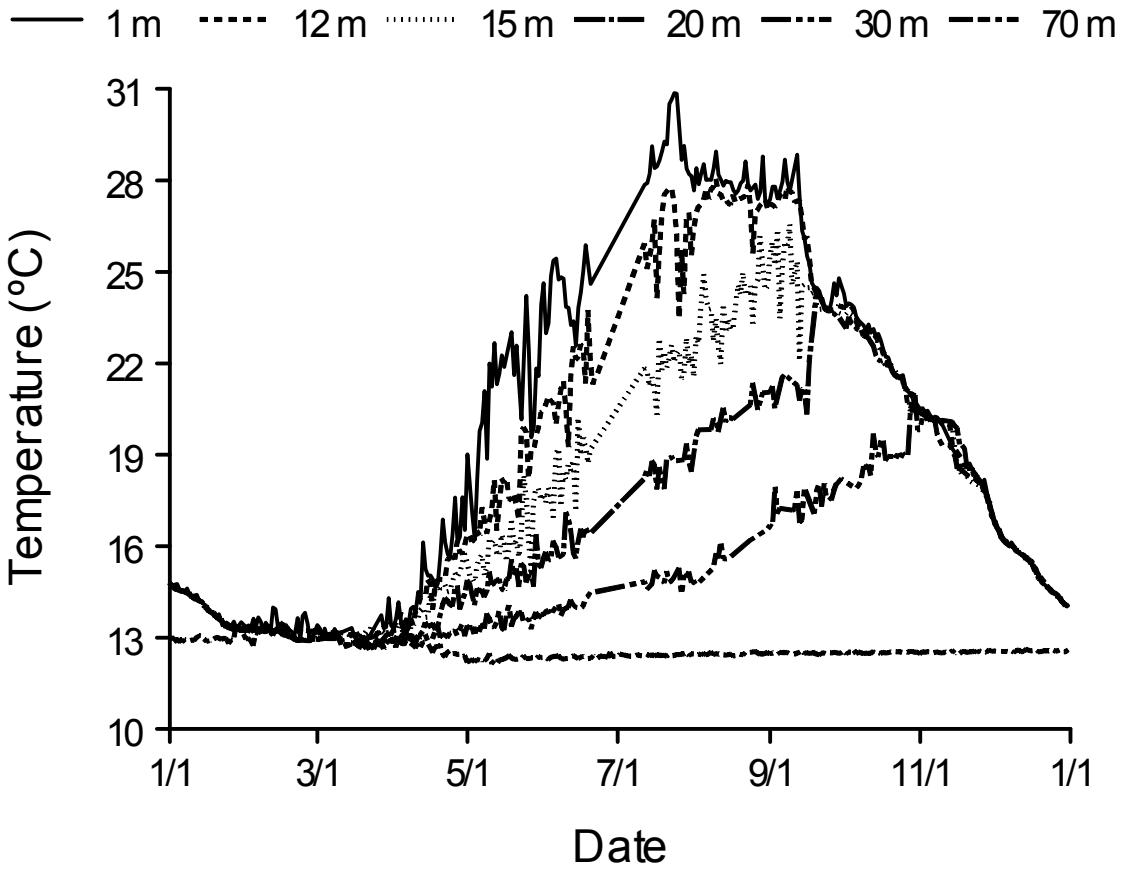

Figure A32. Average daily ambient water temperatures (in °C; y-axis) for Lake Mead (NV/AZ) at various depths during 2006. (Data from the USGS station at Sentinel Island's Ronald Veley).

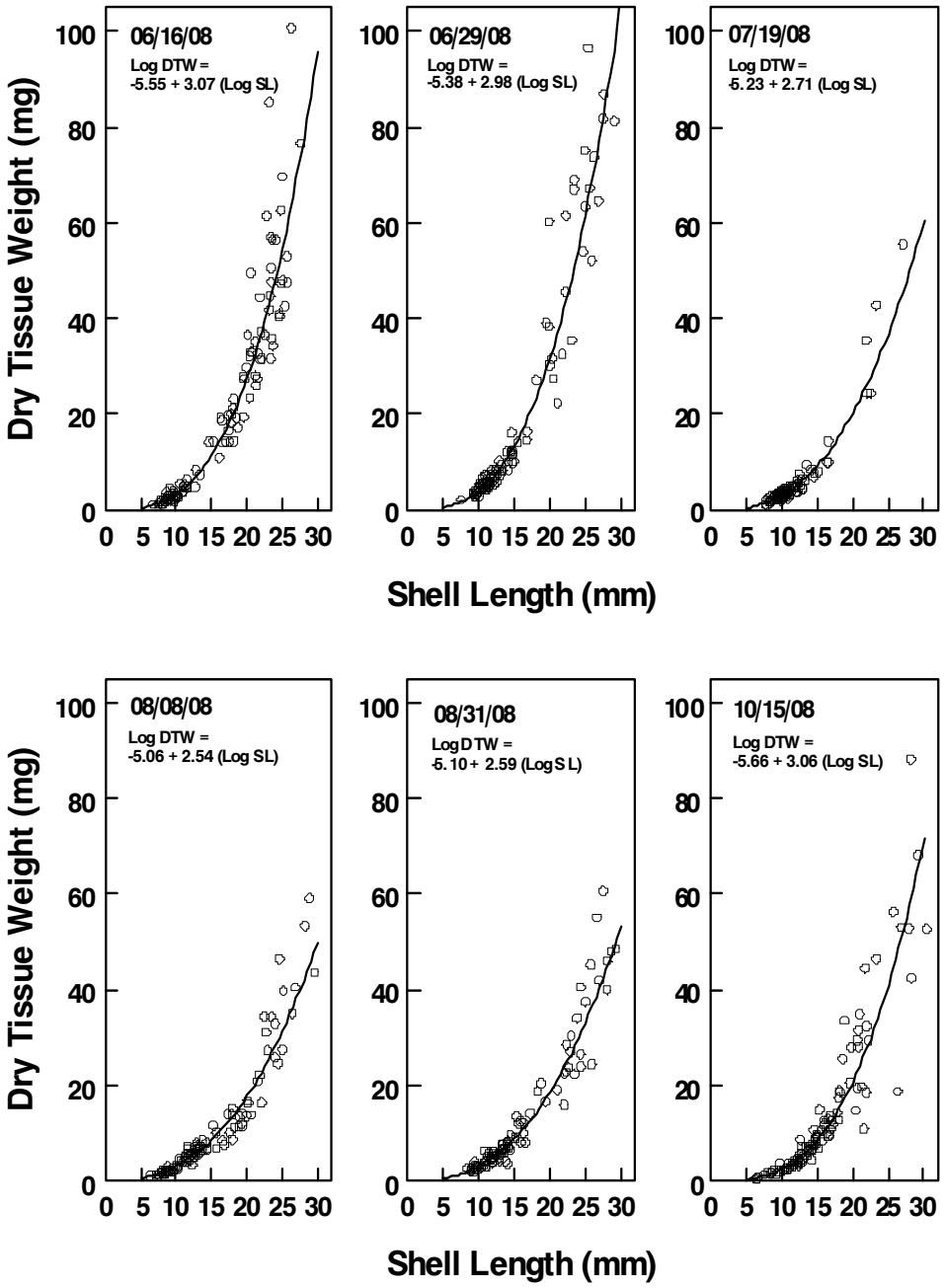

Figure A33. The relationship between the shell length (x axis) and dry tissue weight (y axis) in specimens of zebra mussels (*Dreissena polymorpha*) collected from Winfield City Lake (KS) during the summer and early fall of 2008. The date of collection is indicated in each panel. Open circles are individual DTW values and the solid line represents the fit of a least square linear regression relating the $\log_{10}$ of SL as the independent variable to the $\log_{10}$ of DTW as the dependent variable. All regressions were significant at $P < 0.00001$.

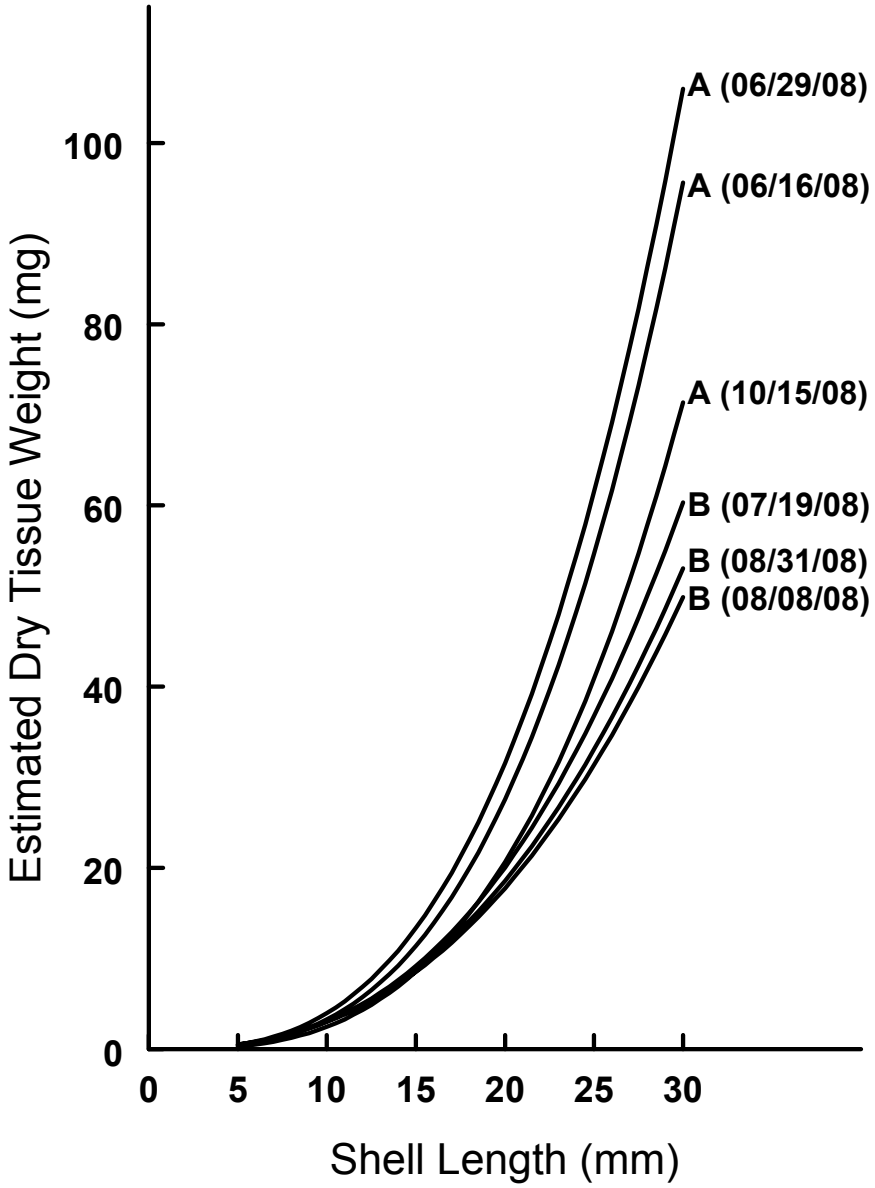

Figure A34. Best fit least square linear regressions relating logarithmic transformations of dry tissue weight (y axis, dependent variable) to the logarithmic transformation of shell length (x axis, independent variable) for six samples (n =100) of zebra mussels (*Dreissena polymorpha*) taken from Winfield City Lake (KS) during the summer and fall of 2008. Sampling dates are indicated for each regression line. Regression lines with similar letters have insignificantly different ($P < 0.05$) slope values.

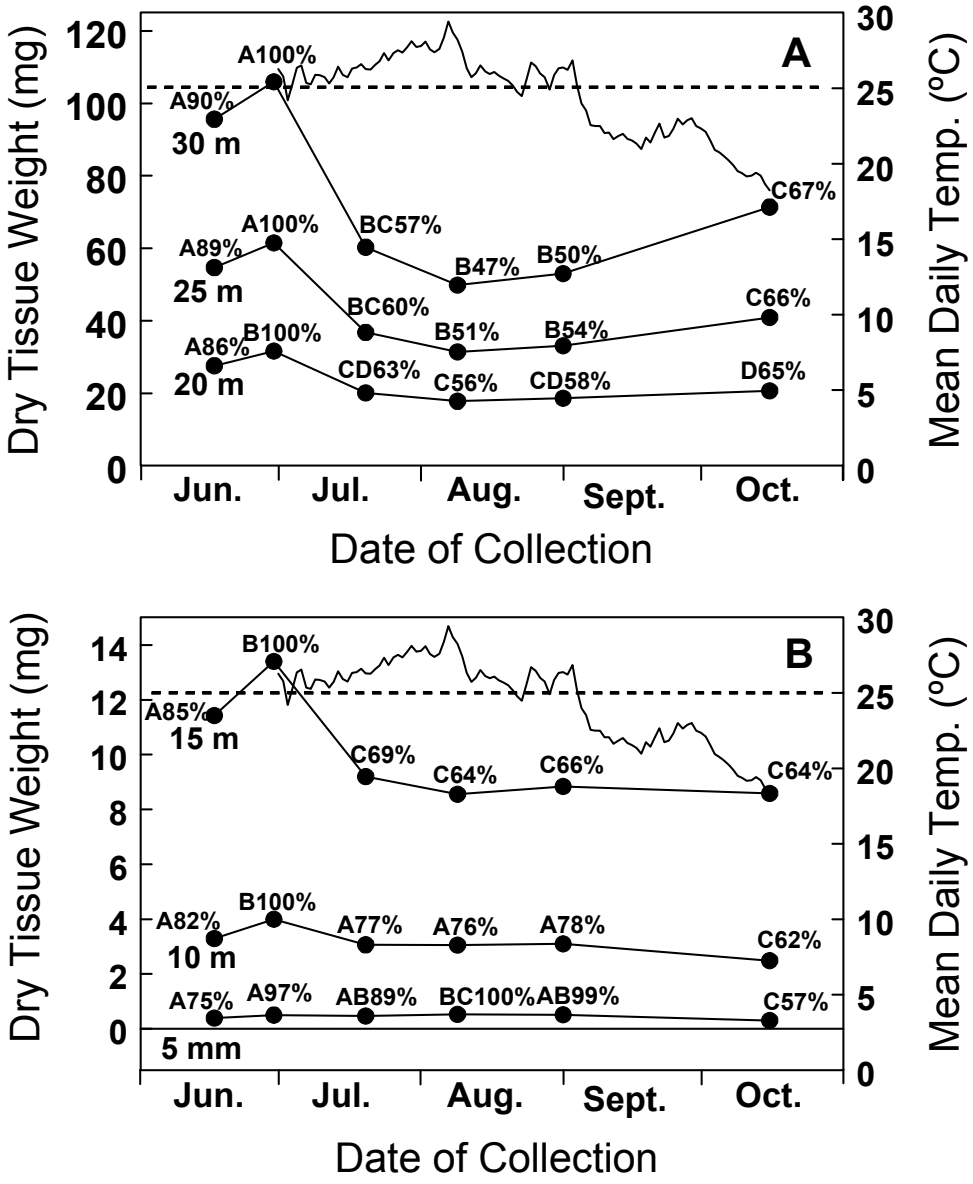

Figure A35. The impact of date of collection (x-axis) and mean daily ambient water temperature averaged across depths of 1, 2, and 4 m (right y-axis, line without points) on the dry tissue weight (DTW, left y-axis) of standard shell length specimens of zebra mussels (*Dreissena polymorpha*) from Winfield City Lake (KS). A) Individuals with standard shell lengths of 20, 25 and 30 mm. B) Individuals with standard shell lengths of 5, 10 and 15 mm. Similar letters above points indicate no significant difference ($P > 0.05$) between values. Percent values indicate the percent of the greatest DTW (100%) recorded. Dashed line indicates 25°C ambient temperature above which mussels are in negative energy balance.

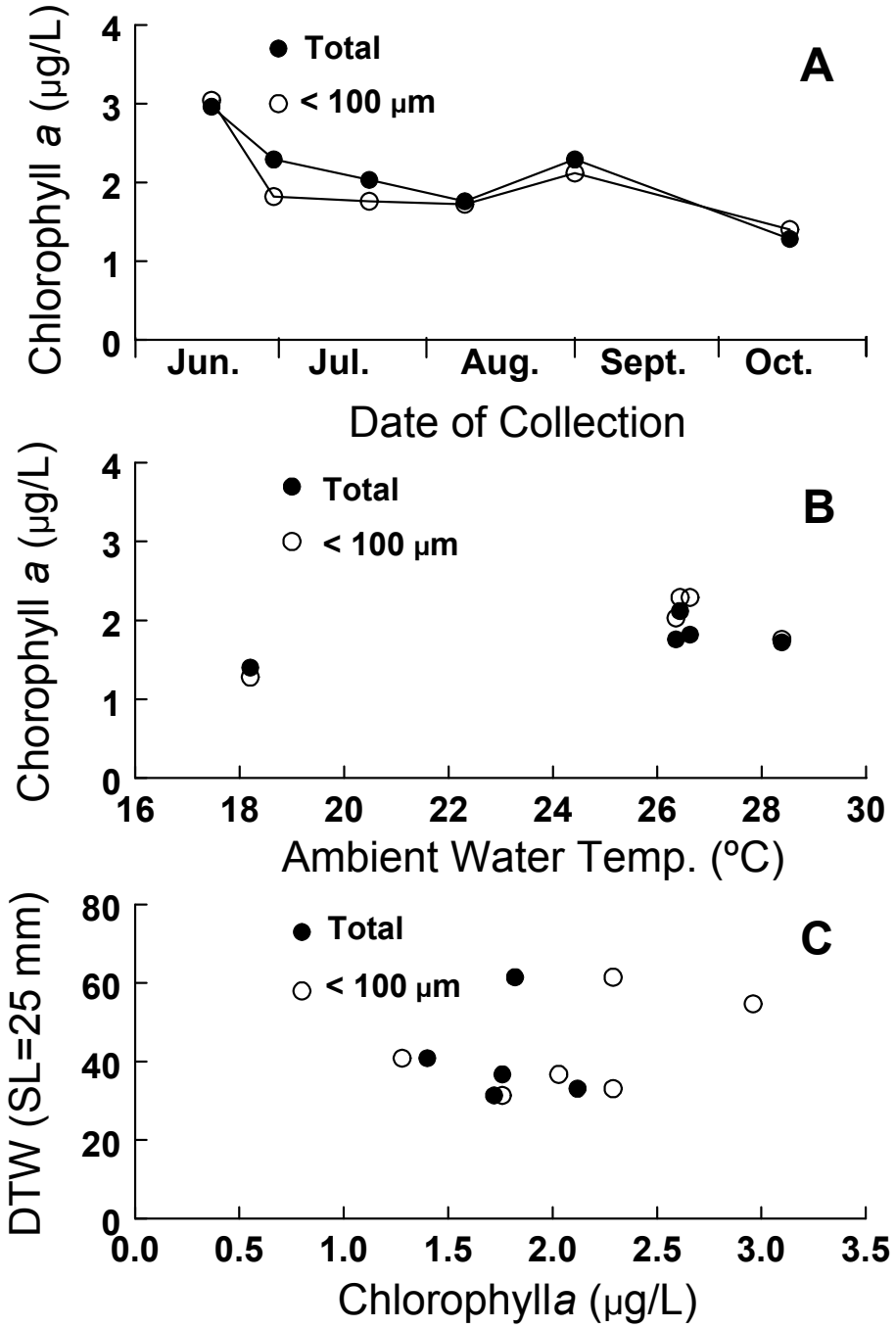

Figure A36. Chlorophyll *a* concentration in Winfield City Lake (KS) relative to date of collection (A), ambient water temperature (B) and the estimated mean dry tissue weight (DTW) of a zebra mussel (*Dreissena polymorpha*) with a standard shell length (SL) of 25 mm (C). In each graph, total Chlorophyll *a* concentration data are depicted as solid circles and when the sample was filtered through a 100 µm filter, as open circles.

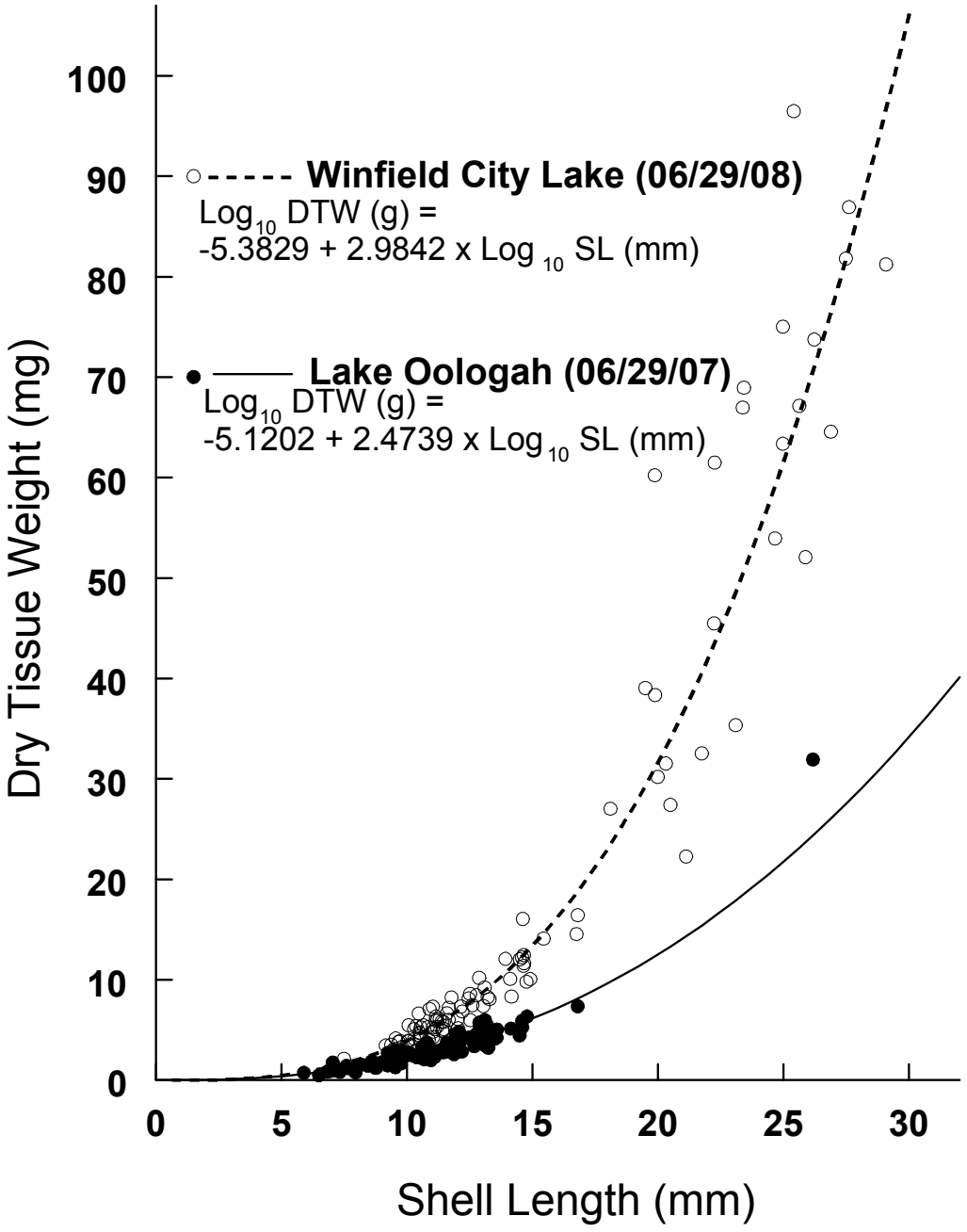

Figure A37. Comparison of the dry tissue weights (DTW) relative to shell length (SL) of specimens of *Dreissena polymorpha* collected on 29 June 2008 from Winfield City Lake (KS) (open circles), to that of specimens collected on 29 June 2007 from Lake Oologah (OK) (solid circles). The dashed line represents the best fit of a least square linear regression relating $\log_{10}$ DTW as the dependent variable to $\log_{10}$ SL for the Winfield City Lake (KS) collection and the solid line, for the Lake Oologah (OK) collection. Regression parameters are indicated on the figure.

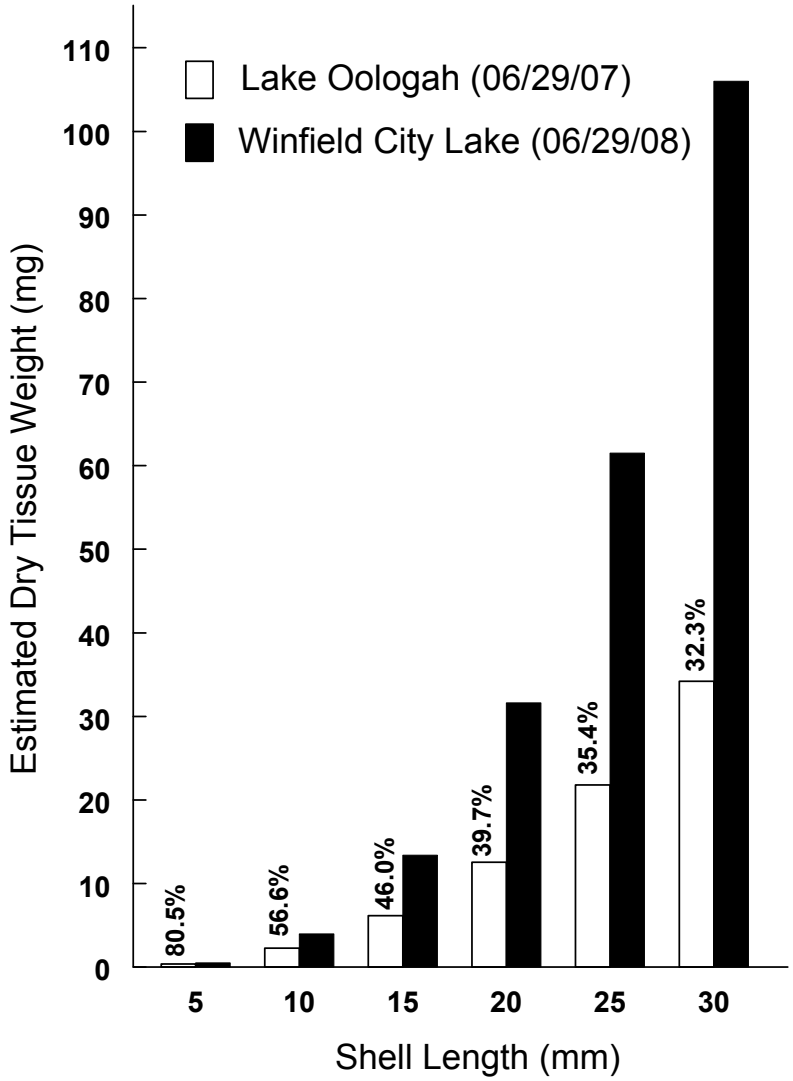

Figure A38. Comparison of the dry tissue weights (DTW, vertical axis) of standard sized individuals of *Dreissena polymorpha* (shell length [SL] = 5, 10, 15, 20, 25 or 30 mm, horizontal axis) as estimated from least square linear regressions relating $\log_{10}$ DTW as the dependent variable to $\log_{10}$ SL as the independent variable for collections taken from Winfield City Lake (KS) (solid bars), on 29 June 2008 and Lake Oologah (OK) (open bars), on 29 June 2007. Percentage values above open bars express the DTW of standard sized specimens from Lake Oologah (OK) as a percent of the DTW of same sized specimens from Winfield City Lake (KS).

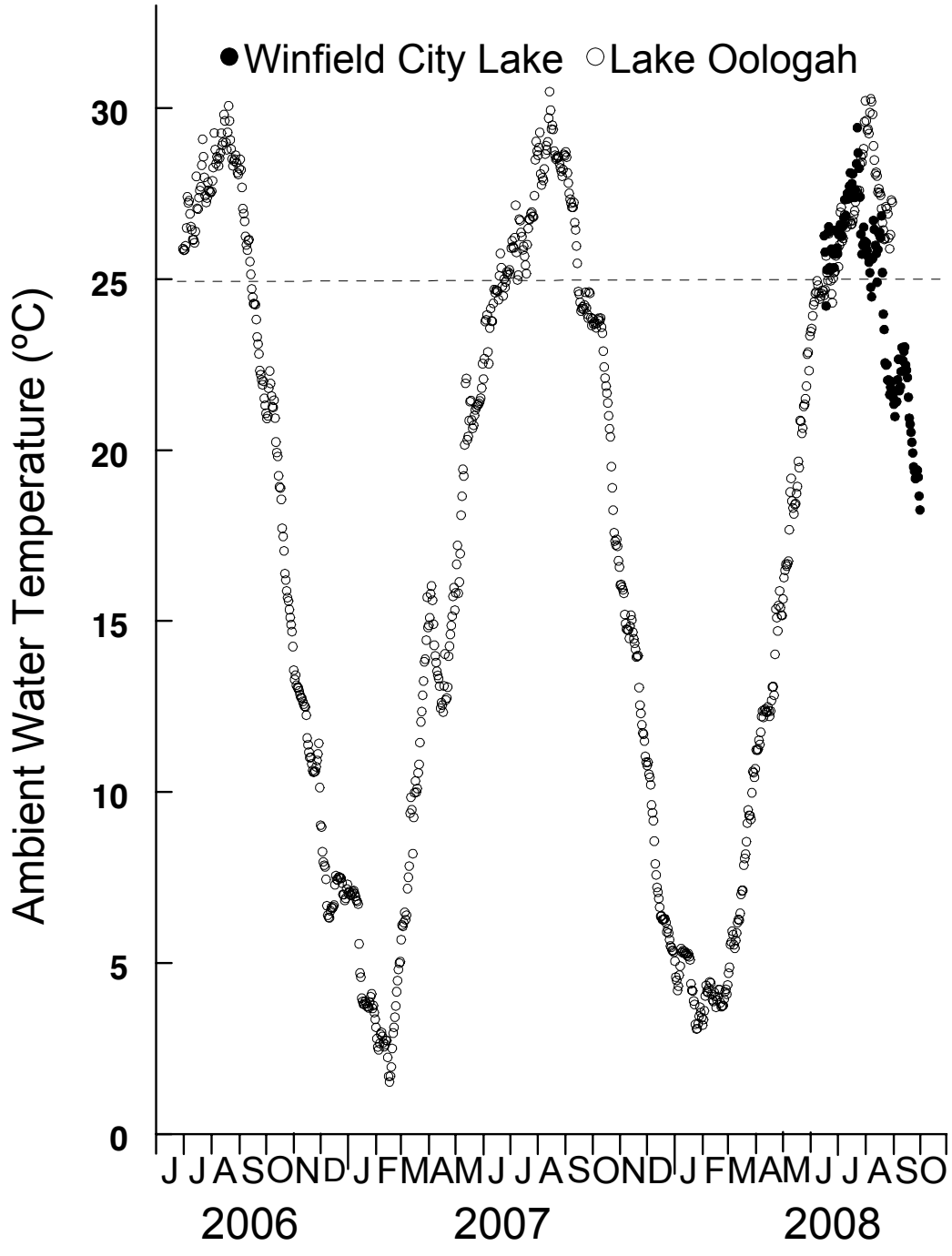

Figure A39. Effect of season on mean daily ambient water temperatures averaged across depths for Lake Oologah (OK) (open circles, averaged for depths of 1, 2, 4 and 8 m) and Winfield City Lake (KS) (solid circles, averaged for depths of 1, 2 and 4 m). The dashed horizontal line indicates an ambient water temperature of 25°C.

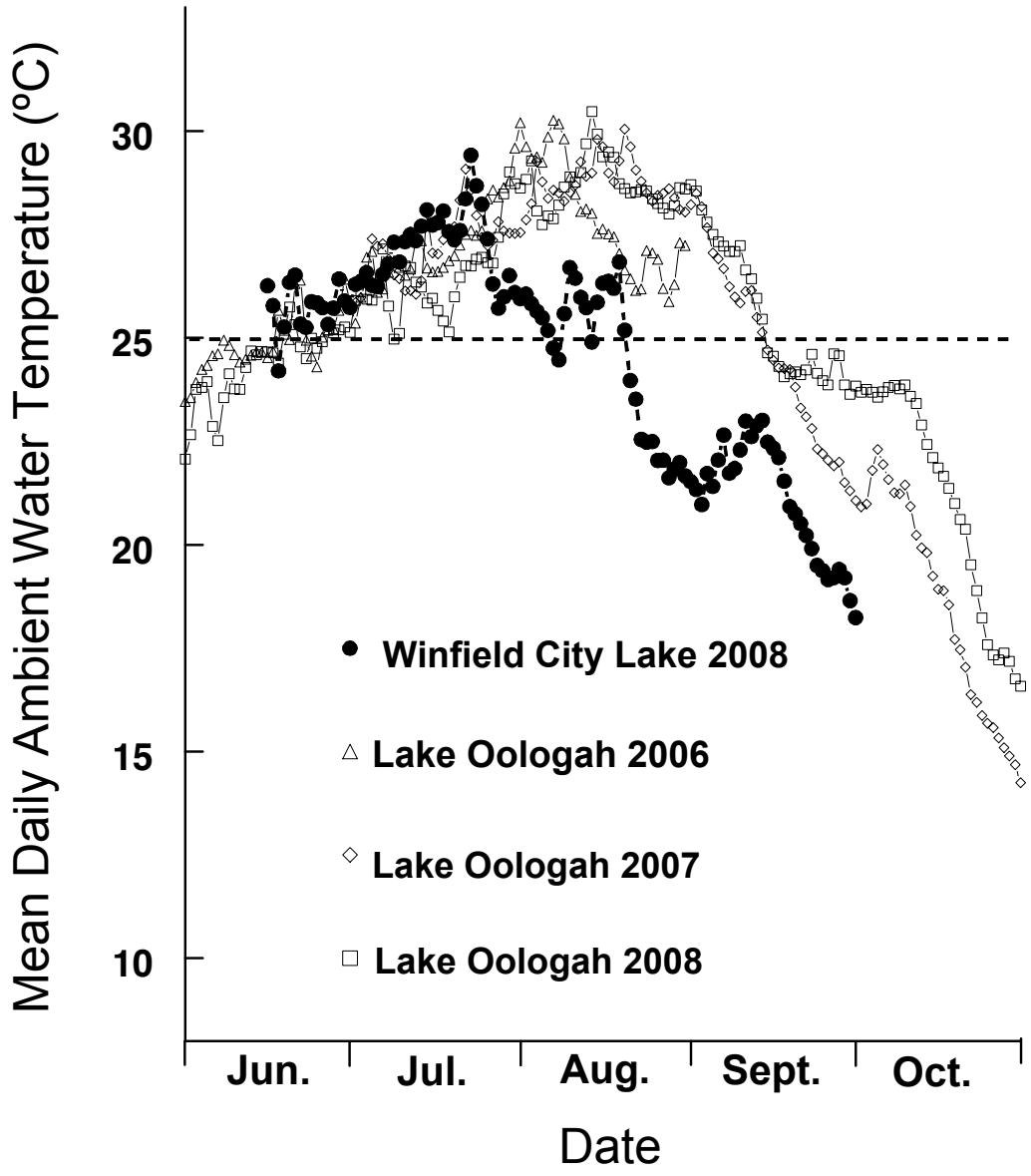

Figure A40. Mean daily ambient water temperatures (in °C; y-axis) during summer and early fall for Lake Oologah (OK) averaged across depths of 1, 2, 4 and 8 m during 2006 (open squares), 2007 (open diamonds) and 2008 (open triangles) and Winfield City Lake (KS) averaged across depths of 1, 2 and 4 m during 2008 (solid circles). The dashed horizontal line indicates an ambient water temperature of 25°C.

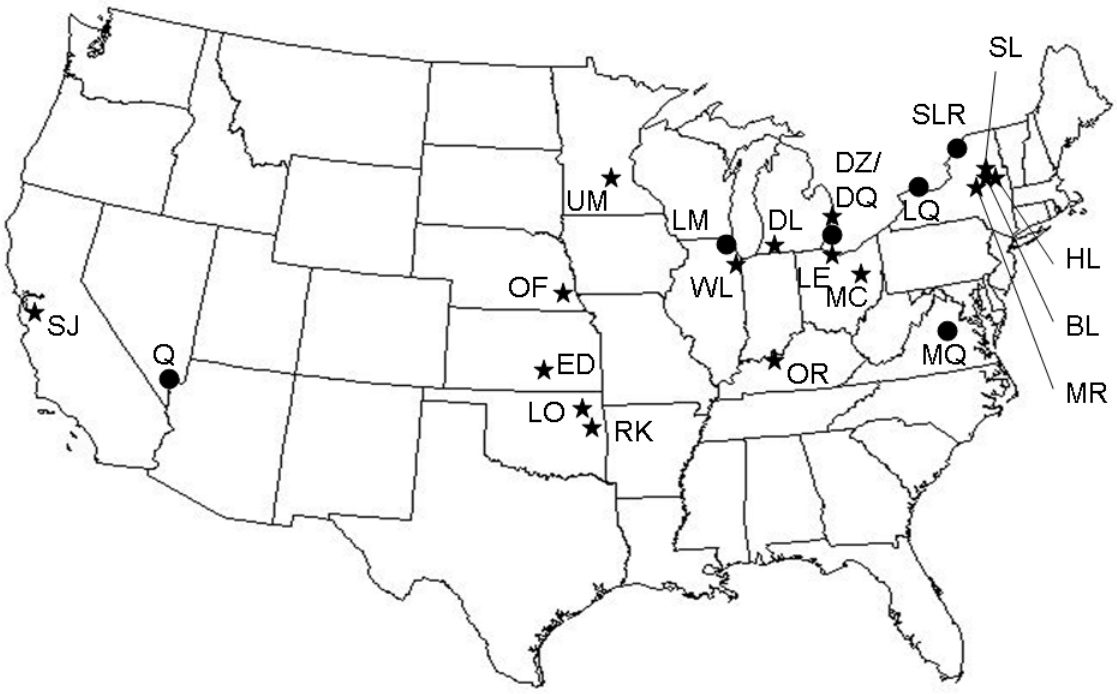

Figure A41. Map of the United States with stars indicating collections sites for AFLP analysis. *Dreissena polymorpha* collection sites (solid stars): OF = Offutt AFB, UM = Upper Mississippi River, SJ = San Justo Reservoir, HL = Hedges Lake, LO = Lake Oologah, MR = Mohawk River, BL = Ballston Lake, SL = Saratoga Lake, DL = Diamond Lake, ED = El Dorado Lake, WL = Wolf Lake, DZ = Detroit River, RK = Robert S. Kerr Reservoir, OR = Ohio River, LE = Lake Erie, MC = Mosquito Creek Lake. *D. Rostriformis bugensis* collection sites (solid circles): SLR = St. Lawrence River, LQ = Lake Ontario, MQ = Millbrook Quarry, LM = Lake Michigan, DQ = Detroit River, Q = Lake Mead.

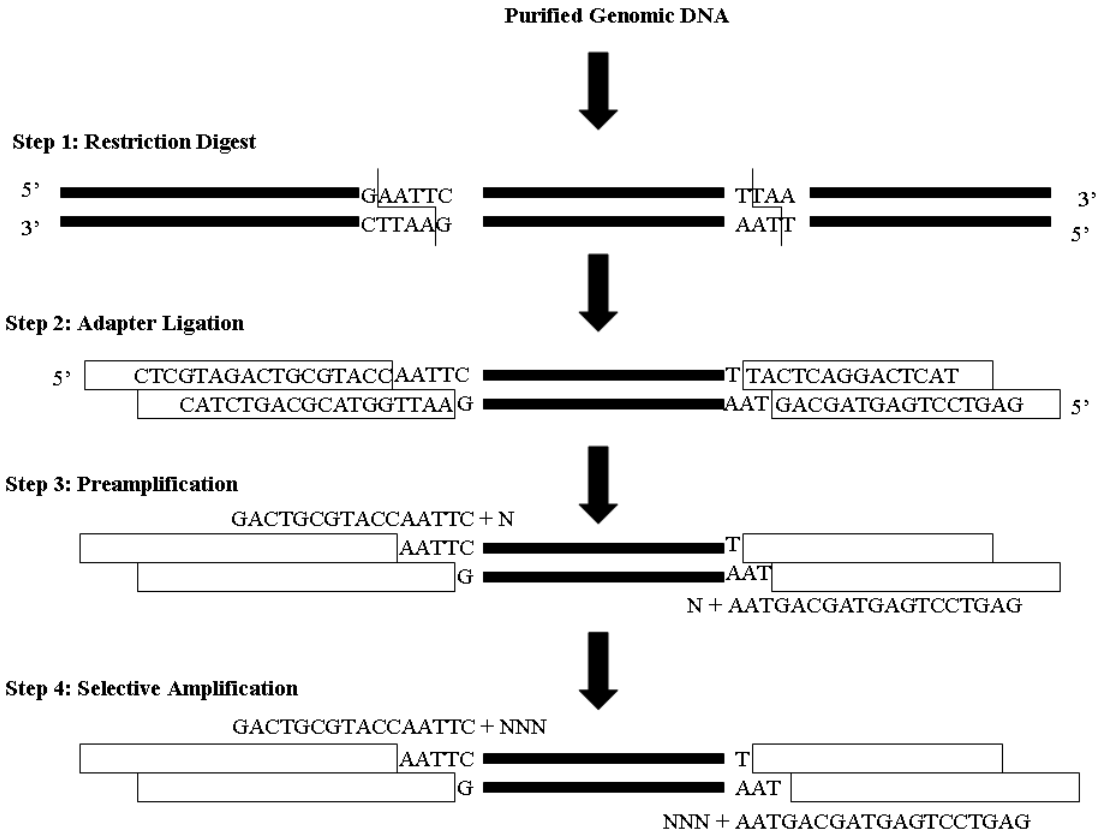

Figure A42. Summary of the AFLP process utilized to genetically analyze specimens of *Dreissena polymorpha* and *D. rostriformis bugensis*. See Sections 4.2.2–4.2.6 for details of the AFLP methodology utilized.

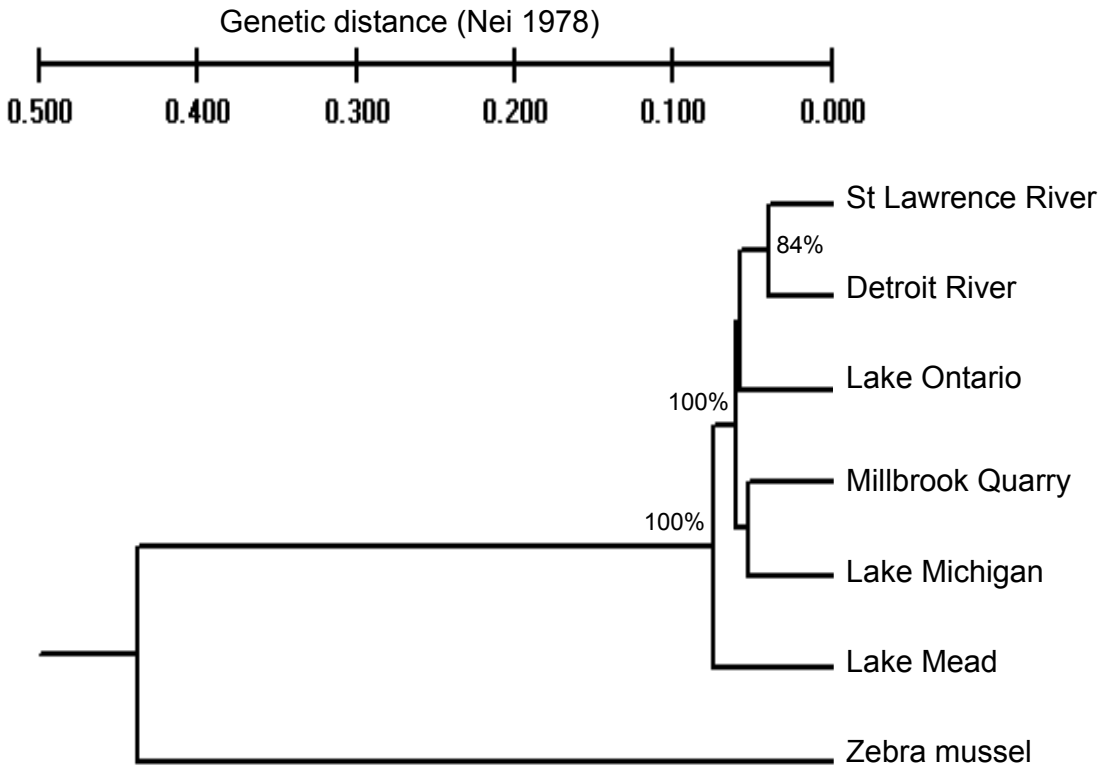

Figure A43. Cluster analysis tree of North American *Dreissena rostriformis bugensis* populations including a *D. polymorpha* population from Lake Oologah (OK). Node support greater than 70% from bootstrapping (iterations = 5000) is indicated on the tree.

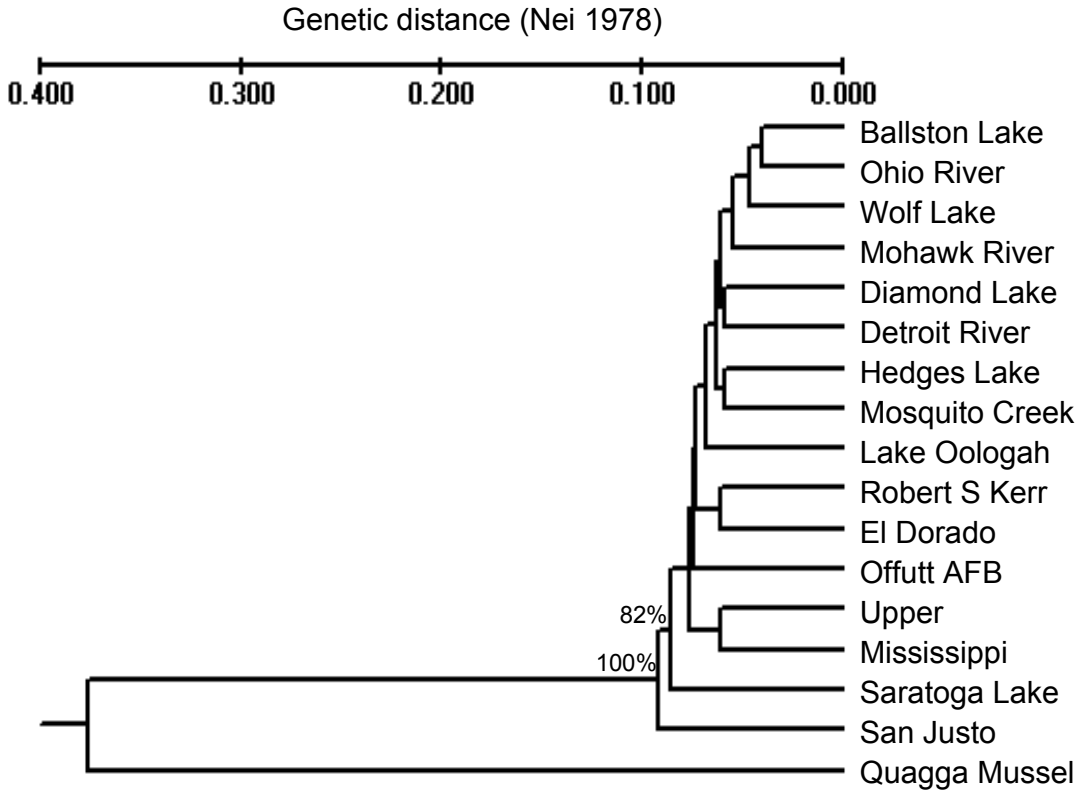

Figure A44. Cluster analysis tree of North American *Dreissena polymorpha* populations including a *D. rostriformis bugensis* population from Lake Mead (NV/AZ). Node support greater than 70% from bootstrapping (iterations = 5000) is indicated on the tree.

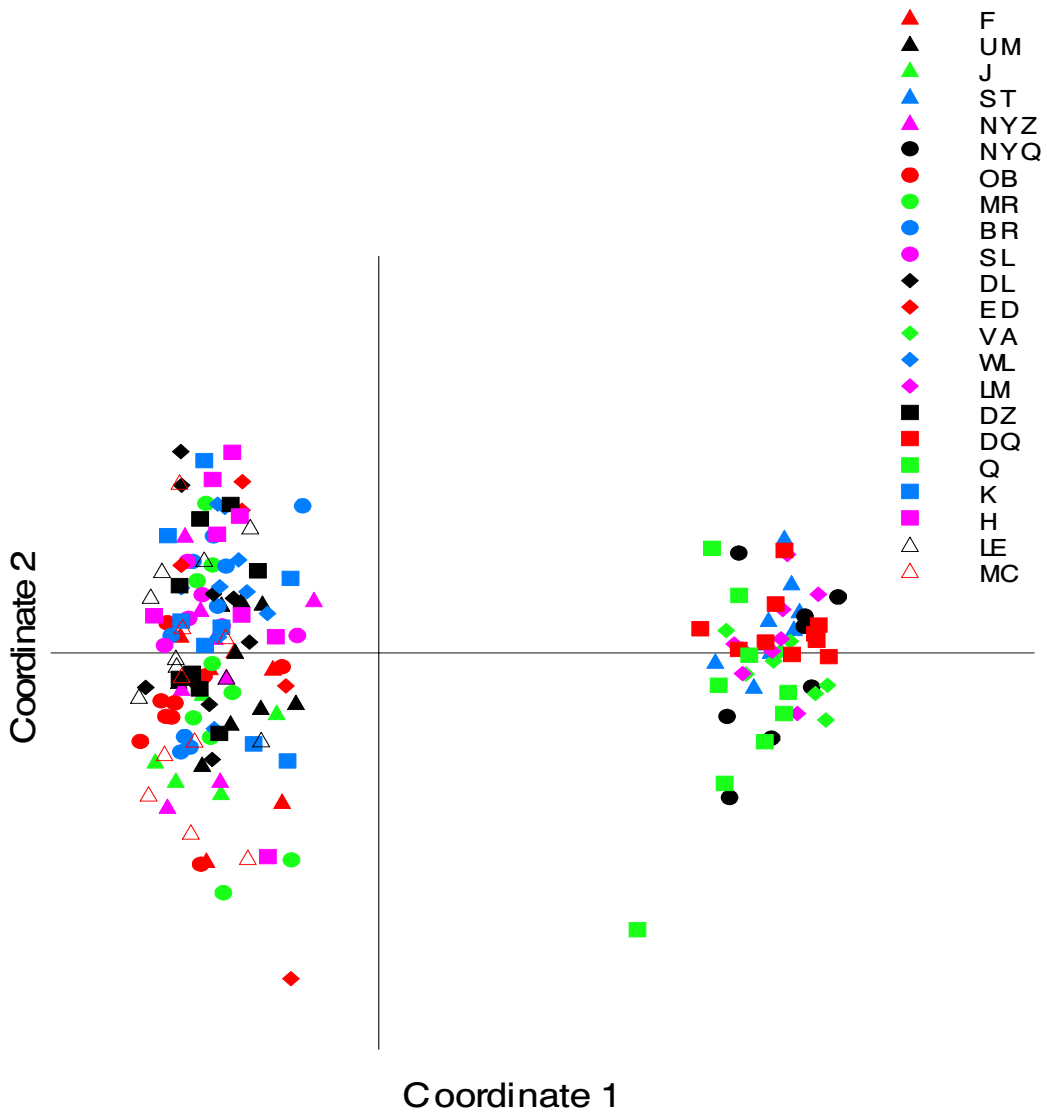

Figure A45. Principle coordinate analysis of AFLP data containing 16 populations of *Dreissena polymorpha* and six populations of *D. rostriformis bugensis* from North America. The two species differentiated along coordinate axis 1, with all specimens of *D. polymorpha* aligning on the left, and all specimens of *D. rostriformis bugensis* aligning on the right. *D. polymorpha* populations: OF = Offutt AFB, UM = Upper Mississippi River, SJ = San Justo, HL = Hedges Lake, MR = Mohawk River, BL = Ballston Lake, SL = Saratoga Lake, DL = Diamond Lake, ED = El Dorado Lake, WL = Wolf Lake, DZ = Detroit River, RK = Robert S. Kerr Reservoir, OR = Ohio River, LE = Lake Erie, MC = Mosquito Creek Lake. *D. rostriformis bugensis* populations: SLR = St. Lawrence River, LQ = Lake Ontario, MQ = Millbrook Quarry, LM = Lake Michigan, DQ = Detroit River, Q = Lake Mead.

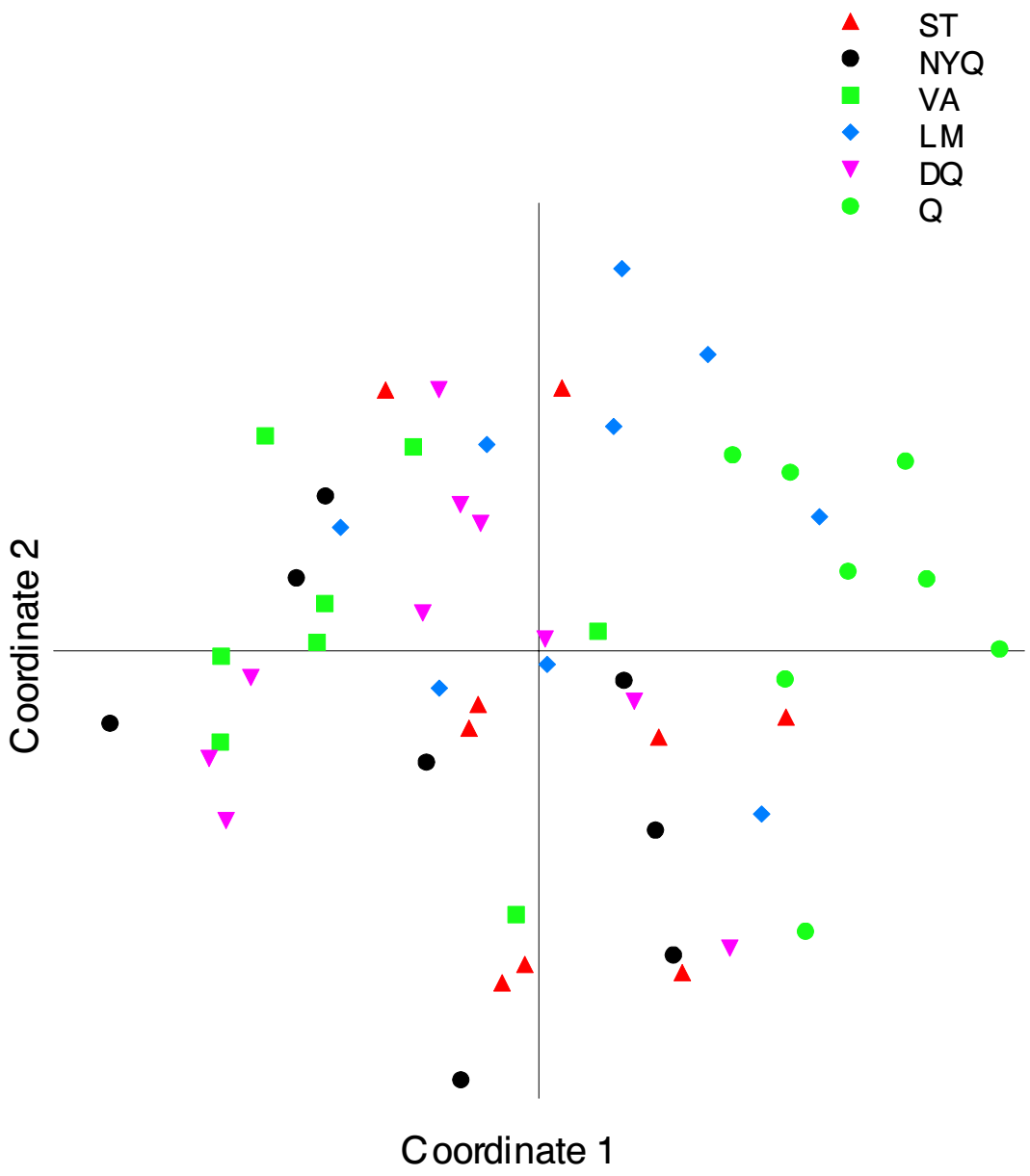

Figure A46. Principle coordinate analysis of AFLP data for 52 specimens sampled from six North American populations of *Dreissena rostriformis bugensis*. SLR = St. Lawrence River, LQ = Lake Ontario, MQ = Millbrook Quarry, LM = Lake Michigan, DQ = Detroit River, Q = Lake Mead.

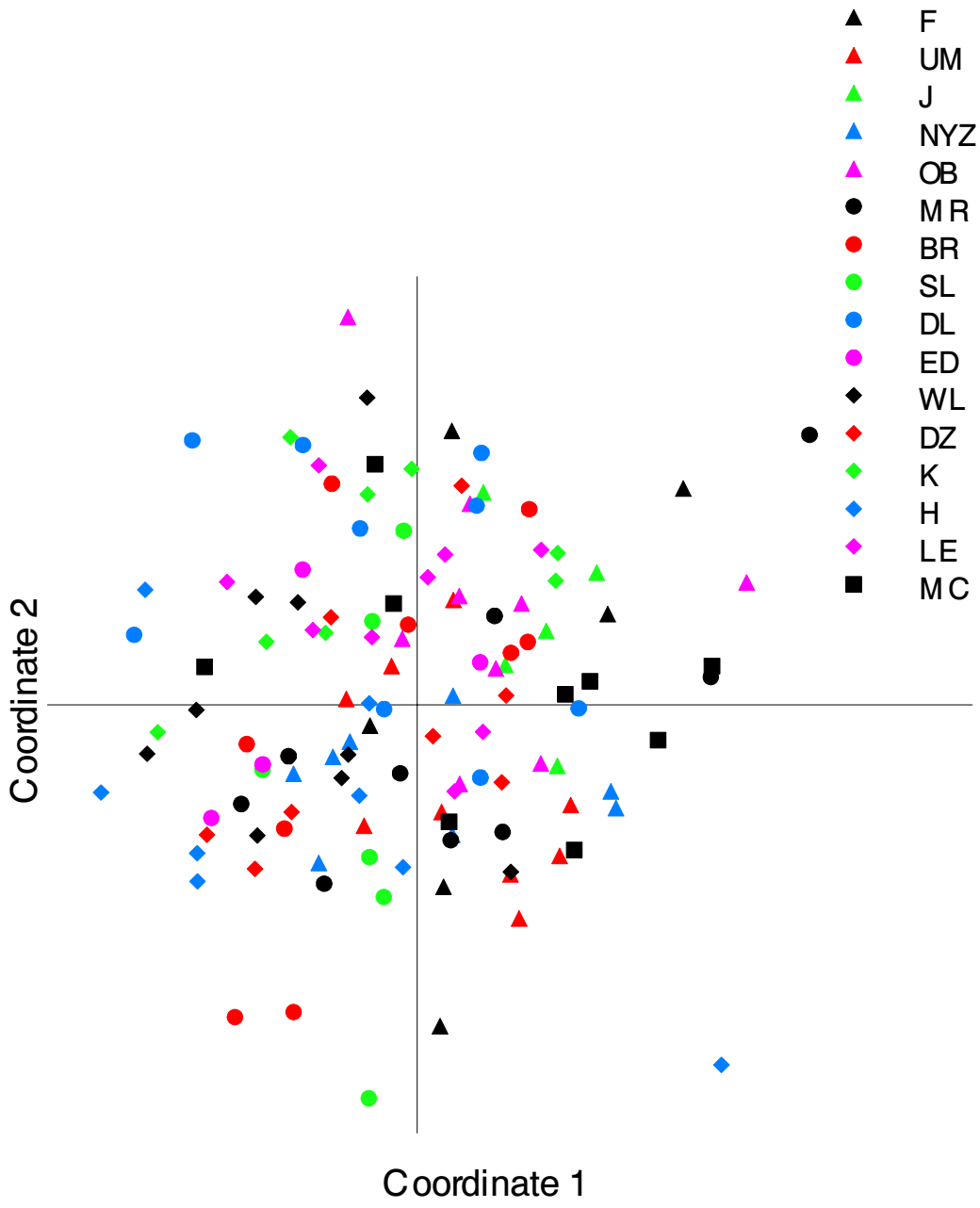

Figure A47. Principle coordinate analysis of AFLP data for 126 specimens from 16 North American populations of *Dreissena polymorpha*. OF = Offutt AFB, UM = Upper Mississippi River, SJ = San Justo, HL = Hedges Lake, MR = Mohawk River, BL = Ballston Lake, SL = Saratoga Lake, DL = Diamond Lake, ED = El Dorado Lake, WL = Wolf Lake, DZ = Detroit River, RK = Robert S. Kerr Reservoir, OR = Ohio River, LE = Lake Erie, MC = Mosquito Creek Lake.

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APPENDIX B

TABLES

Table B1. Summary of the 2006 chronic upper-thermal tolerance test for specimens of *Dreissena polymorpha* from Lake Oologah (OK) and Hedges Lake (NY). Mortality was assessed for 28 d.

Population	Acclimation Temperature (°C)	Test Temperatures (°C)	# Mussels Needed
New York	5	28, 29, 30, 31, 32, 33, 34	140
	10		140
	15		140
	20		140
	25		140
	30		140
			NY Total: 840
Oklahoma	5	28, 29, 30, 31, 32, 33, 34	140
	10		140
	15		140
	20		140
	25		140
	30		140
			OK Total: 840
			Grand Total: 1680

Table B2. Summary of the 2007 chronic upper-thermal tolerance test for specimens of *Dreissena polymorpha* from Lake Oologah (OK) and Hedges Lake (NY). Mortality was assessed for 28 d.

Population	Feeding Regime	Acclimation Temperature (°C)	Test Temperatures (°C)	# Mussels Needed
New York	Fed	20	26, 27, 28, 29, 30, 31, 32, 33, 34	180
		25		180
		30		180
	Starved	20		180
		25		180
		30		180
				NY Total: 1080
Oklahoma	Fed	20	26, 27, 28, 29, 30, 31, 32, 33, 34	180
		25		180
		30		180
	Starved	20		180
		25		180
		30		180
				OK Total: 1080
				Grand Total: 2160

Table B3. Summary of the 2008 chronic upper-thermal tolerance test for specimens of *Dreissena polymorpha* from Winfield City Lake (KS). Mortality was assessed for 28 d.

Population	**Acclimation Temperature (°C)**	**Test Temperatures (°C)**	**# Mussels Needed**
Winfield Early summer	20	26, 27, 28, 29, 30, 31, 32, 33, 34	405
Winfield Late Summer	20	26, 27, 28, 29, 30, 31, 32, 33, 34	405
			Total: 810

Table B4. Summary of the 2007 chronic upper-thermal tolerance test for specimens of *Dreissena rostriformis bugensis* from Lake Mead (NV/AZ). Mortality was assessed for 28 d.

Population	Acclimation Temperature (°C)	Test Temperatures (°C)	Feeding Regime	# Mussels Needed
Lake Mead	20°C	20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33	Starved	280
			Fed	280
				Grand Total: 560

Table B5. Pairwise Wald Chi-square tests of median survival time for a standard 15-mm SL Hedges Lake (NY) specimen of *Dreissena polymorpha* collected in 2006 at different acclimation temperatures and exposure temperatures. Values above the diagonal are *P*-values while values below the diagonal are Wald Chi-square statistics. *P*-values ≤ 0.0017 (in bold) were significant after Bonferroni adjustment.

28°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.6056	0.5412	0.7854	0.4683	0.0517
10	0.2667		0.9497	0.3822	0.2084	0.1240
15	0.3733	0.0040		0.3007	0.1540	0.1212
20	0.0741	0.7634	1.0711		0.6071	0.0121
25	0.5259	1.5822	2.0326	0.2644		0.0060
30	3.7852	2.3655	2.4022	6.2993	7.5422	

29°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0331	0.1134	0.0048	**<0.0001**	0.1354
10	4.5421		0.4365	0.4546	0.0061	0.4589
15	2.5058	0.6054		0.1535	**0.0012**	1.0000
20	7.9713	0.5592	2.0369		0.0259	0.1727
25	24.1552	7.5207	10.4580	0.9570		0.0019
30	2.2292	0.5487	0.0000	1.8589	0.5761	

30°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0221	**0.0002**	**<0.0001**	**<0.0001**	0.0775
10	5.2345		0.1569	0.0273	0.0551	0.5562
15	13.6895	2.0037		0.4079	0.5236	0.0691
20	20.6806	4.8701	0.6848		0.8996	0.0118
25	15.9974	3.6804	0.4068	0.0159		0.0241
30	3.1169	0.3463	3.3035	6.3473	5.0867	

31°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0145	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
10	5.9713		0.0689	**<0.0001**	**<0.0001**	**<0.0001**
15	36.2544	3.3070		0.0018	**<0.0001**	**0.0007**
20	41.0286	16.2333	9.7445		0.0027	0.7229
25	31.4063	24.5204	20.3326	9.0143		0.0341
30	39.6393	17.8220	11.4277	0.1258	4.4882	

Table B5 Continued.

32°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0191	0.0065	**<0.0001**	**<0.0001**	**<0.0001**
10	5.4901		0.7866	**<0.0001**	**<0.0001**	**<0.0001**
15	7.4072	0.0733		**<0.0001**	**<0.0001**	**<0.0001**
20	31.4224	21.0327	21.4991		**<0.0001**	**<0.0001**
25	74.2969	66.0244	66.0256	36.2000		0.0991
30	128.4808	116.8866	119.7325	41.6434	2.7193	

33°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		**0.0005**	1.0000	0.0059	**<0.0001**	**<0.0001**
10	12.2063		**<0.0001**	0.5872	**<0.0001**	**<0.0001**
15	0.0000	29.5858		**0.0001**	**<0.0001**	**<0.0001**
20	7.5711	0.2948	14.4458		**<0.0001**	**<0.0001**
25	155.7093	149.5270	247.1773	129.9444		**<0.0001**
30	131.3489	116.6567	136.0684	117.3198	58.3773	

34°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		**0.0006**	**0.0006**	**<0.0001**	**<0.0001**	**<0.0001**
10	11.8343		1.0000	0.1739	**<0.0001**	**<0.0001**
15	11.8343	0.0000		0.1739	**<0.0001**	**<0.0001**
20	26.6272	1.8491	1.8491		**<0.0001**	**<0.0001**
25	312.5000	59.9112	59.9112	36.2426		**<0.0001**
30	815.4586	213.7574	213.7574	166.4201	118.3432	

Table B6. Pairwise Wald Chi-square tests of median survival time for a standard 15-mm SL Lake Oologah (OK) specimen of *Dreissena polymorpha* collected in 2006 at different acclimation temperatures and treatment temperatures. Values above the diagonal are *P*-values while values below the diagonal are Wald Chi-square statistics. *P*-values ≤ 0.0017 (in bold) were significant after Bonferroni adjustment.

28°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.6047	0.0477	**<0.0001**	**<0.0001**	**<0.0001**
10	0.2679		0.1111	**0.0001**	**<0.0001**	**<0.0001**
15	3.9222	2.5383		0.0085	**<0.0001**	**<0.0001**
20	15.9478	14.3844	6.9330		0.0196	0.0197
25	32.1030	30.6373	21.2360	5.4465		0.9113
30	29.2138	27.6584	19.4158	5.4321	0.0124	

29°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0327	0.5423	0.1180	0.0115	**0.0010**
10	4.5618		0.0526	**<0.0001**	**<0.0001**	**<0.0001**
15	0.3714	3.7559		0.0136	**<0.0001**	**<0.0001**
20	2.4433	15.3694	6.0787		0.2867	0.1971
25	6.3927	35.6195	15.9399	1.1349		0.1913
30	10.7914	26.9280	16.9400	5.4372	1.7072	

30°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0179	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**
10	5.6042		0.3199	0.2380	0.1462	**<0.0001**
15	14.2733	0.9892		0.9245	0.7324	**0.0007**
20	17.2502	1.3922	0.0089		0.7734	**0.0004**
25	16.9357	2.1111	0.1169	0.0829		**0.0056**
30	34.8284	20.2667	11.5919	12.6622	7.6801	

31°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0179	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**
10	5.6042		0.3199	0.2380	0.1462	**<0.0001**
15	14.2733	0.9892		0.9245	0.7324	**0.0007**
20	17.2502	1.3922	0.0089		0.7734	**0.0004**
25	16.9357	2.1111	0.1169	0.0829		**0.0056**
30	34.8284	20.2667	11.5919	12.6622	7.6801	

Table B6 Continued.

32°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
10	25.8679		0.0504	**<0.0001**	**<0.0001**	**<0.0001**
15	49.5928	3.8266		**<0.0001**	**<0.0001**	**<0.0001**
20	73.0969	32.4875	24.9338		**<0.0001**	**<0.0001**
25	173.6111	124.5675	116.6132	25.9492		0.2708
30	84.7751	65.0827	59.6319	25.6268	1.2128	

33°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.0299	0.5872	**<0.0001**	**<0.0001**	**<0.0001**
10	4.7169		0.0414	**<0.0001**	**<0.0001**	**<0.0001**
15	0.2948	4.1605		**<0.0001**	**<0.0001**	**<0.0001**
20	57.7830	46.2278	78.1250		**<0.0001**	**<0.0001**
25	171.6567	159.0651	183.1946	98.4954		**<0.0001**
30	108.2111	100.8964	107.5108	81.1326	19.8962	

34°C Exposure Temperature

Acc. Temp.	5	10	15	20	25	30
5		0.3826	0.3826	**<0.0001**	**<0.0001**	**<0.0001**
10	0.7622		1.0000	**<0.0001**	**<0.0001**	**<0.0001**
15	0.7622	0.0000		**<0.0001**	**<0.0001**	**<0.0001**
20	61.1287	69.4404	69.4404		**<0.0001**	**<0.0001**
25	416.0503	641.0061	641.0061	62.4468		0.1739
30	473.3728	732.4695	732.4695	80.1094	1.8491	

Table B7. Pairwise Wald Chi-square tests of median survival time for a standard 15-mm SL Hedges Lake (NY) specimen of *Dreissena polymorpha* collected in 2007, at different acclimation temperatures, and exposed to temperatures ranging 26–34°C. Values above the diagonal are *P*-values, values below the diagonal are Wald Chi-square statistics. *P*-values ≤ 0.0083 (in bold) were significant after Bonferroni adjustment.

28°C Exposure Temperature

Acc. Temp.	20	25	30
20		0.1952	0.3214
25	1.6779		0.5449
30	0.9833	0.3665	

29°C Exposure Temperature

Acc. Temp.	20	25	30
20		0.0242	0.3065
25	5.0795		**0.0002**
30	1.0457	13.4544	

30°C Exposure Temperature

Acc. Temp.	20	25	30
20		1.0000	0.0555
25	0.0000		0.1789
30	3.6682	1.8059	

31°C Exposure Temperature

Acc. Temp.	20	25	30
20		0.7417	**0.0047**
25	0.10886		0.0200
30	8.0090	5.4074	

Table B7 Continued.

32°C Exposure Temperature			
Acc. Temp.	20	25	30
20		1.0000	**0.0058**
25	0.0000		**0.0058**
30	7.6182	7.6182	

33°C Exposure Temperature			
Acc. Temp.	20	25	30
20		0.0088	**<0.0001**
25	3.8628		**<0.0001**
30	59.3664	53.2339	

34°C Exposure Temperature			
Acc. Temp.	20	25	30
20		0.0494	**<0.0001**
25	3.8628		**<0.0001**
30	30.7574	18.6959	

Table B8. Pairwise Wald Chi-square tests of median survival time for a standard 15-mm SL Lake Oologah (OK) specimen of *Dreissena polymorpha* collected in 2007, at different acclimation temperatures, and exposed to temperatures ranging 26–34°C. Values above the diagonal are *P*-values, values below the diagonal are Wald Chi-square statistics. *P*-values ≤ 0.0083 (in bold) were significant after Bonferroni adjustment.

28°C Exposure Temperature

Acc. Temp.	20	25	30
20		0.1275	0.2301
25	2.3229		0.0111
30	1.4403	6.4483	

29°C Exposure Temperature

Acc. Temp.	20	25	30
20		0.5412	0.3346
25	0.3733		0.1248
30	0.9310	2.3559	

30°C Exposure Temperature

Acc. Temp.	20	25	30
20		0.0299	0.0109
25	4.7121		**0.0001**
30	6.4849	15.1192	

31°C Exposure Temperature

Acc. Temp.	20	25	30
20		1.0000	0.0571
25	0.0000		0.0438
30	3.6203	4.0629	

Table B8 Continued.

32°C Exposure Temperature			
Acc. Temp.	20	25	30
20		0.5327	0.0658
25	0.3892		0.2127
30	3.3841	1.5529	

33°C Exposure Temperature			
Acc. Temp.	20	25	30
20		0.0115	**0.0008**
25	6.3810		0.1433
30	11.2924	2.1425	

34°C Exposure Temperature			
Acc. Temp.	20	25	30
20		0.4073	**<0.0001**
25	0.6866		**<0.0001**
30	18.5208	18.2918	

Table B9. Pairwise Wald Chi-square analysis, by collection site, of differences between median survival times of standard 15-mm unfed individuals, acclimated to 20°C and exposed to different temperatures. WCL = *Dreissena polymorpha* from Winfield City Lake (KS) early summer 2008, LM = *D. rostriformis bugensis* from Lake Mead (NV/AZ) 2007, HL = *D. polymorpha* from Hedges Lake (NY) 2007, LO = *D. polymorpha* from Lake Oologah (OK) 2007. Values above the diagonal represent *P*-values while values below the diagonal represent the Wald Chi-square statistic. Bold *P*-values were significant ($P < 0.0017$) after Bonferroni correction. A "na" indicates that the median survival time for one of the collection sites was not available for comparison.

28°C Exposure

	WCL	LM	HL	LO
WCL		na	na	na
LM	na		0.0766	**<0.0001**
HL	na	3.135		**<0.0001**
LO	na	17.0446	42.4131	

29°C Exposure

	WCL	LM	HL	LO
WCL		na	na	na
LM	na		**0.0013**	0.2437
HL	na	10.3576		**0.0005**
LO	na	1.3591	11.8217	

30°C Exposure

	WCL	LM	HL	LO
WCL		na	na	na
LM	na		**<0.0001**	0.2812
HL	na	22.6959		**<0.0001**
LO	na	1.1612	26.2053	

31°C Exposure

	WCL	LM	HL	LO
WCL		**<0.0001**	**<0.0001**	**<0.0001**
LM	744.9306		**<0.0001**	0.6761
HL	328.9686	182.0715		**<0.0001**
LO	697.3441	0.1745	37.3692	

Table B9 Continued.

32°C Exposure

	WCL	LM	HL	LO
WCL		**<0.0001**	**<0.0001**	**<0.0001**
LM	103.4636		**<0.0001**	0.0249
HL	61.3579	165.9495		**<0.0001**
LO	81.6603	5.0342	16.172	

33°C Exposure

	WCL	LM	HL	LO
WCL		**<0.0001**	**<0.0001**	**<0.0001**
LM	222.8199		**<0.0001**	0.0124
HL	149.5674	200		0.0206
LO	130.7176	6.249	5.3637	

34°C Exposure

	WCL	LM	HL	LO
WCL		na	**0.0008**	**<0.0001**
LM			na	na
HL	11.1826	na		0.1028
LO	17.3213	na	2.6616	

Table B10. Analysis of main effects within the logistic model for specimens of *Dreissena polymorpha* from Winfield City Lake (KS) in response to acute thermal testing.

Effect	*df*	Wald Chi-square	*P*
Collection	4	16.1620	0.0029
Temp	1	90.6164	<0.0001
SL	1	14.5185	0.0001

Table B11. Parameter estimates for the Winfield City Lake (KS) *Dreissena polymorpha* acute thermal response logistic model.

Parameter	*df*	Parameter Estimate	Standard Error	Wald Chi-square	*P*
Intercept	1	-147.5	15.5084	90.5178	<0.0001
Coll 6/29/08	1	1.0595	0.4108	6.6529	0.0099
Coll 7/19/08	1	0.3815	0.4286	0.7921	0.3735
Coll 8/8/08	1	-1.3045	0.4175	9.7620	0.0018
Coll 8/31/08	1	-0.7557	0.4069	3.4492	0.0633
Temp	1	3.7868	0.3978	90.6164	<0.0001
SL	1	-0.1874	0.0492	14.5185	0.0001

Table B12. ANOVA and R^2 for the saturated least square linear regression model of the logarithmic transformation of shell length (SL) and collection date on the logarithmic transformation dry tissue weight for individuals of *D. polymorpha* collected from Winfield City Lake (KS) and Lake Oologah (OK).

Source	***df***	***SS***	***MS***	***F***	***P***
Model	13	147.3640	11.3357	1290.94	<0.0001
SL	1	93.3953	93.3953	10636.1	<0.0001
Collection Date	6	0.5682	0.0947	10.78	<0.0001
SL*Collection Date	6	0.8065	0.1378	15.69	<0.0001
Error	686	6.0238	0.0088		
Total	699	153.3878			

R^2= 0. 9607

Table B13. Pair-wise Bonferroni corrected ($P = 0.0024$) differences between least square regression slope shifters (i.e., SL*collection date interactions) by Winfield City Lake (KS) collection date. The Lake Oologah (OK) sample was collected on 29 June 2007. T-values are above the diagonal while P values are below. Significant differences after Bonferroni correction are in bold.

Collection	**6/16/08**	**6/29/08**	**7/19/08**	**8/08/08**	**8/31/08**	**10/15/08**	**Oologah**
6/16/08		-0.98	**-3.34**	**-6.72**	**-5.80**	-0.11	**-5.51**
6/29/08	0.3260		-2.42	**-5.11**	**-4.39**	0.80	**-4.48**
7/19/08	**0.0009**	0.0159		-1.57	-1.14	**3.08**	-1.82
8/08/08	**<0.0001**	**<0.0001**	0.1180		0.49	**6.02**	-0.64
8/31/08	**<0.0001**	**<0.0001**	0.2563	0.6224		**5.24**	-0.99
10/15/08	0.9092	0.4228	**0.0021**	**<0.0001**	**<0.0001**		**-5.16**
Oologah	**<0.0001**	**<0.0001**	0.0687	0.5240	0.3229	**<0.0001**	

Table B14. Pair-wise Tukey-Kramer adjusted Least Square Mean differences between model estimated dry tissue weights at the six different Winfield City Lake (KS) collection dates for standard length 20, 25, and 30-mm individuals. The Lake Oologah (OK) sample was collected on 29 June 2007. T-statistics are above the diagonals while *P*-values are below the diagonal. Bold values indicate significant differences.

20-mm individuals

Collection	6/16/08	6/29/08	7/19/08	8/08/08	8/31/08	10/15/08	Oologah
6/16/08		**-3.2950**	**4.9709**	**11.0428**	**10.0486**	7.4654	**11.6970**
6/29/08	**0.0178**		**6.7895**	**12.9908**	**12.1062**	9.8102	**13.1768**
7/19/08	**<0.0001**	**<0.0001**		1.8385	1.1731	-0.4488	**5.4998**
8/08/08	**<0.0001**	**<0.0001**	0.5224		-1.0411	**-3.5958**	**5.0371**
8/31/08	**<0.0001**	**<0.0001**	0.9041	0.9443		-2.5670	**5.6971**
10/15/08	**<0.0001**	**<0.0001**	0.9994	**0.0064**	0.1379		**7.2768**
Oologah	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

25-mm individuals

Collection	6/16/08	6/29/08	7/19/08	8/08/08	8/31/08	10/15/08	Oologah
6/16/08		-2.1241	**4.6731**	**10.5890**	**9.4949**	**5.5924**	**10.3620**
6/29/08	0.3396		**5.7449**	**11.2794**	**10.3204**	**6.8909**	**11.1294**
7/19/08	**<0.0001**	**<0.0001**		1.8115	1.1973	-1.2119	**4.6203**
8/08/08	**<0.0001**	**<0.0001**	0.5407		-0.9344	**-4.6860**	**3.9808**
8/31/08	**<0.0001**	**<0.0001**	0.8952	0.9669		**-3.7135**	**4.5550**
10/15/08	**<0.0001**	**<0.0001**	0.8896	**<0.0001**	**0.0041**		**6.8967**
Oologah	**<0.0001**	**<0.0001**	**<0.0001**	**0.0015**	**<0.0001**	**<0.0001**	

30-mm individuals

Collection	6/16/08	6/29/08	7/19/08	8/08/08	8/31/08	10/15/08	Oologah
6/16/08		-1.4987	**4.4826**	**10.0945**	**8.9661**	**4.5178**	**9.5980**
6/29/08	0.7457		**5.1801**	**10.2331**	**9.2597**	**5.3498**	**10.0188**
7/19/08	**0.0002**	**<0.0001**		1.7852	1.1999	-1.5840	**4.1550**
8/08/08	**<0.0001**	**<0.0001**	0.5585		-0.8626	**-5.1133**	**3.4149**
8/31/08	**<0.0001**	**<0.0001**	0.8942	0.9778		**-4.1689**	**3.9431**
10/15/08	**0.0001**	**<0.0001**	0.6927	**<0.0001**	**0.0007**		**6.6519**
Oologah	**<0.0001**	**<0.0001**	**0.0007**	**0.0120**	**0.0017**	**<0.0001**	

Table B15. Pair-wise Tukey-Kramer adjusted Least Square Mean differences between model estimated dry tissue weights at the six different Winfield City Lake (KS) collection dates for standard length 5, 10, and 15-mm individuals. The Lake Oologah (OK) sample was collected on 29 June 2007. T-statistics are above the diagonals while *P*-values are below the diagonal. Bold values indicate significant differences.

5-mm individuals

Collection	6/16/08	6/29/08	7/19/08	8/08/08	8/31/08	10/15/08	Oologah
6/16/08		-2.6728	-1.7411	**-3.2345**	-2.8468	2.8874	-0.3392
6/29/08	0.1068		0.7590	-0.3823	-0.2128	**5.2691**	2.1273
7/19/08	0.5885	0.9886		-1.1577	-0.9504	**4.2773**	1.3001
8/08/08	**0.0216**	0.9998	0.9095		0.1513	**5.9161**	2.5983
8/31/08	0.0679	1	0.9640	1		**5.3940**	2.2994
10/15/08	0.0608	**<0.0001**	**0.0004**	**<0.0001**	**<0.0001**		-2.9783
Oologah	0.9999	0.3377	0.8519	0.1280	0.2457	0.0471	

10-mm individuals

Collection	6/16/08	6/29/08	7/19/08	8/08/08	8/31/08	10/15/08	Oologah
6/16/08		**-4.4560**	1.8753	1.8322	1.3886	**6.2350**	**9.8090**
6/29/08	**0.0002**		**6.9848**	**6.5474**	**5.8007**	**10.6868**	**15.1971**
7/19/08	0.4975	**<0.0001**		0.1077	-0.2766	**5.1824**	**9.3501**
8/08/08	0.5267	**<0.0001**	1.0000		-0.3495	**4.7340**	**8.3022**
8/31/08	0.8080	**<0.0001**	1.0000	0.9999		**4.7914**	**8.0322**
10/15/08	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		2.3949
Oologah	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.2022	

15-mm individuals

Collection	6/16/08	6/29/08	7/19/08	8/08/08	8/31/08	10/15/08	Oologah
6/16/08		**-5.1434**	**5.2191**	**9.3407**	**8.3899**	**9.3570**	**14.0898**
6/29/08	**<0.0001**		**8.9950**	**14.2927**	**13.4040**	**14.3567**	**17.6026**
7/19/08	**<0.0001**	**<0.0001**		1.7390	0.9760	1.6788	**7.7142**
8/08/08	**<0.0001**	**<0.0001**	0.5899		-1.0351	-0.0962	**7.4553**
8/31/08	**<0.0001**	**<0.0001**	0.9590	0.9458		0.9504	**8.2239**
10/15/08	**<0.0001**	**<0.0001**	0.6305	1.0000	0.9640		**7.5674**
Oologah	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

Table B16. ANOVA table for least square linear regressions of Winfield City Lake (KS) ambient water temperature (averaged across depths of 1, 2 and 4 m) on total chlorophyll *a* and chlorophyll *a* after <100 µm filtration.

Total Chl. *a*					
Source	***df***	***SS***	***MS***	***F***	***P***
Regression Model	1	15.0041	15.0041	1.2261	0.3303
Error	4	48.9483	12.2371		
Total	5	63.9523			
<100 µm Chl. *a*					
Source	***df***	***SS***	***MS***	***F***	***P***
Regression Model	1	4.5358	4.5358	0.3054	0.6000
Error	4	59.4165	14.8541		
Total	5	63.9523			

Table B17. ANOVA table for least square linear regressions of Winfield City Lake (KS) total chlorophyll *a* and model-estimated dry tissue weight of standard length *D. polymorpha* individuals.

5-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.1922	0.1921	0.5441	0.5016
Error	4	1.4125	0.3531		
Total	5	1.6047			

10-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.7105	0.7105	3.1786	0.1492
Error	4	0.8941	0.2235		
Total	5	1.6046			

15-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.6369	0.6369	2.6328	0.1799
Error	4	0.9677	0.2419		
Total	5	1.6046			

20-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.4515	0.4515	1.5662	0.2789
Error	4	1.1531	0.2882		
Total	5	1.6046			

25-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.3944	0.3944	1.3039	0.3172
Error	4	1.2101	0.3025		
Total	5	1.6046			

30-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.3375	0.3375	1.0653	0.3603
Error	4	1.2671	0.3167		
Total	5	1.6046			

Table B18. ANOVA table for least square linear regressions of Winfield City Lake (KS) chlorophyll *a* concentration after <100 µm filtration and model-estimated dry tissue weight of standard length *D. polymorpha* individuals.

5-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.0097	0.0097	0.0241	0.8840
Error	4	1.6114	0.4028		
Total	5	1.6211			

10-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.2171	0.2171	0.6185	0.4755
Error	4	1.4040	0.3510		
Total	5	1.6211			

15-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.2922	0.2922	0.8795	0.4014
Error	4	1.3289	0.3322		
Total	5	1.6211			

20-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.2521	0.2521	0.7366	0.4391
Error	4	1.3690	0.3422		
Total	5	1.6211			

25-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.2492	0.2492	0.7267	0.4419
Error	4	1.3718	0.3429		
Total	5	1.6211			

30-mm individuals

Source	*df*	*SS*	*MS*	*F*	*P*
Regression Model	1	0.2320	0.2320	0.6681	0.4595
Error	4	1.3891	0.3472		
Total	5	1.6211			

Table B19. Collection sites for samples of *Dreissena polymorpha* and *D. rostriformis bugensis* utilized in an AFLP analysis. The state indicates the state from which samples were collected if the body of water spans across state boundaries. N/A indicates that the date of collection is unknown.

Location	State	Collection Date	*n*
Oologah Lake	OK	7/1/2006	9
El Dorado	KS	9/9/2005	5
Saratoga Lake	NY	9/16/2006	6
Hedges Lake	NY	7/1/2006	8
Ballston Lake	NY	10/1/2006	9
Wolf Lake	IL	8/16/2006	9
San Justo Reservoir	CA	N/A	5
Diamond Lake	MI	12/30/2006	9
Mosquito Creek Reservoir	OH	8/17/2006	9
Mohawk River	NY	8/16/2006	9
Offutt AFB	NE	10/02/2007	6
Lake Erie	OH	8/17/2006	9
Ohio River	OH	1/15/2008	8
Robert S. Kerr Reservoir	OK	8/20/2007	8
Mississippi River	MN	11/5/2007	9
St. Lawrence River*	NY	10/05/2007	9
Lake Ontario*	NY	2/1/2007	8
Lake Michigan*	MI	8/16/2006	9
Millbrook Quarry*	VA	1/27/2006	8
Detroit River**	MI	N/A	8/10*
Lake Mead*	NV/AZ	5/17/2007	8

* *Dreissena rostriformis bugensis*.

** *D. polymorpha* and *D. rostriformis bugensis*

Table B20. Fisher's Exact Tests (*df* = 322) of population differentiation between collections of *Dreissena rostriformis bugensis* and *D. polymorpha*. Values below the diagonal represent Chi-square statistics while the values above the diagonal represent the *P*-value testing significant differentiation between the populations. Bold values indicate significant differences. *D. rostriformis bugensis* populations: SLR = St. Lawrence River, LQ = Lake Ontario, MQ = Millbrook Quarry, LM = Lake Michigan, DQ = Detroit River, Q = Lake Mead. *D. polymorpha* population: ZM = *D. polymorpha* from Lake Oologah.

	SLR	LQ	MQ	LM	DQ	Q	ZM
SLR		1.0000	1.0000	1.0000	1.0000	0.9995	**<0.0001**
LO	187.23		1.0000	1.0000	1.0000	1.0000	**<0.0001**
MQ	199.74	109.21		1.0000	1.0000	1.0000	**<0.0001**
LM	137.28	210.39	171.86		1.0000	1.0000	**<0.0001**
DR	169.24	190.84	176.77	203.29		0.9830	**<0.0001**
Mead	244.96	166.26	155.57	177.55	270.52		**<0.0001**
ZM	**997.04**	**1073.65**	**1078.62**	**1005.69**	**1192.00**	**989.41**	

Table B21. Fisher's Exact Tests (*df* = 322) of population differentiation between collections of *Dreissena polymorpha* and *D. rostriformis bugensis*. Values below the diagonal represent Chi-square statistics while the values above the diagonal represent the *P*-value testing significant differentiation between the populations. Bold values indicate significant differences. *D. polymorpha* populations: OF = Offutt AFB, UM = Upper Mississippi River, SJ = San Justo Reservoir, HL = Hedges Lake, LO = Lake Oologah, MR = Mohawk River, BL = Ballston Lake, SL = Saratoga Lake, DL = Diamond Lake, ED = El Dorado Lake, WL = Wolf Lake, DZ = Detroit River, RK = Robert S. Kerr Reservoir, OR = Ohio River, LE = Lake Erie, MC = Mosquito Creek Lake. *Dreissena rostriformis bugensis* population: Q = Lake Mead.

	OF	UM	SJ	HL	LO	MR	BL	SL	DL	ED	WL	DZ	RK	OR	LE	MC	Q
OF		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**<0.0001**
UM	151.60		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.9998	1.00	1.00	1.00	**<0.0001**
SJ	114.10	176.66		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**<0.0001**
HL	143.13	200.05	151.98		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**<0.0001**
LO	168.93	181.55	171.37	214.32		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.9999	1.00	1.00	**<0.0001**
MR	142.20	144.35	161.85	205.26	140.82		1.00	0.9983	1.00	1.00	1.00	1.00	0.9982	1.00	1.00	1.00	**<0.0001**
BL	141.43	136.77	159.37	182.49	131.04	106.49		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**<0.0001**
SL	115.27	240.68	206.10	161.97	228.95	252.71	171.41		0.9999	1.00	0.9999	1.00	1.00	1.00	1.00	1.00	**<0.0001**
DL	190.22	170.94	183.94	219.69	141.01	144.24	126.45	233.15		1.00	1.00	1.00	1.00	1.00	1.00	1.00	**<0.0001**
ED	146.05	153.79	80.83	139.52	179.80	185.25	137.07	157.16	160.19		1.00	1.00	1.00	1.00	1.00	1.00	**<0.0001**
WL	192.60	205.28	187.41	224.23	166.32	160.41	116.19	233.25	143.88	179.34		1.00	1.00	1.00	1.00	1.00	**<0.0001**
DR	176.63	221.37	151.89	112.30	203.66	200.52	186.00	192.53	180.66	133.12	210.93		1.00	1.00	1.00	1.00	**<0.0001**
RK	151.37	239.72	161.11	136.50	205.95	253.19	183.54	183.62	193.15	139.24	207.30	134.45		1.00	1.00	1.00	**<0.0001**
OR	181.16	198.82	176.75	122.97	238.05	177.69	139.42	172.55	220.19	110.88	180.12	124.39	131.34		1.00	1.00	**<0.0001**
LE	174.80	161.35	187.38	228.75	126.33	194.14	118.67	193.95	141.92	157.37	180.79	222.20	186.78	209.33		1.00	**<0.0001**
MC	152.63	171.65	158.61	179.75	127.06	154.69	131.31	192.80	149.56	160.40	158.03	201.31	227.90	199.71	123.12		**<0.0001**
Q	**681.31**	**870.22**	**699.10**	**713.80**	**984.88**	**903.91**	**909.66**	**755.46**	**946.75**	**594.84**	**921.96**	**754.64**	**716.39**	**709.12**	**986.47**	**948.82**	

Table B22. Nei's (1978) unbiased genetic differences between *Dreissena polymorpha* and *D. rostriformis bugensis* populations. *D. polymorpha* populations: OF = Offutt AFB, UM = Upper Mississippi River, SJ = San Justo Reservoir, HL = Hedges Lake, LO = Lake Oologah, MR = Mohawk River, BL = Ballston Lake, SL = Saratoga Lake, DL = Diamond Lake, ED = El Dorado Lake, WL = Wolf Lake, DZ = Detroit River, RK = Robert S. Kerr Reservoir, OR = Ohio River, LE = Lake Erie, MC = Mosquito Creek Lake. *D. rostriformis bugensis* populations: SLR = St. Lawrence River, LQ = Lake Ontario, MQ = Millbrook Quarry, LM = Lake Michigan, DQ = Detroit River, Q = Lake Mead.

	OF	UM	SJ	HL	LO	MR	BL	SL	DL	ED	WL	DZ	RK	OR	LE	MC	SLR	LQ	MQ	LM	DQ
OF																					
UM	0.039																				
SJ	0.058	0.071																			
HL	0.041	0.039	0.054																		
LO	0.056	0.065	0.066	0.050																	
MR	0.034	0.047	0.064	0.042	0.049																
BL	0.040	0.045	0.063	0.041	0.037	0.029															
SL	0.074	0.080	0.082	0.066	0.068	0.073	0.041														
DL	0.062	0.064	0.075	0.051	0.039	0.044	0.037	0.082													
ED	0.062	0.057	0.063	0.044	0.060	0.056	0.039	0.061	0.053												
WL	0.050	0.071	0.079	0.054	0.046	0.043	0.027	0.073	0.044	0.056											
DZ	0.054	0.064	0.064	0.043	0.058	0.046	0.038	0.074	0.041	0.045	0.040										
RK	0.057	0.070	0.078	0066	0.051	0.074	0.042	0.063	0.046	0.052	0.044	0.059									
OR	0.057	0.049	0.079	0.044	0.055	0.035	0.020	0.057	0.045	0.039	0.027	0.041	0.052								
LE	0.057	0.065	0.083	0.065	0.052	0.069	0.043	0.060	0.053	0.057	0.056	0.061	0.044	0.049							
MC	0.050	0.069	0.063	0.040	0.046	0.049	0.035	0.057	0.053	0.050	0.047	0.050	0.058	0.038	0.046						
SLR	0.350	0.344	0.404	0.416	0.371	0.359	0.364	0.373	0.373	0.341	0.357	0.359	0.361	0.343	0.391	0.416					
LQ	0.374	0.383	0.435	0.431	0.395	0.383	0.393	0.409	0.400	0.369	0.368	0.395	0.399	0.360	0.428	0.438	0.044				
MQ	0.361	0.384	0.446	0.438	0.404	0.383	0.392	0.403	0.405	0.387	0.388	0.413	0.395	0.378	0.426	0.450	0.036	0.036			
LM	0.364	0.354	0.428	0.420	0.388	0.369	0.384	0.391	0.373	0.363	0.373	0.395	0.370	0.359	0.412	0.426	0.053	0.045	0.035		
DQ	0.336	0.343	0.393	0.391	0.369	0.361	0.349	0.362	0.362	0.345	0.332	0.358	0.357	0.336	0.381	0.410	0.023	0.036	0.036	0.050	
Q	0.332	0.317	0.398	0.375	0.344	0.331	0.344	0.368	0.352	0.330	0.353	0.371	0.358	0.342	0.393	0.389	0.063	0.059	0.058	0.034	0.061

Table B23. Average heterozygosity and the % polymorphic loci (total loci = 161) for each *Dreissena polymorpha* and *D. rostriformis bugensis* population. *D. polymorpha* populations: OF = Offutt AFB, UM = Upper Mississippi River, SJ = San Justo Reservoir, HL = Hedges Lake, LO = Lake Oologah, MR = Mohawk River, BL = Ballston Lake, SL = Saratoga Lake, DL = Diamond Lake, ED = El Dorado Lake, WL = Wolf Lake, DZ = Detroit River, RK = Robert S. Kerr Reservoir, OR = Ohio River, LE = Lake Erie, MC = Mosquito Creek Lake. *D. rostriformis bugensis* populations: SLR = St. Lawrence River, LQ = Lake Ontario, MQ = Millbrook Quarry, LM = Lake Michigan, DQ = Detroit River, Q = Lake Mead.

Population	*n*	Average Heterozygosity	% Polymorphic Loci
OF	6	0.2270	62.1118
UM	9	0.2271	66.4596
SJ	5	0.1799	49.0683
HL	7	0.2134	59.0062
LO	10	0.2317	57.1429
MR	9	0.2335	66.4596
BL	9	0.2434	68.3230
SL	6	0.1766	50.9317
DL	9	0.2223	62.1118
ED	5	0.2030	59.6273
WL	9	0.2342	65.8385
DZ	8	0.2126	65.2174
RK	8	0.2247	63.9752
OR	8	0.2273	65.8385
LE	9	0.1985	57.1429
MC	9	0.2288	65.8385
ALL ZM	126	0.2877	78.2609
SLR	9	0.2143	64.5963
LQ	8	0.2316	65.2174
MQ	8	0.2207	64.5963
LM	9	0.2182	63.9752
DQ	10	0.2324	55.9006
Q	8	0.2333	67.7019
ALL QM	52	0.2759	75.7764

APPENDIX C

CHRONIC THERMAL TESTING MODELS AND TESTS OF GENERAL LINEAR HYPOTHESES

Table C1. 2006 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 28°C exposed individuals and tests of the General Linear Hyothesis (n = 360).

Term	Estimate	S.E.	Chi-Square	*P*
Population	1.45126	2.64744	0.3005	0.5836
SL	0.10196	0.05014	4.0549	0.0440
Acclimation	0.01740	0.53039	0.0011	0.9738
Pop*SL	-0.22399	0.07842	8.1589	0.0043
Pop*Acc	-0.27828	0.78538	0.1256	0.7231
Acc*SL	-0.00762	0.00289	6.9305	0.0085
Pop*SL*Acc	0.01021	0.00412	6.1495	0.0131
Acc^2	0.01176	0.05578	0.0444	0.8330
$Pop*Acc^2$	0.02292	0.08189	0.0783	0.7796
Acc^3	-0.00086	0.00232	0.1371	0.7112
$Pop*Acc^3$	-0.00108	0.00341	0.0996	0.7523
Acc^4	0.00002	0.00003	0.2540	0.6143
$Pop*Acc^4$	0.00002	0.00005	0.1312	0.7172

All terms containing:	Wald Chi-Square	*df*	*P*
Population	98.8247	7	<0.0001
Acclimation	68.8389	10	<0.0001
SL	16.1832	4	0.0028

Table C2. 2006 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 29°C exposed individuals and tests of the General Linear Hyothesis (n = 361).

Term	Estimate	S.E.	Chi-Square	*P*
Population	7.42150	2.42152	9.3930	0.0022
SL	0.04505	0.04409	1.0442	0.3068
Acclimation	1.14862	0.53315	4.6414	0.0312
Pop*SL	-0.04015	0.06697	0.3595	0.5488
Pop*Acc	-2.43942	0.75697	10.3852	0.0013
Acc*SL	-0.00602	0.00236	6.5157	0.0107
Pop*SL*Acc	0.00265	0.00349	0.5800	0.4463
Acc^2	-0.09841	0.05568	3.1241	0.0771
$Pop*Acc^2$	0.24141	0.07980	9.1518	0.0025
Acc^3	0.00351	0.00231	2.3033	0.1291
$Pop*Acc^3$	-0.00976	0.00333	8.6033	0.0034
Acc^4	-0.00004	0.00003	1.7801	0.1821
$Pop*Acc^4$	0.00014	0.00005	8.3696	0.0038

All terms containing:	Wald Chi-Square	*df*	*P*
Population	53.7445	7	<0.0001
Acclimation	58.6963	10	<0.0001
SL	22.5889	4	0.0002

Table C3. 2006 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 30°C exposed individuals and tests of the General Linear Hyothesis (n = 359).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.52103	2.30608	0.0510	0.8213
SL	0.01304	0.03393	0.1477	0.7007
Acclimation	-0.15153	0.51625	0.0862	0.7691
Pop*SL	0.04732	0.06544	0.5228	0.4697
Pop*Acc	-0.23562	0.76370	0.0952	0.7577
Acc*SL	-0.00338	0.00188	3.2363	0.0720
Pop*SL*Acc	0.00035	0.00341	0.0108	0.9173
Acc^2	0.00134	0.05500	0.0006	0.9806
$Pop*Acc^2$	0.02993	0.08086	0.1370	0.7113
Acc^3	0.00048	0.00232	0.0419	0.8378
$Pop*Acc^3$	-0.00167	0.00337	0.2452	0.6205
Acc^4	-0.00001	0.00003	0.1522	0.6965
$Pop*Acc^4$	0.00003	0.00005	0.4274	0.5132

All terms containing:	Wald Chi-Square	*df*	*P*
Population	38.6107	7	<0.0001
Acclimation	10.0497	10	<0.0001
SL	10.0497	4	0.0396

Table C4. 2006 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 31°C exposed individuals and tests of the General Linear Hyothesis (n = 360).

Term	Estimate	S.E.	Chi-Square	*P*
Population	4.54472	2.41560	3.5397	0.0599
SL	0.02571	0.03990	0.4153	0.5193
Acclimation	0.54962	0.53548	1.0535	03047
Pop*SL	0.05161	0.06322	0.6665	0.4143
Pop*Acc	-1.51239	0.76268	3.9323	0.0474
Acc*SL	-0.00110	0.00213	0.2666	0.6056
Pop*SL*Acc	-0.00125	0.00336	0.1375	0.7107
Acc^2	-0.04916	0.05568	0.7796	03773
$Pop*Acc^2$	0.14610	0.07964	3.3656	0.0666
Acc^3	0.00152	0.00230	0.4388	0.5077
$Pop*Acc^3$	-0.00580	0.00329	3.1104	0.0778
Acc^4	-0.00002	0.00003	0.2469	0.6192
$Pop*Acc^4$	0.00008	0.00005	3.0710	0.0797

All terms containing:	Wald Chi-Square	*df*	*P*
Population	15.0671	7	0.0351
Acclimation	158.0385	10	<0.0001
SL	4.4609	4	0.3472

Table C5. 2006 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 32°C exposed individuals and tests of the General Linear Hyothesis (n = 358).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.27080	2.88430	0.0088	0.9252
SL	0.08841	0.04516	3.8332	0.0502
Acclimation	-1.38788	0.61686	5.0621	0.0245
Pop*SL	-0.00201	0.07000	0.0008	0.9771
Pop*Acc	-0.32531	0.86122	0.1427	0.7056
Acc*SL	-0.00646	0.00239	7.3239	0.0068
Pop*SL*Acc	0.00254	0.00363	0.4883	0.4847
Acc^2	0.14014	0.06311	4.9300	0.0264
$Pop*Acc^2$	0.05694	0.08843	0.4146	0.5196
Acc^3	-0.00590	0.00256	5.3226	0.0211
$Pop*Acc^3$	-0.00311	0.00360	0.7477	0.3872
Acc^4	0.00009	0.00004	5.7143	0.0168
$Pop*Acc^4$	0.00005	0.00005	1.0649	0.3021

All terms containing:	Wald Chi-Square	*df*	*P*
Population	13.2331	7	0.0666
Acclimation	250.2815	10	<0.0001
SL	10.6752	4	0.0305

Table C6. 2006 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 33°C exposed individuals and tests of the General Linear Hyothesis (n = 360).

Term	Estimate	S.E.	Chi-Square	*P*
Population	7.04477	2.83845	6.1599	0.0131
SL	0.03518	0.04870	0.5217	0.4701
Acclimation	-1.44697	0.64097	5.0962	0.0240
Pop*SL	-0.05600	0.07814	0.5136	0.4736
Pop*Acc	-1.94826	0.90534	4.6310	0.0314
Acc*SL	-0.00349	0.00265	1.7362	0.1876
Pop*SL*Acc	0.00321	0.00414	0.6013	0.4381
Acc^2	0.17462	0.06696	6.8005	0.0091
$Pop*Acc^2$	0.19556	0.09440	4.2917	0.0383
Acc^3	-0.00814	0.00276	8.7041	0.0032
$Pop*Acc^3$	-0.00758	0.00387	3.8353	0.0502
Acc^4	0.00012	0.00004	9.8539	0.0017
$Pop*Acc^4$	0.00010	0.00005	3.2308	0.0723

All terms containing:	Wald Chi-Square	*df*	*P*
Population	41.2209	7	<0.0001
Acclimation	197.0431	10	<0.0001
SL	3.6981	4	0.4484

Table C7. 2006 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 34°C exposed individuals and tests of the General Linear Hyothesis (n = 360).

Term	Estimate	S.E.	Chi-Square	*P*
Population	2.40193	3.39621	0.5002	0.4794
SL	0.12306	0.06325	3.7858	0.0517
Acclimation	-1.81431	0.72047	6.3414	0.0118
Pop*SL	-0.17438	0.09830	3.1469	0.0761
Pop*Acc	0.07655	1.01394	0.0057	0.9398
Acc*SL	-0.00573	0.00328	3.0454	0.0810
Pop*SL*Acc	0.00672	0.00526	1.6299	0.2017
Acc^2	0.21638	0.07688	7.9217	0.0049
$Pop*Acc^2$	-0.05342	0.10717	0.2485	0.6181
Acc^3	-0.00996	0.00324	9.4240	0.0021
$Pop*Acc^3$	0.00369	0.00452	0.6662	0.4144
Acc^4	0.00015	0.00005	10.3746	0.0013
$Pop*Acc^4$	-0.00007	0.00007	1.1758	0.2782

All terms containing:	Wald Chi-Square	*df*	*P*
Population	18.6662	7	0.0093
Acclimation	119.5822	10	<0.0001
SL	5.0115	4	0.2861

Table C8. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 26°C exposed individuals and tests of the General Linear Hyothesis (n = 239).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.10515	0.15973	0.4334	0.5103
SL	-2.60416	2.26179	1.3257	0.2496
Acclimation 20	-2.76239	1.93205	2.0442	0.1528
Acclimation 25	-3.72314	2.07841	3.2089	0.0732
Feeding	1.71356	2.13874	0.6419	0.423
Acc20*SL	0.22597	0.19152	1.3921	0.2381
Acc20*Feed	2.08378	2.86001	0.5308	0.4663
Acc20*Pop	2.41248	2.82357	0.73	0.3929
Acc25*SL	0.35206	0.20491	2.952	0.0858
Acc25*Feed	3.49057	3.13601	1.2389	0.2657
Acc25*Pop	4.57463	2.79084	2.6868	0.1012
Pop*SL	0.01971	0.18035	0.0119	0.913
Pop*Feed	0.3663	3.00736	0.0148	0.9031
SL*Feed	-0.15885	0.20495	0.6007	0.4383
Pop*SL*Feed	0.00383	0.23954	0.0003	0.9872
Acc20*Pop*SL	-0.23648	0.22241	1.1305	0.2877
Acc20*Pop*Feed	-5.49809	4.02528	1.8657	0.172
Acc20*SL*Feed	-0.23018	0.28199	0.6663	0.4143
Acc20*Pop*SL*Feed	0.44442	0.32583	1.8604	0.1726
Acc25*Pop*SL	-0.39806	0.22822	3.0423	0.0811
Acc25*Pop*Feed	-6.69297	4.07703	2.6949	0.1007
Acc25*SL*Feed	-0.32906	0.30673	1.1509	0.2834
Acc25*Pop*SL*Feed	0.53744	0.34146	2.4773	0.1155

All terms containing:	Wald Chi-Square	*df*	*P*
Population	80.9012	12	<0.0001
Feeding	14.3966	10	0.1557
SL	28.9928	12	0.0039
Acclimation (20 or 25)	19.5688	15	0.1891

Table C9. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 27°C exposed individuals and tests of the General Linear Hyothesis (n = 238).

Term	Estimate	S.E.	Chi-Square	*P*
Population	0.00117	0.11952	0.0001	0.9922
SL	-2.45876	2.20394	1.2446	0.2646
Acclimation 20	1.59776	1.6522	0.9352	0.3335
Acclimation 25	-1.56589	1.64899	0.9017	0.3423
Feeding	3.72493	2.02112	3.3967	0.0653
Acc20*SL	-0.14743	0.16544	0.7941	0.3729
Acc20*Feed	-5.7195	2.90187	3.8847	0.0487
Acc20*Pop	-0.6699	3.04082	0.0485	0.8256
Acc25*SL	0.23066	0.16017	2.0738	0.1498
Acc25*Feed	-2.55584	2.54914	1.0053	0.316
Acc25*Pop	4.88451	2.89463	2.8475	0.0915
Pop*SL	-0.02728	0.16041	0.0289	0.865
Pop*Feed	-2.72201	3.06084	0.7909	0.3738
SL*Feed	-0.30375	0.18953	2.5683	0.109
Pop*SL*Feed	0.24441	0.23215	1.1084	0.2924
Acc20*Pop*SL	-0.00357	0.23262	0.0002	0.9878
Acc20*Pop*Feed	4.64474	4.44456	1.0921	0.296
Acc20*SL*Feed	0.46545	0.2791	2.7811	0.0954
Acc20*Pop*SL*Feed	-0.38506	0.35072	1.2054	0.2722
Acc25*Pop*SL	-0.40816	0.21265	3.6843	0.0549
Acc25*Pop*Feed	1.96568	3.9491	0.2478	0.6187
Acc25*SL*Feed	0.16513	0.23951	0.4753	0.4905
Acc25*Pop*SL*Feed	-0.12795	0.29824	0.184	0.6679

All terms containing:	Wald Chi-Square	*df*	*P*
Population	103.9483	12	<0.0001
Feeding	8.5174	10	0.5784
SL	20.2487	12	0.0026
Acclimation (20 or 25)	47.4152	15	<0.0001

Table C10. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 28°C exposed individuals and tests of the General Linear Hyothesis (n = 240).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.16433	0.14346	1.3122	0.252
SL	-4.12391	1.97744	4.3492	0.037
Acclimation 20	-2.76391	1.836	2.2662	0.1322
Acclimation 25	-2.04052	1.72397	1.401	0.2366
Feeding	3.37063	2.51741	1.7927	0.1806
Acc20*SL	0.24347	0.19124	1.6209	0.203
Acc20*Feed	-1.38939	3.03906	0.209	0.6475
Acc20*Pop	1.80004	2.72447	0.4365	0.5088
Acc25*SL	0.26402	0.17787	2.2033	0.1377
Acc25*Feed	-1.27405	3.07921	0.1712	0.6791
Acc25*Pop	3.60146	2.85056	1.5962	0.2064
Pop*SL	0.14339	0.17024	0.7095	0.3996
Pop*Feed	-3.25251	3.07472	1.119	0.2901
SL*Feed	-0.30419	0.24392	1.5552	0.2124
Pop*SL*Feed	0.29954	0.26908	1.2393	0.2656
Acc20*Pop*SL	-0.20449	0.22688	0.8123	0.3674
Acc20*Pop*Feed	-0.39672	4.06166	0.0095	0.9222
Acc20*SL*Feed	0.10595	0.29608	0.128	0.7205
Acc20*Pop*SL*Feed	0.0405	0.33794	0.0144	0.9046
Acc25*Pop*SL	-0.34629	0.21993	2.4792	0.1154
Acc25*Pop*Feed	1.47617	4.10086	0.1296	0.7189
Acc25*SL*Feed	0.09416	0.29811	0.0998	0.7521
Acc25*Pop*SL*Feed	-0.07458	0.33705	0.049	0.8249

All terms containing:	Wald Chi-Square	*df*	*P*
Population	97.0769	12	<0.0001
Feeding	8.5728	10	0.5731
SL	16.7490	12	0.1593
Acclimation (20 or 25)	27.1604	15	0.0275

Table C11. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 29°C exposed individuals and tests of the General Linear Hyothesis (n = 238).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.08977	0.10754	0.6968	0.4039
SL	-2.77093	1.72214	2.5889	0.1076
Acclimation 20	0.63761	1.66227	0.1471	0.7013
Acclimation 25	1.36697	1.70732	0.641	0.4233
Feeding	-4.6123	1.84403	6.256	0.0124
Acc20*SL	0.00593	0.16364	0.0013	0.9711
Acc20*Feed	6.86587	2.67464	6.5896	0.0103
Acc20*Pop	0.43692	2.50184	0.0305	0.8614
Acc25*SL	-0.02863	0.15747	0.0331	0.8557
Acc25*Feed	1.785	2.5214	0.5012	0.479
Acc25*Pop	0.79554	2.32279	0.1173	0.732
Pop*SL	0.05994	0.13009	0.2123	0.645
Pop*Feed	4.88249	2.39489	4.1563	0.0415
SL*Feed	0.55933	0.18139	9.5088	0.002
Pop*SL*Feed	-0.57373	0.20225	8.0473	0.0046
Acc20*Pop*SL	-0.05187	0.19407	0.0714	0.7892
Acc20*Pop*Feed	-8.36254	3.57127	5.4832	0.0192
Acc20*SL*Feed	-0.78821	0.26835	8.6277	0.0033
Acc20*Pop*SL*Feed	0.9023	0.30071	9.0032	0.0027
Acc25*Pop*SL	-0.03222	0.18116	0.0316	0.8588
Acc25*Pop*Feed	-2.67668	3.50291	0.5839	0.4448
Acc25*SL*Feed	-0.3109	0.24032	1.6737	0.1958
Acc25*Pop*SL*Feed	0.36487	0.27733	1.7309	0.1883

All terms containing:	Wald Chi-Square	*df*	*P*
Population	103.8005	12	<0.0001
Feeding	23.6127	10	0.0087
SL	22.8000	12	0.0295
Acclimation (20 or 25)	39.2035	15	0.0006

Table C12. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 30°C exposed individuals and tests of the General Linear Hyothesis (n = 237).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.08225	0.13557	0.3681	0.544
SL	-0.24189	1.92118	0.0159	0.8998
Acclimation 20	-2.03336	1.91971	1.1219	0.2895
Acclimation 25	-3.48093	2.12155	2.692	0.1009
Feeding	-0.71954	1.96315	0.1343	0.714
Acc20*SL	0.25178	0.17902	1.978	0.1596
Acc20*Feed	4.98405	2.67086	3.4823	0.062
Acc20*Pop	1.57299	2.82599	0.3098	0.5778
Acc25*SL	0.46179	0.20548	5.0508	0.0246
Acc25*Feed	5.143	2.6556	3.7507	0.0528
Acc25*Pop	1.28867	2.85407	0.2039	0.6516
Pop*SL	-0.07007	0.15072	0.2161	0.642
Pop*Feed	-2.00644	2.49966	0.6443	0.4222
SL*Feed	-0.0145	0.18263	0.0063	0.9367
Pop*SL*Feed	0.15584	0.20108	0.6007	0.4383
Acc20*Pop*SL	-0.17605	0.21961	0.6427	0.4227
Acc20*Pop*Feed	-4.78055	3.66645	1.7001	0.1923
Acc20*SL*Feed	-0.45757	0.26248	3.0389	0.0813
Acc20*Pop*SL*Feed	0.42637	0.30143	2.0009	0.1572
Acc25*Pop*SL	-0.27675	0.22936	1.4559	0.2276
Acc25*Pop*Feed	-1.54317	3.66466	0.1773	0.6737
Acc25*SL*Feed	-0.45077	0.25461	3.1346	0.0766
Acc25*Pop*SL*Feed	0.26252	0.28907	0.8247	0.3638

All terms containing:	Wald Chi-Square	*df*	*P*
Population	61.4532	12	<0.0001
Feeding	23.7685	10	0.0082
SL	20.1358	12	0.0646
Acclimation (20 or 25)	60.9553	15	<0.0001

Table C13. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 31°C exposed individuals and tests of the General Linear Hyothesis (n = 239).

Term	Estimate	S.E.	Chi-Square	*P*
Population	0.06751	0.07739	0.761	0.383
SL	-0.8389	1.34629	0.3883	0.5332
Acclimation 20	0.17067	1.53621	0.0123	0.9115
Acclimation 25	1.91254	1.35379	1.9958	0.1577
Feeding	-1.91862	1.64391	1.3621	0.2432
Acc20*SL	0.06651	0.15074	0.1947	0.6591
Acc20*Feed	1.74072	2.18989	0.6319	0.4267
Acc20*Pop	-0.34939	2.14447	0.0265	0.8706
Acc25*SL	-0.04778	0.12731	0.1408	0.7075
Acc25*Feed	1.25773	2.48145	0.2569	0.6123
Acc25*Pop	-2.26446	2.40742	0.8848	0.3469
Pop*SL	-0.09859	0.10142	0.945	0.331
Pop*Feed	1.23921	2.19437	0.3189	0.5723
SL*Feed	0.10133	0.15626	0.4206	0.5167
Pop*SL*Feed	-0.05559	0.1777	0.0979	0.7544
Acc20*Pop*SL	0.01032	0.17445	0.0035	0.9528
Acc20*Pop*Feed	0.4848	2.99751	0.0262	0.8715
Acc20*SL*Feed	-0.12104	0.21148	0.3276	0.5671
Acc20*Pop*SL*Feed	-0.02158	0.24235	0.0079	0.929
Acc25*Pop*SL	0.12782	0.17168	0.5543	0.4566
Acc25*Pop*Feed	-0.11755	3.44523	0.0012	0.9728
Acc25*SL*Feed	-0.11504	0.23994	0.2298	0.6316
Acc25*Pop*SL*Feed	0.01262	0.27579	0.0021	0.9635

All terms containing:	Wald Chi-Square	*df*	*P*
Population	81.9181	12	<0.0001
Feeding	11.2737	10	0.3366
SL	8.6071	12	0.7361
Acclimation (20 or 25)	60.0579	15	<0.0001

Table C14. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 32°C exposed individuals and tests of the General Linear Hyothesis (n = 240).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.04886	0.16899	0.0836	0.7725
SL	0.43679	2.21277	0.039	0.8435
Acclimation 20	0.63443	2.51466	0.0637	0.8008
Acclimation 25	2.20568	2.12473	1.0777	0.2992
Feeding	0.14892	2.064	0.0052	0.9425
Acc20*SL	0.06003	0.24509	0.06	0.8065
Acc20*Feed	0.42757	3.30391	0.0167	0.897
Acc20*Pop	-4.43932	3.12719	2.0152	0.1557
Acc25*SL	-0.08007	0.20959	0.146	0.7024
Acc25*Feed	-0.99637	2.74494	0.1318	0.7166
Acc25*Pop	-3.99619	2.79738	2.0407	0.1531
Pop*SL	-0.11129	0.19086	0.34	0.5598
Pop*Feed	-3.7337	2.68947	1.9273	0.1651
SL*Feed	-0.08872	0.2001	0.1966	0.6575
Pop*SL*Feed	0.26932	0.22865	1.3874	0.2389
Acc20*Pop*SL	0.24612	0.2724	0.8164	0.3662
Acc20*Pop*Feed	2.32775	4.08473	0.3247	0.5688
Acc20*SL*Feed	0.09939	0.32318	0.0946	0.7584
Acc20*Pop*SL*Feed	-0.22473	0.35754	0.3951	0.5297
Acc25*Pop*SL	0.26227	0.23614	1.2335	0.2667
Acc25*Pop*Feed	5.17992	3.75232	1.9057	0.1674
Acc25*SL*Feed	0.19004	0.26705	0.5064	0.4767
Acc25*Pop*SL*Feed	-0.41803	0.30839	1.8375	0.1752

All terms containing:	Wald Chi-Square	*df*	*P*
Population	84.0215	12	<0.0001
Feeding	17.8324	10	0.0579
SL	16.9886	12	0.1500
Acclimation (20 or 25)	109.4209	15	<0.0001

Table C15. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 33°C exposed individuals and tests of the General Linear Hyothesis (n = 233).

Term	Estimate	S.E.	Chi-Square	*P*
Population	0.22512	0.14836	2.3027	0.1291
SL	1.04507	2.04425	0.2614	0.6092
Acclimation 20	-1.10998	2.07657	0.2857	0.593
Acclimation 25	4.86335	2.00589	5.8783	0.0153
Feeding	1.98379	1.80116	1.2131	0.2707
Acc20*SL	0.25799	0.19482	1.7536	0.1854
Acc20*Feed	2.01511	2.66555	0.5715	0.4497
Acc20*Pop	2.80313	2.58104	1.1795	0.2775
Acc25*SL	-0.27752	0.18247	2.3131	0.1283
Acc25*Feed	-1.14094	2.7319	0.1744	0.6762
Acc25*Pop	-2.47882	2.60185	0.9077	0.3407
Pop*SL	-0.25668	0.17099	2.2535	0.1333
Pop*Feed	-1.45346	2.46343	0.3481	0.5552
SL*Feed	-0.33157	0.17484	3.5963	0.0579
Pop*SL*Feed	0.28606	0.2035	1.976	0.1598
Acc20*Pop*SL	-0.0969	0.21759	0.1983	0.6561
Acc20*Pop*Feed	-0.82654	3.49538	0.0559	0.8131
Acc20*SL*Feed	-0.08455	0.25356	0.1112	0.7388
Acc20*Pop*SL*Feed	-0.0614	0.28883	0.0452	0.8316
Acc25*Pop*SL	0.33445	0.20933	2.5527	0.1101
Acc25*Pop*Feed	-1.14341	3.53323	0.1047	0.7462
Acc25*SL*Feed	0.21612	0.26536	0.6633	0.4154
Acc25*Pop*SL*Feed	-0.14094	0.29625	0.2263	0.6343

All terms containing:	Wald Chi-Square	*df*	*P*
Population	34.0707	12	0.0007
Feeding	26.9140	10	0.0027
SL	27.3025	12	0.0070
Acclimation (20 or 25)	156.0308	15	<0.0001

Table C16. 2007 Lake Oologah (OK) and Hedges Lake (NY) *Dreissena polymorpha* treatment model parameter estimates for 34°C exposed individuals and tests of the General Linear Hyothesis (n = 240).

Term	Estimate	S.E.	Chi-Square	*P*
Population	-0.21601	0.12033	3.2224	0.0726
SL	-2.23107	1.61752	1.9025	0.1678
Acclimation 20	1.09083	1.78814	0.3721	0.5418
Acclimation 25	0.15587	1.84897	0.0071	0.9328
Feeding	-2.34544	2.03383	1.3299	0.2488
Acc20*SL	0.18631	0.17585	1.1225	0.2894
Acc20*Feed	0.18889	3.03597	0.0039	0.9504
Acc20*Pop	1.00399	2.29402	0.1915	0.6616
Acc25*SL	0.2746	0.17889	2.3561	0.1248
Acc25*Feed	1.79219	2.63339	0.4632	0.4961
Acc25*Pop	1.3254	2.50841	0.2792	0.5972
Pop*SL	0.15117	0.13361	1.2802	0.2579
Pop*Feed	2.28177	2.63321	0.7509	0.3862
SL*Feed	0.22173	0.19405	1.3057	0.2532
Pop*SL*Feed	-0.19796	0.21554	0.8435	0.3584
Acc20*Pop*SL	-0.11111	0.19338	0.3301	0.5656
Acc20*Pop*Feed	-1.60761	3.90239	0.1697	0.6804
Acc20*SL*Feed	-0.16909	0.28819	0.3443	0.5574
Acc20*Pop*SL*Feed	0.14217	0.32187	0.1951	0.6587
Acc25*Pop*SL	-0.21929	0.20216	1.1766	0.278
Acc25*Pop*Feed	-2.39151	3.64699	0.43	0.512
Acc25*SL*Feed	-0.30799	0.24884	1.5319	0.2158
Acc25*Pop*SL*Feed	0.27668	0.28543	0.9397	0.3324

All terms containing:	Wald Chi-Square	*df*	*P*
Population	19.0961	12	0.0862
Feeding	12.0999	10	0.2784
SL	4.8776	12	0.9619
Acclimation (20 or 25)	111.3177	15	<0.0001

Table C17. 2008 Winfield City Lake (OK) *Dreissena polymorpha* early summer treatment model parameter estimates and tests of the General Linear Hyothesis (n = 317).

Term	Estimate	S.E.	Chi-Square	*P*
Temperature	-13.74550	2.41579	32.3744	<0.0001
SL	-0.67874	0.33207	4.1777	0.0410
Temp*SL	0.01631	0.01005	2.6341	0.1046
$Temp^2$	0.25428	0.03964	41.1520	<0.0001
SL^2	0.00504	0.00300	2.8301	0.0925

All terms containing:	Wald Chi-Square	*df*	*P*
Temperature	273.9561	3	<0.0001
SL	7.1535	3	0.0672

Table C18. 2008 Winfield City Lake (OK) *Dreissena polymorpha* late summer treatment model parameter estimates and tests of the General Linear Hyothesis (n = 360).

Term	Estimate	S.E.	Chi-Square	*P*
Temperature	209.51607	67.66933	9.5863	0.0020
SL	10.94334	4.33416	6.3751	0.0116
Temp*SL	-0.49826	0.20451	5.9357	0.0148
$Temp^2$	-6.74660	2.13284	10.0058	0.0016
$Temp^3$	0.07338	0.02252	10.6176	0.0011
$Temp^3$*SL	0.00015	0.00007	5.1904	0.0227

All terms containing:	Wald Chi-Square	*df*	*P*
Temperature	243.6047	5	<0.0001
SL	22.1400	3	<0.0001

Table C19. 2007 Lake Mead (NV/AZ) *Dreissena rostriformis bugensis* treatment model parameter estimates and tests of the General Linear Hypotheses (n = 225).

Term	Estimate	S.E.	Chi-Square	*P*
Temperature	1.29469	0.84440	2.3509	0.1252
Feeding	0.11561	0.16808	0.4731	0.4916
SL	-0.94841	3.13655	0.0914	0.7624
Temp*SL	0.01623	0.10281	0.0249	0.8746
SL^2	0.01484	0.00313	<0.0001	0.9974
SL^2*Temp	-0.00001	0.09463	0.0246	0.8754

All terms containing:	Wald Chi-Square	*df*	*P*
Temperature	197.2432	3	<0.0001
Feeding	0.4731	1	0.4916
SL	3.0253	4	0.5536

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BIOGRAPHICAL INFORMATION

John Morse was born in Syracuse, New York, on 4 October 1977. He earned his Bachelor of Science in Biology with Honors and his Master of Science in Biology from the University of Texas at Arlington (UTA) in 1999 and 2001, respectively. It was during his time as a UTA undergraduate that he first met his wife, Roshi Mehdibeigi, who never fails to support his dreams. He apparently enjoyed UTA enough to give it another go and earned his Doctorate in Quantitative Biology from there in 2009. Both his Master and Doctorate degrees were completed while performing invasive mollusk research in the Center for Biological Macrofouling under the tutelage of Dr. Robert F. McMahon. Throughout his academic and professional career John became involved in many biological disciplines including field and laboratory studies of population genetics, neuroscience, aquatic ecology, and aquatic insect biology. However, it was his work on invasive species, particularly the invasive bivalves *Dreissena polymorpha* and *D. rostriformis bugensis* that he enjoyed the most. Between earning his two graduate degrees he moved to Cambridge, NY where he was involved in the development of a novel biopesticide designed to eradicate dreissenid mussels using the ubiquitous soil bacterium, *Pseudomonas fluorescens*. This research was performed under the guidance of Dr. Daniel Molloy while employed by the New York State

Museum. John began his career with the United States Fish and Wildlife Service in April of 2009.