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Upper Thermal Limits Of Freshwater Mussels (Bivalvia, Unionoida) In Ramped Temperature Exposures

Kathryn Rae Cottrell Martin

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**UPPER THERMAL LIMITS OF FRESHWATER MUSSELS (BIVALVIA,
UNIONOIDA) IN RAMPED TEMPERATURE EXPOSURES**

A Masters Thesis

Presented to

The Graduate College of
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree
Master of Science, Biology

By

Kathryn Cottrell Martin

July 2016

UPPER THERMAL LIMITS OF FRESHWATER MUSSELS (BIVALVIA, UNIONOIDA) IN RAMPED TEMPERATURE EXPOSURES

Biology

Missouri State University, July 2016

Master of Science

Kathryn Cottrell Martin

ABSTRACT

The purpose of this study was to determine the upper thermal limits of unionids. Three species of juvenile freshwater mussels were tested in lab experiments mimicking a diel pattern of temperature change (increasing 6 h, peak 2 h, and decreasing 8 h). The peak temperature fatal to half of the exposed population (LT50) was tested with respect to species, population, age, and seasonal acclimation. Mortality was monitored for 2 weeks after exposure. The smallest size classes were tested in a thermal cyler instrument, a novel application for testing mussels. LT50s for juveniles less than 3 weeks old were within 2-3°C higher or lower compared to juveniles 1-2 years older. LT50s for peak temperature in summer-acclimated mussels were 33.2, 39.1, and 38.9°C for Western pearlshell, Fatmucket, and Washboard juveniles less than 3 weeks old compared to LT50s of 36.1 and 40.8°C for Fatmucket and Washboard 1-2 years of age. These results are several degrees higher than previously reported for continuous temperature exposures lasting 1- several days. LT50s for summer acclimated mussels immersed in water were 2-3°C higher than for those emersed in damp sand. LT50s for winter acclimated mussels immersed in water were 1°C lower than those emersed in damp sand. Winter acclimated Washboard had LT50s 2-4°C lower than summer acclimated animals. These data can be used to predict the impacts on threatened mussel species of increased temperatures resulting from anthropogenic factors including climate change.

KEYWORDS: Unionoida, LT50, temperature tolerance, thermal cyler method, *Lampsilis siliquoidea*, *Megaloniais nervosa*, *Margaritifera falcata*

This abstract is approved as to form and content

M. Christopher Barnhart, PhD
Chairperson, Advisory Committee
Missouri State University

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INTRODUCTION

Freshwater mussels (Order Unionoida) are an important component of lotic ecosystems in eastern North America. Mussels are benthic organisms that filter-feed on bacteria, algae, and detritus. When abundant, mussels can provide an important food source for certain mammals and fish. Historically, their shells were an important raw material for manufacture of buttons and jewelry. North America contains the most diverse freshwater mussel fauna in the world (Graf and Cummings 2007). Many of these species have been lost in historical times, and many others are in danger of extinction. For this reason, 88 mussel species are protected in the USA under the federal Endangered Species Act (U.S. Fish and Wildlife Service 2016a). These species are significant in the context of aquatic environmental conservation, because not only the species, but their habitats, are protected (U.S. Fish and Wildlife Service 2016b).

Many factors may affect the presence and the abundance of mussels. These factors include the stability and type of benthic substrate present (Neves et al. 1997, Layzer and Madison 1995, Strayer 1999), the effects of pollutants, including copper and ammonia (Augspurger et al. 2003, Wang et al. 2009, Wang et al. 2011), and the availability of host fish, which support the development of the parasitic mussel larvae (Watters 1996, Haag and Warren 1998). Another potentially important habitat factor is temperature. Mussels are ectothermic animals whose body temperature is entirely dependent on environmental temperature. Temperature influences a variety of rate processes of mussels including oxygen consumption, food clearance, and biodeposition (Spooner and Vaughn 2008). Temperature may play a role in limiting the current

geographic range of some freshwater mussel species. Although some species such as the threeridge, *Amblema plicata*, are widely distributed among latitudinal gradients, others have a smaller latitudinal range, suggesting that seasonal temperatures might be restrictive (Haag 2012). For example, *Lasmigona compressa* is chiefly found in the Upper Mississippi River and northern Ohio River tributaries areas where the mean daily air temperature in July is lower than 24°C (Haag 2012). The distribution of *L. compressa* does not appear to be inhibited by physical barriers or degraded habitat, and many species whose ranges overlap with *L. compressa* are found further south, all of which suggests that high temperatures may limit the southern distribution of *L. compressa*. Conversely, the distribution of *Potamilus purpuratus* suggests that it may be limited by low temperature (Haag 2012).

Human activity has directly and indirectly altered the natural range and variation of temperature in freshwater systems in many ways, ranging from local impacts of deforestation, industry and utilities, to hydrologic alterations, to climate change (Caissie 2006; Heino et al. 2009; Woodward et al. 2010; Domisch et al. 2011; O'Reilly et al. 2015). Humans can increase the effect of solar heating by removal of riparian trees. It has been shown that clear-cutting up to a riverbank can increase the average monthly maximum water temperature as much as 15.5°C (Brown and Krygier 1970). Urban areas add heat loads including power generation, industrial energy use, and water treatment. Urbanization has increased water temperature as much as 5.5°C in some areas in 40 years (Kinouchi 2007). Kinouchi found effluent from wastewater treatment plants to be the primary cause of increased temperatures in waterbodies near areas of increasing urbanization. Additionally, power generators often use surface water to cool process

water. Most cooling systems do not recirculate the water and instead use a once-through design where the water is withdrawn at an intake, absorbs the heat from the process, and is then released back into the natural setting.

Besides industrial heat loads, other factors can affect water temperature by interrupting flow or reducing water level (Caissie 2006). These factors include drought, water removal for irrigation, industry or other human use, and flow fluctuations from dams during cycles of water retention and power generation. Flow interruption and reduced water level increase temperature fluctuations from solar heating and potentially expose relatively immobile benthic organisms such as mussels to consequent heating or cooling in shallow water or during air emersion. Large dams releasing from the hypolimnetic layer of the reservoir releases cold water in to the river, which can alter the water temperature downstream.

Given that temperature can fluctuate widely on a short-term basis, it is likely that acute effects of extreme temperatures, rather than climate temperature averages, may often limit the survival of organisms. Such acute effects are often quantified experimentally by determining the temperature at which a particular endpoint, such as death or loss of equilibrium, occurs in a specified fraction of the tested population. One common metric for temperature stress is the LT50, the upper temperature that is lethal to fifty percent of the tested group. In order to be useful for comparison, LT50 must be measured under a defined set of circumstances that control for other factors that affect the outcome, such as the rate and duration of temperature exposure, the species and life stage tested, the previous thermal history of the tested organisms, and so on (Beitinger 2000).

A few studies have examined LT50 of freshwater mussel species. These studies have generally used relatively prolonged (24-96 hour or longer) exposures at constant temperatures (Pandolfo et al. 2010; Galbraith et al. 2012; Ganser et al. 2013; Archambault 2013; Archambault 2014a,b). In contrast, the present study examined the LT50 of mussels exposed to ramped temperature excursions, with the temperature rising slowly to a peak, held at the peak for 2 hours, and then declining slowly back to baseline over the course of a 16 hour period. These temperature changes were meant to represent those that might be experienced in summer due to solar heating in shallow water or air emersion as a result of drought, water removal, or reservoir operations. LT50 was measured as the peak temperature that caused 50% mortality. LT50 was examined with respect to species, population latitude, age class, season, and acclimation temperature.

METHODS

Animals

Mussel species tested were Fatmucket [*Lampsilis siliquoidea* (Barnes)], Washboard [*Megaloniaias nervosa* (Rafinesque)] and Western pearlshell [*Margaritifera falcata* (Gould)]. Fatmucket larvae were collected from four locations: 1) Silver Fork of Perche Creek (39.0675, -92.3915, Boone County, MO) 2) Bourbeuse River (38.1817, -91.5704, Gasconade County, MO), 3) the St. Croix River (44.9689, -92.7279, St. Croix County, WI) and 4) Pool 10 of the Upper Mississippi Rivers (43.0517, -91.1412, Crawford County, WI). Washboard were from two locations: 1) the Sac River (37.8697, -93.8031, Cedar County, MO) and 2) Pool 10 of the Upper Mississippi River (43.0517, -91.1412, Crawford County, WI). Western pearlshell were from the Upper Columbia River (48.8146, -117.9478, Stevens County, WA).

All juveniles tested were cultured from glochidia at Missouri State University or Genoa National Fish Hatchery using standard methods (Barnhart 2006). Newly metamorphosed juveniles were cultured in the laboratory using artificial foods and were kept at ambient laboratory temperature (21-23°C). Mussels intended for use at larger size and with seasonal winter acclimatization (fatmucket and washboard) were transferred to upweller systems at the Kansas City Zoo, where they were supplied with natural water and food from the Swope Park lagoon. At the zoo, the mussels were exposed to ambient seasonal temperature variations. Animals acclimatized to “winter conditions” overwintered at the zoo and were taken to the lab in February, where they were held at 7°C until testing later that month. Animals acclimated to “summer conditions” were

removed from the lab in August and held in the lab at 23°C until tested in October.

Temperatures were recorded hourly using I-Button temperature loggers (see below).

Field Temperatures

Temperature loggers were deployed in the field to determine the daily cycle of temperature in mussel habitats in the Sac River in southwestern Missouri in midsummer. This river reach supports populations of more than 20 species of freshwater mussels (McMurray et al. 2012). Temperature data loggers (iButton® model DS1922L Maxim Integrated Products, Inc.) recorded temperature at 15 minute intervals with a precision of ± 0.5 degrees C. Accuracy of each logger was tested before deployment. Each iButton was placed within a sealed 50-ml plastic tube, which was secured to a length of rebar as an anchor. Six of these assemblies were then placed at intervals on a transect across the width of the Sac River. Loggers were placed to represent the position of mussels buried at 4-5 cm depth in substrate, both submerged and above the water line in moist substrate and dry substrate. Temperatures were recorded for a 7-day period (August 21 – 28, 2014) to determine the daily pattern of environmental temperature change. The seven diel records were averaged at each time of day. The transect locations (Figure 1) were: 1.) West bank: buried 4-5 cm, in dry sand, 2 m from water line. 2.) West bank: buried 4-5 cm in moist sand, 1 m from water line. 3.) Channel: Submerged 15 cm, buried in 4-5 cm in substrate. 4.) Channel: Submerged 50 cm, buried in 4-5 cm in substrate. 5.) East bank: buried 4-5 cm in moist sand, 0.5 m from water line. 6.) East bank: in air at about 1-m above ground level.

Laboratory Temperature Exposures

Laboratory temperature exposures were arranged to mimic the timing of the field diel temperature changes (Figure 2). Over 16-hours, temperature was elevated from the control (lowest) temperature linearly over 6 h to a peak temperature, which was maintained constant for 2 h, then lowered linearly over 8 hours to control. Separate groups of mussels were exposed simultaneously to 4-6 different peak temperatures. Each group experienced only a single 16-hour exposure. Tested mussels and controls were observed for mortality during a two week period following experimental exposure. Three approaches were used for the laboratory temperature exposures. Juveniles less than 3 weeks of age were tested in 96-conical well plates using a thermal cycler to control temperature. Older juveniles were exposed in larger chambers within temperature-controlled water baths or environmental cabinets. Each temperature exposure included replicate sets of animals in water and in damp sand paired in the same chambers (Figure 3). These approaches are described in detail below.

Thermal Cycler. Mussels less than 3 weeks of age were tested with an Applied Biosystems® Veriti® 96-well Thermal Cycler. The thermal cycler contained 6 independently programmable heating zones, which were each programmed with a different peak temperature and rate of temperature change. The manufacturer's stated accuracy of the instrument is 0.25°C. Mussels were either unfed newly metamorphosed individuals, or if fed, were starved for 24 hours to depurate prior to testing. The mussels were pipetted individually into wells of 96-well plates. Each conical well contained 200 µL of filtered (0.45µm) river water. Following the temperature exposure, the mussels were pipetted individually into larger 24-well plates, with 500 µL of filtered river water,

and kept between 19 - 26°C. The mussels were not fed following the trials. The mussels were observed for mortality with a VWR VistaVision dissecting microscope daily for 3 days following the experiment and again at days 7 and 14. Mussels were classified as dead if they failed to show foot movement or heartbeat during a 1 minute observation period. Partial water changes were performed three times per week during this observation period.

Data for 0-3 week old mussels were derived from 16 thermal cyclers trials. The tests included 3 species, 6 populations, and 1,724 individuals. Mussel species and populations tested with this method were Fatmucket (Silver Fork and Bourbeuse Rivers in Missouri, St. Croix and Pool 10 of the Upper Mississippi Rivers in Wisconsin), Washboard (Pool 10 of the Upper Mississippi River), and Western Pearlshell (Upper Columbia River in Washington). In each trial, 16 different individuals were exposed to each of six temperature excursions with peak temperatures differing by 1°C. Controls were also placed into a 96-well plate, which was kept at laboratory temperature (average 22.1°C, range 19.6-26.6). Mortality was assessed immediately after the temperature exposure and at intervals over the next 2 weeks (i.e. on Days 0, 1, 2, 7, and 14).

Water Baths. One-to two-year-old animals (1-2 cm) were tested using calibrated Fisher Isotemp 10 L water baths, which are accurate to $\pm 0.5^\circ\text{C}$. Five water baths, each with a different peak temperature, were used. Mussels were tested in both immersed and emersed conditions (Figure 3). In each water bath, four 600-mL beakers were filled with 400 mL of river water. A covered plastic cup containing 1-cm of damp sand was fitted inside of each beaker. Each beaker contained 5 mussels from each population in both types of temperature exposures. The water in each beaker was gently aerated with air

that was humidified by bubbling through a jar of deionized water kept in the water bath. Seachem Ammonia Alert cards were used to monitor unionized ammonia. In order to minimize changes in water quality, 100 ml of water was replaced approximately every 3 hours from a reservoir of fresh river water at similar temperature in each bath. Water changes occurred more often if increased ammonia levels were detected. Temperature was recorded with calibrated i-Button temperature loggers and National Institute of Standards and Technology (NIST) certified thermometers. Following the experiment, the mussels were housed in a modified mucket bucket (Barnhart 2006) recirculating culture system, fed, and observed for mortality periodically for two weeks. Mussels were classified as dead if they had visibly decayed tissue or were gaping and unresponsive to touch. Mussels were pooled during the monitoring period but were identified to population by marks on their shell.

The water bath set-up was used to test animals that had been in culture for over 1 year and which were 1 – 2 cm in length. The mussels were acclimatized at the Kansas City Swope Park Zoo Lagoon and then brought to the lab and held at seasonal temperature (23°C for summer conditions and 7°C for winter conditions) until temperature exposures were performed. Washboard mussels from the Sac and UMR populations were tested after both summer and winter acclimatization. Sac and UMR Fatmucket were tested only after winter acclimatization. In these tests, five mussels were tested in each treatment group. Four groups were tested at each temperature for a total of 20 animals from each population. Treatments included medium (immersed in aerated water or emersed in damp sand), populations, and temperature (5 different temperature maxima). Control groups were immersed and emersed at constant temperature.

Environmental Chambers. Animals two to three years of age (4-7 cm) were tested within programmable Percival I-36VL environmental chambers, which are accurate to $\pm 0.5^{\circ}\text{C}$. Four environmental chambers, each with a different peak temperature and slope, were used for these trials. The mussels were placed in 6-quart plastic storage boxes that were filled with 2 liters of river water for immersed trials or 3 centimeters of damp sand for emersed conditions. Each container held 2 mussels from each tested population for a total of 4 mussels per box. Five boxes were tested with immersed conditions and five with emersed conditions for a total of 10 mussels per species in each condition. Calibrated iButton temperature loggers and National Institute of Standards and Technology (NIST) certified thermometers were randomly placed in at least one immersed and one emersed container per chamber. At least one Seachem Ammonia Alert card was placed in a water-filled container in each temperature group for monitoring throughout the treatment. In order to minimize changes in water quality, 500 ml of water was replaced approximately every 3 hours from a reservoir of fresh river water in environmental chamber. Following the experiment, the mussels were housed in a modified mucket bucket (Barnhart 2006) recirculating culture system, fed, and observed for mortality for two weeks. Criteria were similar to those described for the water bath exposures.

Calculation and comparison of LT₅₀. LT₅₀ (peak temperature resulting in 50% mortality) was calculated using the Probit analysis function in Minitab. This function calculates a binomial regression which is commonly used to model responses in toxicology. Probit analysis requires at least two partial survival results in order to calculate an estimate of LT₅₀ and standard error. After each experiment, mortality was

monitored periodically for 2 weeks. In some cases delayed mortality was evident, so the final measurement was considered to be the most meaningful result.

Statistical comparisons of LT_{50} s were made using the ratios method of Wheeler et al. (2006). This method calculates a confidence interval for the ratio of two measurements of LT_{50} , based on the SE of the LT_{50} estimates from the probit analysis. If the confidence interval of the ratio includes 1.0 (i.e. the expected ratio if the two measures are equal) or if the confidence intervals of the natural log of the ratio includes 0 then the difference is deemed not significant. The ratio test method is a more powerful method with a lower type I error rate than comparison of confidence intervals of each LT_{50} (Wheeler et al. 2006).

RESULTS

Field Temperatures

Temperatures at the Sac River were recorded in air and in river and riparian substrate during 7 consecutive days (August 21-28, 2014). The 24-h recordings were averaged at 15 minute intervals to construct a mean diel temperature pattern (Figure 4). During the week of the recordings, the weather pattern was consistently sunny and temperatures were near seasonal highs. The logger elevated at 1 m in air recorded temperature ranged from a low of 27°C at 7 AM to a high of 51°C at 6 PM, indicating that it experienced full sun in the late afternoon and did not record a true air temperature. The temperature of the logger buried at 4-5 cm in dry sand (position 1) followed most closely and ranged from 26 - 48°C. Temperature buried in moist sand in the sun (position 2) ranged from 24 - 35°C. Moist sand in the shade (position 5) ranged from 27 - 35°C. Substrate temperature in shallow (15 cm) water ranged from 29 - 34°C. Substrate temperature in deeper water (50 cm) varied least over the diel, from 29 - 32°C (Figure 4).

LT₅₀ of 0-3 Week Old Mussels

Fatmucket. Seven experiments were made between August and December 2015 with individuals from the Silver Fork of Perche Creek population of Fatmucket from 0 - 3 weeks old. Peak temperatures used were 36 - 42°C. Survival on Days 0 - 14 following the exposure is presented in Figure 5. Delayed mortality was negligible, meaning little mortality difference was noticed between the initial observation following the

experimental exposure and the observation on Day 14. Mean LT_{50} was 39.0 ± 0.2 at 14 days (Table 1).

One trial was made during August 2015 with mussels from the Bourbeuse River population from 0 - 3 weeks old. Peak temperatures ranged from 40 to 45°C. Survival on Days 0-14 following the exposure is presented in Figure 5. Survival of the 40°C group was 100% survival on Days 0 and 1 and 94% survival on Day 2 and 7. Survival on Day 0 was below 20% for groups exposed to 41 - 45°C. LT_{50} could be only roughly estimated for this group (Table 1) because of insufficient resolution of the survivorship curve (Figure 5).

Two thermal cyclers trials were made during June 2015 with mussels from the St. Croix River population from 0-3 weeks old. Peak test temperatures ranged from 35 - 40°C. This range of temperatures was not high enough to cause mortality in the lower part of the range (Figure 5). On Day 14, the 38°C group survival was 75%; all other group survivals remained above 80%. LT_{50} exceeded 40C but cannot otherwise be estimated accurately (Table 1).

One thermal cycler trial was made during June 2015 with mussels from the Upper Mississippi River, UMR, from 0-3 weeks old. Peak temperatures ranged from 35 to 40°C. All group survival rates were above 80% on Days 0 – 3, the 40°C temperature group survival rate was 69% on Day 7, and no observation was made on Day 14 (Figure 5). LT_{50} clearly exceeded 40°C but the temperature range tested was too low for an accurate estimate of LT_{50} (Table 1).

Washboard. Three thermal cyclers trials were made with 0-2 week old mussels during December of 2015. Peak temperatures used were 36-41°C. Mortality was confined mainly to Day 0 (Figure 6). LT_{50} was $38.6 \pm 0.3^{\circ}\text{C}$ on Day 14 (Table 2).

Western Pearlshell (Upper Columbia River): Two thermal cyclers trials were made with 0-3 week old mussels in June and July of 2015. The two trials used different ranges of peak temperatures, so that 11 peak temperatures (rather than 6 as in the other experiments) were tested from 25 to 35°C. One group of 16 mussels was tested per temperature treatment. Delayed mortality was evident (Figure 7). Survival was high across all temperatures on Day 0. Thereafter, survival dropped gradually in proportion to test temperatures above 25°C (Figure 7). LT_{50} was $33.2 \pm 0.27^{\circ}\text{C}$ on Day 14 (Table 3). Figure 8 shows the calculated LT_{50} and 95% confidence intervals of < 3 week old Fatmucket, Washboard, and Western Pearlshell.

LT_{50} of 1-2 Year Old Mussels

Washboard. Summer acclimatized 1-2 year old Washboard peak test temperatures were 37, 38, 39, 40, and 41°C. Washboard from both Sac River and UMR populations were tested immersed and emersed. Survival was 100% at all temperatures below 39°C (Figure 9). When immersed, Day 14 LT_{50} s were $41.3 \pm 0.4^{\circ}\text{C}$ for the Sac River and $40.8 \pm 0.2^{\circ}\text{C}$ for the UMR (Figure 10). LT_{50} of emersed Washboard was 2-3°C lower than in water. When emersed, delayed mortality was evident (Figure 9). LT_{50} on Day 14 was $38.2 \pm 0.3^{\circ}\text{C}$ for the Sac River and $38.7 \pm 0.2^{\circ}\text{C}$ for the UMR populations (Table 2, Figure 10).

Winter acclimatized 1-2 year old Washboard peak test temperatures were 25, 30, 35, 40, and 45°C. Washboard from the Sac and UMR were tested immersed and emersed. Survival was high when exposed to a peak temperatures of 25, 30, and 35°C, but groups exposed to peak temperatures of 40 and 45°C declined steadily to 100% mortality over the observation period (Figure 11). Winter LT₅₀s were similar among populations and treatments and were about 3-5°C lower than summer LT₅₀s. Immersed LT₅₀ on day 14 was 36.6 ± 0.8°C for the Sac and 36.4 ± 0.8°C for the UMR (Table 5). Emersed LT₅₀ on Day 14 was 35.8 (estimated) for the Sac and 37.4 ± 0.51 for the UMR (Table 5).

Fatmucket. Winter acclimatized 1-2 year old Fatmucket peak test temperatures were 25, 30, 35, 40, and 45°C. Fatmucket from the UMR and St. Croix populations were tested immersed and emersed. LT₅₀ was 36.0 ± 0.8°C for the St. Croix population and 36.1 ± 0.8°C for the UMR population on Day 14. Similar to the Washboard survival results from the same experimental exposure, both Fatmucket populations experienced high survival when treated with 25, 30, or 35°C at peak temperature exposure but groups treated with 40 or 45°C had increasing mortality over the observation period (Figure 12). Emersion had little effect on LT₅₀ but estimates had high variance which may have obscured any difference due to emersion (Table 5). Figures 13 and 14 compares the LT₅₀ of the 1 – 2 year old Washboard and Fatmucket acclimatized to 7°C that were tested in immersed conditions, respectively.

LT₅₀ of 2-3 Year Old Mussels

The environmental chamber set-up was used to test animals that had been in culture for 2 – 3 years and which were 3 – 5 cm in length. The mussels were acclimatized at the Kansas City Swope Park Zoo Lagoon and then brought to the lab and held at seasonal temperature (23°C) until temperature exposures were performed. UMR Washboard and St. Croix Fatmucket were tested after summer acclimatization. In these tests, two mussels of each species were tested in each treatment group. Treatments included immersed in shallow, unaerated water or emersed in damp sand in addition to the 4 different temperature excursions.

Washboard. Peak test temperatures were 36, 38, 40, and 42°C. 2-3 year old Washboard from the UMR were tested immersed and emersed. Survival was 50% or less for both types of treatments exposed to 38°C or higher (Figure 15). LT₅₀s were similar among treatments and decreased slightly with time. On Day 7, the immersed LT₅₀ was $38.6 \pm 0.38^\circ\text{C}$ and the emersed LT₅₀ was roughly 37°C (Table 6). On Day 14, the immersed LT₅₀ was $38.4 \pm 0.39^\circ\text{C}$ and the emersed LT₅₀ remained near 37°C (Table 6). Figure 16 compares the LT₅₀ of Washboard tested in immersed and emersed conditions on Day 0 and Day 1. LT₅₀s could not be calculated with the data from Days 2 – 14 due to limited partial mortality.

Fatmucket. Peak temperature exposures were 36, 38, 40, and 42°C. 2 – 3 year old Fatmucket from the St. Croix were tested immersed and emersed. Survival was low for both types of treatments exposed to 38°C or higher (Figure 15). When immersed in water, the Day 7 and Day 14 LT₅₀ was roughly 36°C (Table 6). When Fatmucket were tested emersed in sand, the LT₅₀ was below 36°C (Table 6). Figures 17 and 18 compare

the LT_{50} s of 2 – 3 year old Washboard and Fatmucket tested in immersed and emersed conditions, respectively.

DISCUSSION

The small sampling of substrate temperatures in this study is representative of the general pattern of daily temperature change that would be experienced by mussels in water or during emersion in midsummer. Because of solar heating, temperatures generally rise over the course of the day from a low before sunrise to a high after midday (Caissie 2006). Heat transfer to water occurs by direct solar heating and by conduction from air and groundwater. Heat loss from water occurs to air by conduction and by evaporation (Mohseni et al. 1999, Caissie 2006). In my measurements, peak temperature exceeded 35°C in emersed wet substrate and approached 48°C in dry substrate. This result indicates the potential for lethal temperatures in emersed conditions. Maximum temperatures that result from solar heating are normally experienced for only a few hours each day, so that the ramped temperature exposures used in this study are more ecologically realistic than the continuous temperatures used in previous studies. Because mortality was recorded for up to 2 weeks following temperature stress, it was documented that most mortality occurred promptly. However there were cases when delayed effects were evident (e.g. Western pearlshell; Figure 7).

Overall, LT₅₀ of mussels in the 16-hour ramped exposures ranged from 33.2 to 41.3 C. All but one result (< 2 week old Western pearlshell) LT₅₀ exceeded 36°C. Not surprisingly, LT₅₀s in the ramped exposures are higher than those reported previously for continuous exposures for longer periods of time. For example, Pandolfo et al. (2010) reported LT₅₀ of 34-35°C in 96 hour exposures of juvenile and subadult Fatmucket and Washboard (Table 4). In the present study, these species had LT₅₀ from 2-6 degrees

higher depending on size and acclimation history (Tables 1, 2, 3). Ganser et al. (2013) tested 3 species of mussels for 28 days, and reported the lowest LT_{50} s for Washboard as 30.3°C and Fatmucket as 25.3°C (Ganser et al. 2013). Those results are questionable, however, because mussels in Midwestern rivers regularly experience sustained water temperatures of 30°C or higher in summer (e.g., Figure 4).

The use of the thermal cyclor for temperature exposures was very efficient because the instrument is programmable and can run 6 magnitudes of temperature change and 96 wells simultaneously. The method is suited only to very small individuals because of the small size of the test wells. However, the use of early juveniles for stress testing is attractive because of the large numbers that can be obtained from captive propagation. In toxicology testing, juvenile mussels are often found to be the most sensitive life stage (Augsburger 2007, Wang et al. 2007). In the present study, LT_{50} did not vary consistently with age and size and early juveniles did not seem to be more sensitive than the other size classes tested. LT_{50} for St. Croix Fatmucket was about 41°C for new juveniles, compared to $\sim 36^{\circ}\text{C}$ for summer acclimated 2-3 year olds. UMR Washboard had LT_{50} = 38.9 , 40.8 , and 38.4°C for new juveniles, 1-2 year olds, and 3-4 year olds, respectively. Differences between calculated LT_{50} s of groups were not found to be significant if the confidence intervals of the natural log of the ratio test included 0, these ratios and confidence intervals can be found in Figure 19. Previous studies of Fatmucket show lower LT_{50} s for juveniles than were found in this study (Table 4). Certainly the thermal cyclor can be a powerful method for exploring effects of species and interactions of other factors with temperature sensitivity. However, before

extrapolating early juvenile results to all life stages, further study with larger, older mussels is advisable to determine whether they exhibit different temperature tolerance.

Different species and populations of mussels might be expected to be adapted to different ranges of environmental temperature. The LT_{50} of Western Pearlshell was 5-6°C lower than that of fatmucket and washboard (Table 1). Western pearlshell and some other members of the family Margaritiferidae have geographic distributions at the highest latitudes known for freshwater mussels, up to 55 degrees N for *M. falcata* (Dall 1905, Williams et al. 1993). Possibly the species is adapted to a lower range of temperature than the unionids tested. However, the north and south populations of Fatmucket and Washboard were generally very similar and did not show any consistent difference in LT_{50} that might be predicted from latitude.

Seasonal acclimation had a pronounced effect on LT_{50} . Winter-acclimated washboard from 7°C had LT_{50} approximately 5°C lower than summer-acclimated mussels from 23°C (Tables 2, 5, 6). Previous measurements of acclimation effects in Washboard and other mussels (Table 4) did not use winter-acclimation or a seasonal range of acclimation temperature. For example, Pandolfo et al. (2010) found no effect on Washboard LT_{50} of acclimation to 22 or 27°C (Table 4). Likewise, Archambault (2013, 2014a, 2014b) reported little difference between Fatmucket LT_{50} after acclimation to 22 or 27°C (Table 4). Studies of acclimation in other animals have demonstrated that both season (Segal 1961, Bulger and Tremaine 1985, Hutchison and Richart 1989, Sharma et al 2015) and acclimation (Todd and Dehnel 1960, Cheper 1980, Zeis et al. 2004, Galbraith 2012) can affect thermal tolerance.

Mussels are sometimes exposed to air emersion and temperature stress as a result of drought or because of reservoir operations. Emersion had significant effects on LT_{50} and these interacted with acclimation effects. Summer acclimated Washboard had 2-3°C lower LT_{50} when emersed than when immersed. Interestingly, in winter-acclimated mussels the difference was reversed and LT_{50} was 1-2°C higher in emersed than immersed mussels (Table 2). Archambault et al. (2014a) also measured the effects of emersion on LT_{50} in several species including Fatmucket (Table 4). In those studies emersion effects were generally insignificant (Archambault 2014a)

Temperature Impacts on Other Organisms

Temperature is widely recognized as a fundamental factor in ecology because of its impacts on rate processes. Temperature has been described as the most important abiotic factor for fish because of effects on development, metabolism, gene expression, locomotion, and orientation (Fry 1947, Feder and Hofmann 1999, Beitinger 2000). Aquatic plants react to temperature changes too. Similar to mussels, aquatic vegetation is unlikely to escape introduced thermal stress. Industrial thermal discharge has been shown to alter the distribution of fishes and aquatic plants (Anderson 1969). Aquatic plants have been found to increase respiration at higher temperatures (Anderson 1969). Two species of pondweeds have shown that each leaf has its own ability to accommodate an increase in temperature and that this ability is developed as the leaf matures (Anderson 1969).

During the monitoring period following this experiment, filamentous bacteria and/or a water fungus, likely *Saprolegnia*, were sometimes observed. It should be noted that neither of these organisms appeared to stress the treated mussels.

Other Possible Temperature Interactions

Mussel larvae (glochidia) are parasitic on fish and mussel populations rely on this parasitic stage for dispersal (Barnhart et al. 2008). Glochidia may have increased metamorphic success in cooler water temperatures, perhaps as a result of temperature effects on the host (Roberts and Barnhart 1999). Glochidia also metamorphose quicker at higher temperatures (Roberts and Barnhart 1999).

Unlike benthic organisms such as mussels, fish can easily move from an unfavorable condition or location to a better situation. Most fishes are ectotherms that are quick to seek temperatures below their critical maximum and will even compete for ideal water temperatures (Mundahl 1990; Beitinger 2000). The upper critical maximum for many fishes are higher than are typically found in nature (Mundahl 1990; Beitinger 2000), but a sustained temperature increase may alter the fish assemblage. An organismal change like this can have impacts on other parts of the ecosystem as well. If a mussel is a host-specialist, reproduction of specific mussel species may be disrupted by changes to the fish community. In addition, competition or predation relationships will likely be altered. Fish and mussels found in temperate zones have regulatory systems in place to help adapt to temperatures within certain limits. In addition to temperature

stresses alone, raised water temperatures can exacerbate other stressors for these organisms such as low dissolved oxygen and algal blooms.

Freshwater mussels are ectothermic invertebrates whose body temperatures are dictated by their surroundings. However, factors such as body size, position, and burrowing probably affect temperature stresses. A study of intertidal mussels showed that larger body size provides a type of thermal barrier because small mussels will heat up faster than larger mussels due to less thermal inertia (Helmuth 1998). Another study showed that body size had little impact on body temperature, but position of the mussel within the sediment was significant. Individuals that were buried in the sediment remained cooler during times of heat stress than individuals at the surface or on top of the sediment (Jost and Helmuth 2007). Similar to body size, high density mussel beds can act as a thermal buffer for individuals due to the aggregate acting as a unit, therefore, increasing thermal inertia (Helmuth 1998).

Acclimation and Acclimatization

Acclimation is the process where an individual organism adjusts to a gradual change in its environment (such as temperature, humidity, photoperiod, or pH), allowing it to maintain performance across a range of environmental conditions. Acclimation occurs in short periods of time (days to weeks), and within an organism's lifetime (compared to adaptation which evolves over generations). Animals that are acclimated are responding to artificial or controlled situations (i.e., temperature exposures in an experiment) while acclimatized animals are responding to a natural seasonal change (i.e. shedding of a winter fur with natural seasonal changes).

As far back as the 1960's, fish that had previously been exposed to temperatures near their maximum were described as experiencing a "temperature hardening" (Hutchison 1961, Hutchison & Maness 1979, Maness & Hutchison 1980). In 1974, heat shock proteins were described in *Drosophila* cells (Schlesinger 1990, Tissieres et al. 1974). Heat-shock proteins act as a chaperone to help protect other proteins from improper folding during times of heat stress (Feder and Hofmann 1999, Kregel 2002). Once heat-shock proteins, or Hsps, are expressed their effects drop off gradually. This gradual drop-off allows for an organism to build a tolerance to high temperature conditions (Moseley 1997).

Yet another major factor potentially influencing water temperature is climate change. Although gradual change in global and local patterns of precipitation and temperature is natural, there is abundant evidence that the current rates of change are being affected by human burning of fossil fuels and biomass to CO₂ and the release of methane from landfills and wastewater treatment (Heino 2009; Bates et al. 2008). Although local effects vary widely, global temperatures are rising on average and weather patterns are becoming more severe (Heino 2009; Bates et al. 2008). The Intergovernmental Panel on Climate Change (IPCC) predicts that the global mean temperature will increase by at least 1.5°C by 2100 (IPCC 2014). The U.S. National Oceanic and Atmospheric Administration, or NOAA, measures global temperatures and reports that the recorded 17 hottest years have all taken place in the past 18 years (U.S. NOAA 2016). NOAA also reports that the mean global temperature for 2015 was 0.9°C higher than the average global temperatures of the 20th century (U.S. NOAA 2016).

It is likely that the lotic diversity and abundance variants along latitudinal bands is related to the corresponding changes in temperature regimes (Heino 2009). As climate changes, fauna is expected to shift to higher latitudes and higher elevations in order to adjust to the projected temperature shifts (Shah 2014, IPCC 2014). Shah et al. argue that many organisms will experience minimal negative impacts until 2080, but by 2080 fewer genera will be found in latitudes between 30 - 45°N while more will be found 51 - 70°N (Shah 2014). In contrast, Burgmer et al. suggests that we are already seeing “profound impacts” in lentic macroinvertebrate communities in as short of time periods as 10 – 15 years (Burgmer et al. 2007). It is also suggested that warm-water lentic zooplankton species will begin shifting their range further north (Heino 2009). Burgmer et al. found that macroinvertebrate communities are significantly negatively impacted by increasing temperatures (Burgmer et al. 2007).

Mohensi et al. (1999) modeled the response of stream temperatures to air temperatures from 166 weather stations and 803 stream gaging stations and predicted weekly average stream temperatures under a climate warming scenario based on doubling atmospheric CO₂. The projections showed that on average, there would be a 1-3°C increase in the maximum weekly stream temperatures. However weekly averages may be less important than daily maxima. Over the past few decades most of North America has experienced more unusually hot days and nights and fewer unusually cold days and nights (U.S. Climate Change Science Program, 2008).

As temperatures rise, species that are limited by high temperatures such as *L. compressa* should be monitored for range reduction and/or advancement to the north. Studies that focus on filling in the gaps of which species are currently present or absent

will continue to be useful going forward. Gaining a stronger understanding of community structure and how physical parameters play a role in these structures will allow us to enhance the accuracy of our predictions and make quicker and more informed decisions going forward.

CONCLUSION

My results indicate that some North American native freshwater mussels are more robust to elevated temperature than previously reported. Age, seasonal acclimation, immersion, species, and population all play a significant role in an individual's ability to tolerate thermal stress. These results also show that a small increase in temperature can have detrimental effects. In the future, studying ramped temperature exposures over multiple days would allow us to further simulate natural heating events and gain understanding of their impacts.

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Table 1. Median lethal temperature (LT₅₀, °C) of 0-2 week-old Fatmucket (FM), Washboard (WB) and Western pearlshell acclimated to 22-23°C and tested in thermal cycler. Populations used for these species were Fatmucket: Silver Fork, Bourbeuse, St. Croix, and UMR; Washboard: UMR; and Western Pearlshell: UCR. Data are calculated LT₅₀ ± SE (number of trials) from probit regressions of survival on 6 peak temperatures tested. Mortality was observed on days 0, 1, 2, 7, and 14 following temperature exposures. LT₅₀ estimate for St. Croix FM used data from peak temperature test groups 37 - 40°C, excluding 35 and 36°C groups due to unexplained low survival in the 36°C group.

Day	Silver Fork FM	Bourbeuse FM	St. Croix FM	UMR FM	UMR WB	Western Pearlshell
0	39.5 ± 0.09 (7)	40.8 ± 0.24 (1)	40.5 ± 0.64 (1)	> 40*	39.8 ± 0.11 (3)	> 35*
1	39.3 ± 0.09 (7)	40 – 41*	41.2 ± 0.87 (2)	> 40*	39.7 ± 0.11 (3)	37.1 ± 1.50 (1)
2	39.2 ± 0.09 (7)	40 – 41*	41.2 ± 0.87 (2)	> 40*	39.6 ± 0.12 (3)	36.7 ± 1.30 (1)
7	38.1 ± 0.11 (6) ^a	40 – 41*	40.8 ± 0.65 (2) ^b	41.6 ± 1.25 (1) ^c	39.3 ± 0.15 (3) ^d	35.2 ± 0.92 (1) ^e
14	39.1 ± 0.16 (5) ^a	40 – 41*	> 40*	40.3 ± 1.11 (1) ^b	38.9 ± 0.17 (3) ^c	33.2 ± 0.27 (1) ^d

*Asterisk indicates LT₅₀ could not be calculated with probit because less than two of the test temperatures caused partial mortality.

^{a-e} Different superscript letters within a row indicate significant differences in LT₅₀.

Table 2. LT₅₀ of 1–2 year old summer acclimated (23°C) Washboard and winter acclimatized (7°C) Washboard and Fatmucket tested in immersed and emersed conditions in water bath chambers. Data are LT₅₀ ± SE from probit regressions of survival. The 5 peak temperatures tested with summer acclimated mussels were 37, 38, 39, 40, and 41°C. Winter acclimated mussels were tested at 25, 30, 35, 40, and 45°C. Mortality was observed on days 0, 1, 2, 7, and 14 following temperature exposures.

Day	Immersed		Emersed	
Summer Acclimated Washboard				
	Sac	UMR	Sac	UMR
1	> 41*	> 41*	> 41*	> 41*
2	> 41*	> 41*	38.8 ± 0.23	39.0 ± 0.19
7	41.5 ± 0.51	40.8 ± 0.16	38.2 ± 0.30	38.7 ± 0.19
14	41.3 ± 0.41 ^a	40.8 ± 0.16 ^b	38.2 ± 0.30 ^c	38.7 ± 0.19 ^d
Winter Acclimated Washboard				
	Sac	UMR	Sac	UMR
1	41.7 ± 1.35	44.5 ± 1.92	55.8 ± 8.60	> 45*
2	38.4 ± 0.90	38.6 ± 0.76	41.9 ± 1.19	52.9 ± 7.15
7	36.6 ± 0.76	35 – 40*	35 – 40*	37.4 ± 0.51
14	36.6 ± 0.76 ^e	36.4 ± 0.75 ^{e,g}	35 – 40*	37.4 ± 0.51 ^f
Winter Acclimated Fatmucket				
	St. Croix	UMR	St. Croix	UMR
1	44.8 ± 2.18	47.8 ± 3.77	42.4 ± 1.08	44.5 ± 1.30
2	38.5 ± 0.87	38.6 ± 0.97	39.5 ± 0.73	40.7 ± 0.57
7	37.4 ± 0.80	36.6 ± 0.80	35 – 40*	35 – 40*
14	36.1 ± 0.76 ^g	36.1 ± 0.78 ^g	35 – 40*	37.7 ± 0.66 ^f

*Asterisk indicates LT₅₀ could not be calculated with probit because less than two of the test temperatures caused partial mortality.

^{a–g} Different superscript letters indicate significant differences in LT₅₀ between species and/or populations on Day 14.

Table 3. LT₅₀ of 2–3 year old Washboard (UMR) and Fatmucket (St. Croix) acclimated to 23°C and tested in immersed and emersed conditions in environmental chambers. Data are calculated LT₅₀ ± SE from probit regressions of survival on 5 peak temperatures tested (25, 30, 35, 40, or 45°C). Different superscript letters within a row indicate significant differences in LT₅₀. Mortality was observed on days 0, 1, 2, 7, and 14 following temperature exposures.

Day	Immersed		Emersed	
	Washboard	Fatmucket	Washboard	Fatmucket
0	42.0 ± 1.12	40 – 42*	40.0 ± 0.61	36.6 ± 0.98
1	39.9 ± 0.42	38.4 ± 0.39	39.3 ± 0.55	36 -38*
2	39.2 ± 0.32	36.7 ± 0.41	36 – 38*	36*
7	38.6 ± 0.38	36*	36 – 38*	< 36*
14	38.4 ± 0.39	36*	36 – 38*	< 36*

*Asterisk indicates LT₅₀ could not be calculated with probit because less than two of the test temperatures caused partial mortality.

Table 4. Previous reports of LT₅₀ and CTM in freshwater mussels. LT₅₀ tests used constant temperature exposures. Critical thermal maximum (CTM) was measured by increasing test temperature 0.35°C per minute until persistent gaping was observed. Age is classified by: m – newly metamorphosed, j – juvenile, s – sub-adult, or a – adult.

Study Pandolfo et al., 2010		Ganser et al., 2013	Galbraith et al., 2014	Archambault et al., 2013 - 2014a,b							
Exposure	96 h	28 d	47 - 92 min. ramped	96 h							
Aerated	48 h (90%) renewal	Yes	Yes	48 h (90%) renewal							
Mean	LT50 (96h)	LT50 (7 d, 14 d, 28 d)	CTM	LT50 (96 h)							
Conditions	Water-only		Water-only	Water-only		Water-only		Water & Sediment		Dewatered Sediment	
Acclimation	22°C	27°C	18°C	15°C	25°C	22°C	27°C	22°C	27°C	22°C	27°C
<i>M. nervosa</i>	34.2-j	34-j	35.6, 30.8, 30.3-j	-	-	-	-	-	-	-	-
<i>L. siliquioidea</i>	35.6-s	34.4-s	32.5, 30.1, 25.3-j	-	-	-	-	33.3-j	36.0-j	35.4-j	35.3-j
<i>P. alatus</i>	35-s	34.1-s	-	-	-	-	-	-	-	-	-
<i>L. recta</i>	32.5-s	35.1-s	-	-	-	-	-	-	-	-	-
<i>E. lineolata</i>	38.8-j	33.1-j	-	-	-	-	-	-	-	-	-
<i>A. varicosa</i>	35-j	36.1-j	-	39.5-a	41.1-a	-	-	-	-	-	-
<i>V. delumbis</i>	34.6-j	34.2-j	-	-	-	-	-	-	-	-	-
<i>L. abrupta</i>	-	-	33.6, 27.2-j	-	-	34.8-m	34.9-m	31.9-j	36.5-j, 33.3-j	35.1-j	35.0-j, 35.6-j
<i>E. complanata</i>	-	-	-	42.7-a	41.4-a	-	-	-	-	-	-
<i>S. undulatus</i>	-	-	-	40.0-a	42.3-a	-	-	-	-	-	-
<i>L. fasciola</i>	-	-	-	-	-	-	-	34.4-a	34.7-a	33.7-a	33.7-a
<i>A. plicata</i>	-	-	-	-	-	36.4-j	36.4-j	-	37.2-j	-	35.3-j
<i>L. cariosa</i>	-	-	-	-	-	36.8-j	35.5-j	-	36.5-j	35.1-j	35.5-j
<i>L. radiata</i>	-	-	-	-	-	-	-	29.9-j	31-j	34.8-j	-

Table 5. Combined LT₅₀ results from this study. I – Immersed, E – Emerged. Asterisk* indicates LT₅₀ could not be calculated with probit because less than two of the test temperatures caused partial mortality. Numbers are mean ± SE (number of experiments).

Mortality was observed on days 0, 1, 2, 7, and 14 following temperature exposures.

Species	Age	Acclimation	Aerated	Condition	Population	Day 0	p	Day 1	p	Day 2	p	Day 7	p	Day 14	p
Fatmucket (<i>L. siliquioidea</i>)	< 21 d	23°C	N	I	Silver Fork	39.5 ± 0.10 (7)	0.00	39.3 ± 0.09 (7)	0.00	39.2 ± 0.09 (7)	0.00	38.1 ± 0.11 (6)	0.00	39.1 ± 0.16 (5)	0.00
					Bourbeuse	40.8 ± 0.24 (1)	0.00	< 41*	-	< 41*	-	< 41*	-	< 41*	-
					St. Croix	40.5 ± 0.64 (1)	0.03	41.2 ± 0.87 (2)	0.01	41.2 ± 0.87 (2)	0.01	40.8 ± 0.65 (2)	0.00	> 40*	-
					UMR	> 40*	-	> 40*	-	> 40*	-	41.6 ± 1.25	0.01	40.3 ± 1.11	0.01
	1 - 2 y	7°C	Y	I	St. Croix	-	-	44.8 ± 2.18	0.00	38.5 ± 0.87	0.00	37.4 ± 0.80	0.00	36.1 ± 0.76	0.00
					UMR	-	-	47.8 ± 3.77	0.00	38.6 ± 0.97	0.00	36.6 ± 0.80	0.00	36.1 ± 0.78	0.00
					St. Croix	-	-	42.4 ± 1.08	0.00	39.5 ± 0.73	0.00	35 - 40*	-	35 - 40*	-
					UMR	-	-	44.5 ± 1.30	0.00	40.7 ± 0.57	0.00	35 - 41*	-	37.7 ± 0.65	0.00
	2-3 y	23°	N	I	St. Croix	40 - 42*	-	38.4 ± 0.39	0.00	36.7 ± 0.41	0.00	~ 36*	-	~ 36*	-
				E	St. Croix	36.6 ± 0.98	0.01	36 - 38*	-	~ 36*	-	< 36*	-	< 36*	-
Washboard (<i>M. nervosa</i>)	< 14 d	22°C	N	I	UMR	39.8 ± 0.11	0.00	39.7 ± 0.11	0.00	39.6 ± 0.12	0.00	39.3 ± 0.15	0.00	38.9 ± 0.17	0.00
					Sac	> 41*	-	> 41*	-	> 41*	-	41.5 ± 0.51	0.00	41.3 ± 0.41	0.00
					UMR	> 41*	-	> 41*	-	> 41*	-	40.8 ± 0.16	0.00	40.8 ± 0.16	0.00
					Sac	> 41*	-	> 41*	-	38.8 ± 0.23	0.00	38.2 ± 0.30	0.00	38.2 ± 0.30	0.00
	1 - 2 y	23°C	Y	E	UMR	> 41*	-	> 41*	-	39.0 ± 0.19	0.00	38.7 ± 0.19	0.00	38.7 ± 0.19	0.00
					Sac	-	-	41.7 ± 1.35	0.00	38.4 ± 0.90	0.00	36.6 ± 0.76	0.00	36.6 ± 0.76	0.00
					UMR	-	-	44.5 ± 1.92	0.00	38.6 ± 0.76	0.00	35 - 40*	-	36.4 ± 0.75	0.00
					Sac	-	-	55.8 ± 8.60	0.01	41.9 ± 1.19	0.00	35 - 40*	-	35 - 40*	-
					UMR	-	-	35 - 40*	-	52.9 ± 7.15	0.01	37.4 ± 0.51	0.00	37.4 ± 0.51	0.00
	2 - 3 y	23°C	N	I	UMR	42.0 ± 1.12	0.01	39.9 ± 0.42	0.00	39.2 ± 0.32	0.00	38.6 ± 0.38	0.00	38.4 ± 0.39	0.00
				E	UMR	40.0 ± 0.61	0.00	39.3 ± 0.55	0.00	36 - 38*	-	36 - 38*	-	36 - 38*	-
Western pearlshell (<i>M. falcata</i>)	< 7 d	22°C	N	I	Upper Columbia	> 35*	-	37.1 ± 1.50	0.00	36.7 ± 1.30	0.00	35.2 ± 0.92	0.00	33.2 ± 0.27	0.00

Table 6. LT₅₀ comparisons. Top numbers are ratio method test statistic of LT₅₀s, bottom numbers are the difference between the paired LT₅₀ values. Data are calculated LT₅₀ ± SE (n) from probit regressions of survival on 6 peak temperatures tested. Species: WP – Western Pearlshell, FM – Fatmucket, WB – Washboard. Populations: UC - Upper Columbia River, St. C - St. Croix, UMR – Upper Mississippi River. Conditions: I – immersed and E – emersed. Mortality measured on Day 14 after test.

Experiment	Age	Species	Population	Acclimation	Conditions	LT ₅₀ (Day 14)	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
A	< 2 wk	WP	UC	23°C	I	33.2 ± 0.27 (1)	-	0.083*	0.084*	0.093*	0.100*	0.121*	0.128*	0.143*	0.147*	0.155*	0.160*	0.164*	0.194*	0.208*	0.219*
B	1 - 2 y	FM	St. C	7°C	I	36.0 ± 0.76 (1)	2.9*	-	0.001	0.010	0.016*	0.037*	0.044*	0.059*	0.064*	0.072*	0.076*	0.080*	0.111*	0.125*	0.135*
C	1 - 2 y	FM	UMR	7°C	I	36.1 ± 0.78 (1)	2.9*	0.0	-	0.009	0.015	0.036*	0.043*	0.058*	0.063*	0.071*	0.075*	0.079*	0.110*	0.124*	0.134*
D	1 - 2 y	WB	UMR	7°C	I	36.4 ± 0.75 (1)	3.2*	0.3	0.3	-	0.007	0.028*	0.035*	0.050*	0.054*	0.062*	0.067*	0.071*	0.101*	0.115*	0.126*
E	1 - 2 y	WB	Sac	7°C	I	36.6 ± 0.76 (1)	3.5*	0.6*	0.6	0.2	-	0.021*	0.028*	0.043*	0.047*	0.055*	0.060*	0.064*	0.095*	0.108*	0.119*
F	1 - 2 y	WB	UMR	7°C	E	37.4 ± 0.51 (1)	4.3*	1.4*	1.3*	1.0*	0.8*	-	0.007	0.022*	0.026*	0.034*	0.039*	0.043*	0.074*	0.087*	0.098*
G	1 - 2 y	FM	UMR	7°C	E	37.7 ± 0.66 (1)	4.5*	1.6*	1.6*	1.3*	1.0*	0.3	-	0.015*	0.019*	0.027*	0.032*	0.036*	0.067*	0.080*	0.091*
H	1 - 2 y	WB	Sac	23°C	E	38.2 ± 0.30 (1)	5.1*	2.2*	2.2*	1.8*	1.6*	0.8*	0.6*	-	0.004	0.012*	0.017*	0.021*	0.052*	0.065*	0.076*
I	2 - 3 y	WB	UMR	23°C	I	38.4 ± 0.39 (1)	5.3*	2.4*	2.3*	2.0*	1.8*	1.0*	0.7*	0.2	-	0.008*	0.013*	0.017*	0.047*	0.061*	0.072*
J	1 - 2 y	WB	UMR	23°C	E	38.7 ± 0.19 (1)	5.6*	2.7*	2.6*	2.3*	2.1*	1.3*	1.0*	0.5*	0.3*	-	0.004*	0.009*	0.039*	0.053*	0.064*
K	< 2 wk	WB	UMR	23°C	I	38.9 ± 0.17 (3)	5.7*	2.9*	2.8*	2.5*	2.3*	1.5*	1.2*	0.7*	0.5*	0.2*	-	0.004*	0.035*	0.048*	0.059*
L	< 2 wk	FM	St. C	23°C	I	39.1 ± 0.16 (5)	5.9*	3.0*	3.0*	2.7*	2.4*	1.7*	1.4*	0.8*	0.7*	0.3*	0.2*	-	0.031*	0.044*	0.055*
M	< 2 wk	FM	UMR	23°C	I	40.3 ± 1.11 (1)	7.1*	4.2*	4.2*	3.9*	3.6*	2.9*	2.6*	2.0*	1.9*	1.6*	1.4*	1.2*	-	0.014	0.024*
N	1 - 2 y	WB	UMR	23°C	I	40.8 ± 0.16 (1)	7.7*	4.8*	4.7*	4.4*	4.2*	3.4*	3.2*	2.6*	2.4*	2.1*	1.9*	1.8*	0.6	-	0.011*
O	1 - 2 y	WB	Sac	23°C	I	41.3 ± 0.41 (1)	8.1*	5.2*	5.2*	4.9*	4.6*	3.9*	3.6*	3.0*	2.9*	2.5*	2.4*	2.2*	1.0*	0.4*	-

*Asterisk indicates significant difference (p < 0.05)

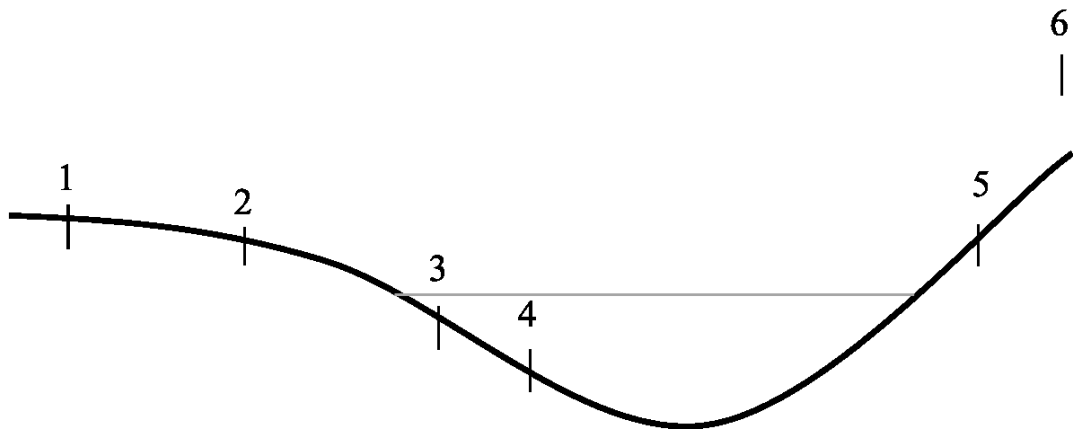


Figure 1. Placement of data loggers across the Sac River in August 2014. 1.) West bank: buried 4-5 cm, in dry sand, 2 m from water line. 2.) West bank: buried 4-5 cm in moist sand, 1 m from water line. 3.) Channel: Submerged 15 cm, buried in 4-5 cm in substrate. 4.) Channel: Submerged 50 cm, buried in 4-5 cm in substrate. 5.) East bank: buried 4-5 cm in moist sand, 0.5 m from water line. 6.) East bank: in air at about 1-m above ground level.

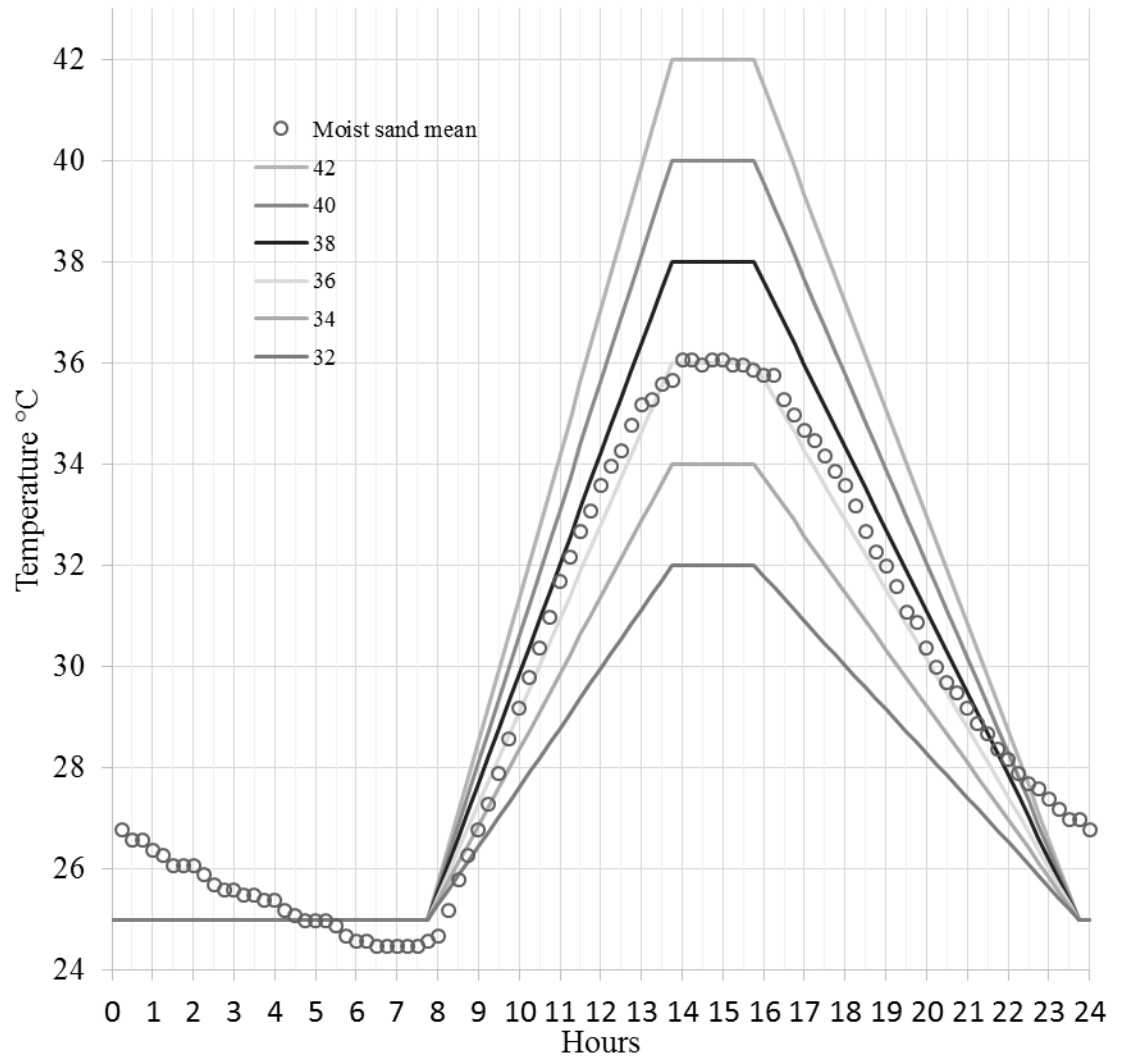


Figure 2. Moist sand field temperature compared to experimental peak temperature range.

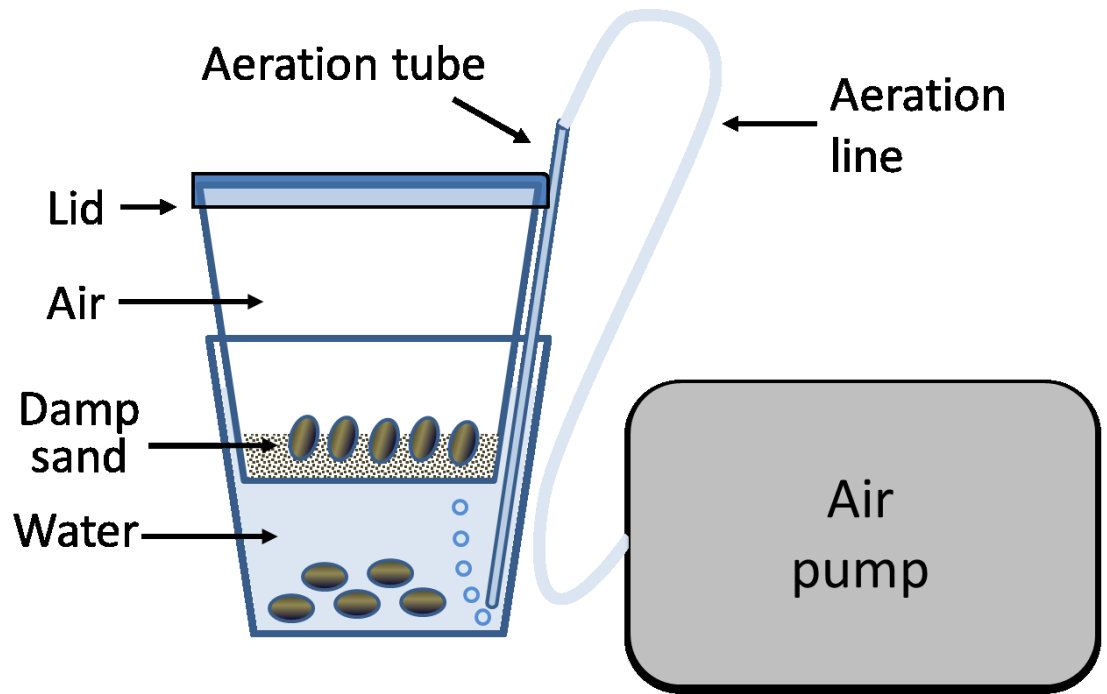


Figure 3. Diagram of nested chambers used to provide immersed and emersed conditions in the water bath experiments. The chamber assemblies were partly submerged in covered, temperature-controlled water baths to provide temperature control.

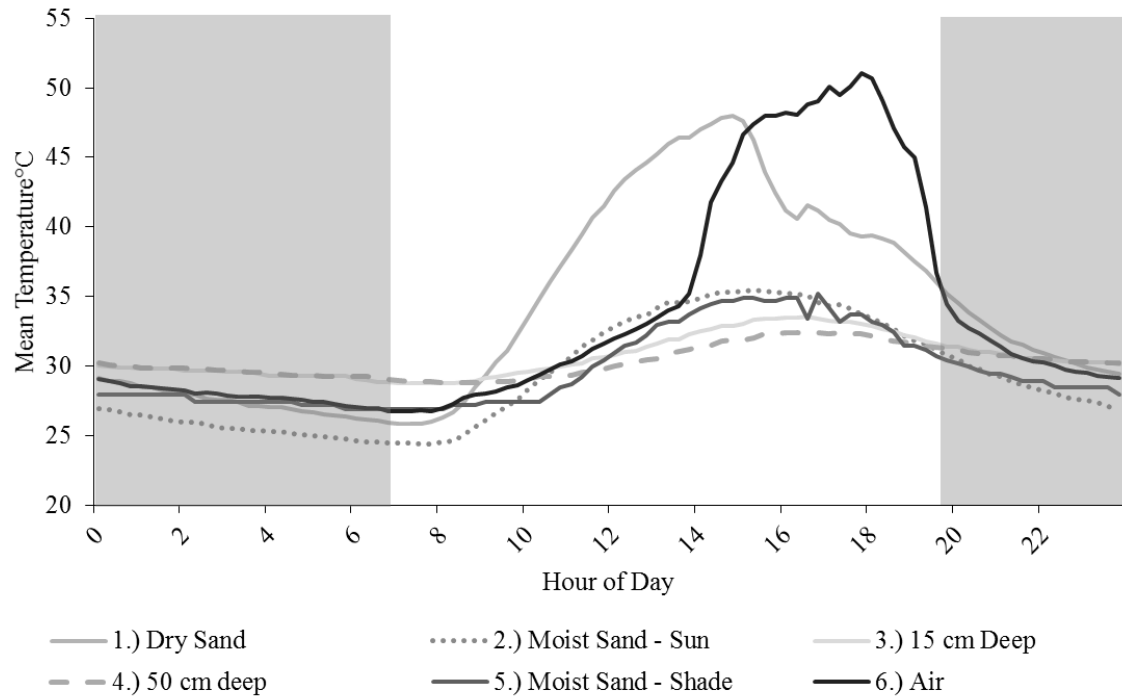


Figure 4. Temperature recordings at transect intervals along the Sac River. Temperature data loggers placed at six locations along a transect of the Sac River during August 21 - 28, 2014. Temperature was recorded for 7 days and nights at 15 minute intervals. The seven diel records were averaged at each time interval. Median sunrise was 6:38 a.m. and median sundown was 7:53 p.m. during this week and is noted by shading. The transect locations are described here: 1.) West bank: buried 4-5 cm, in dry sand, 2 m from water line. 2.) West bank: buried 4-5 cm in moist sand, 1 m from water line. 3.) Channel: Submerged 15 cm, buried in 4-5 cm in substrate. 4.) Channel: Submerged 50 cm, buried in 4-5 cm in substrate. 5.) East bank: buried 4-5 cm in moist sand, 0.5 m from water line. 6.) East bank: in air at about 1-m above ground level.

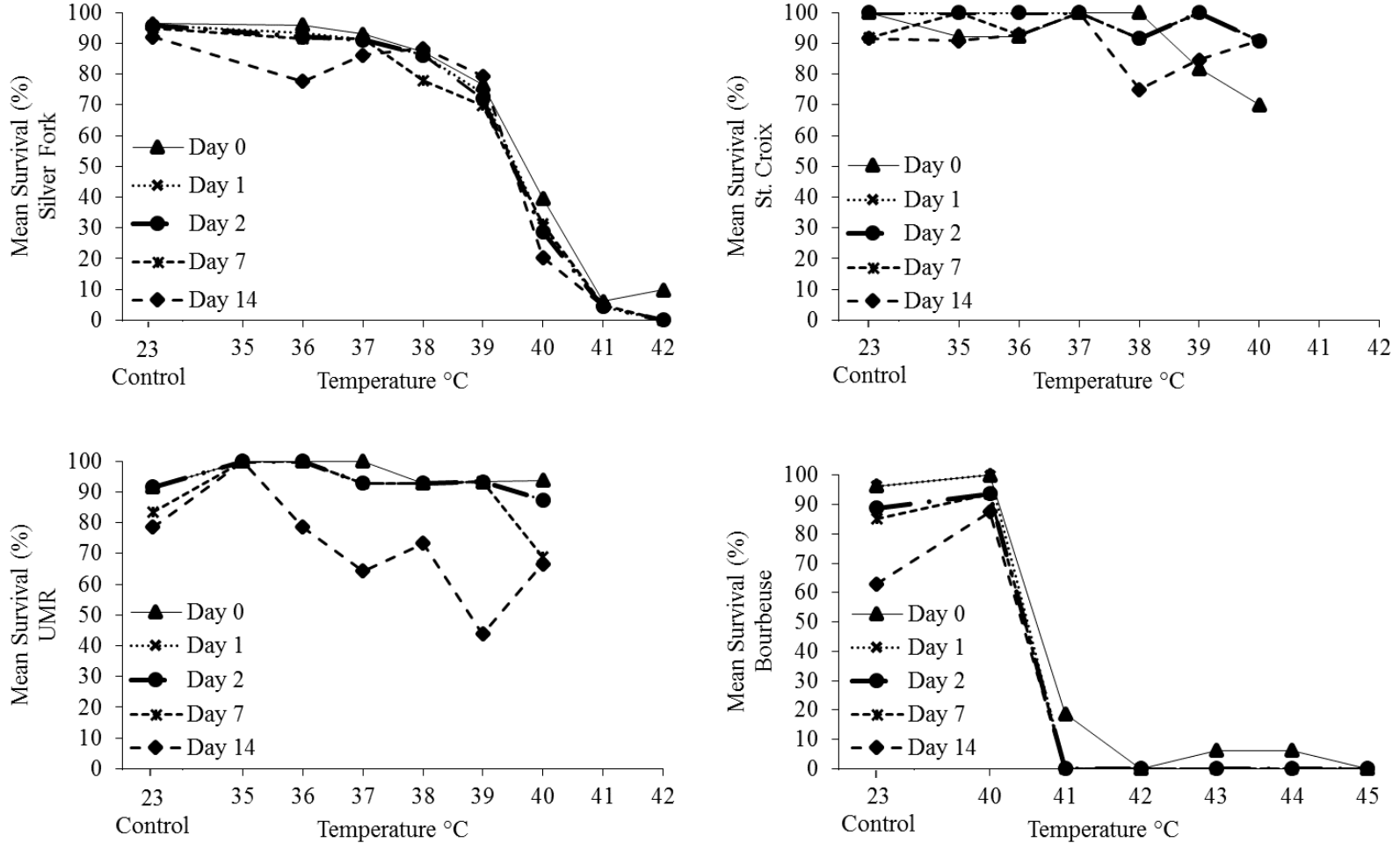


Figure 5. Fatmucket (0-3 week old) acclimated to 23°C from population (n): Silver Fork (7), St. Croix (2), UMR (1), and Bourbeuse (1). Each point represents the mean survival of 14 - 28 individuals in 7 test groups. Symbols indicate the day of observation following the temperature exposure.

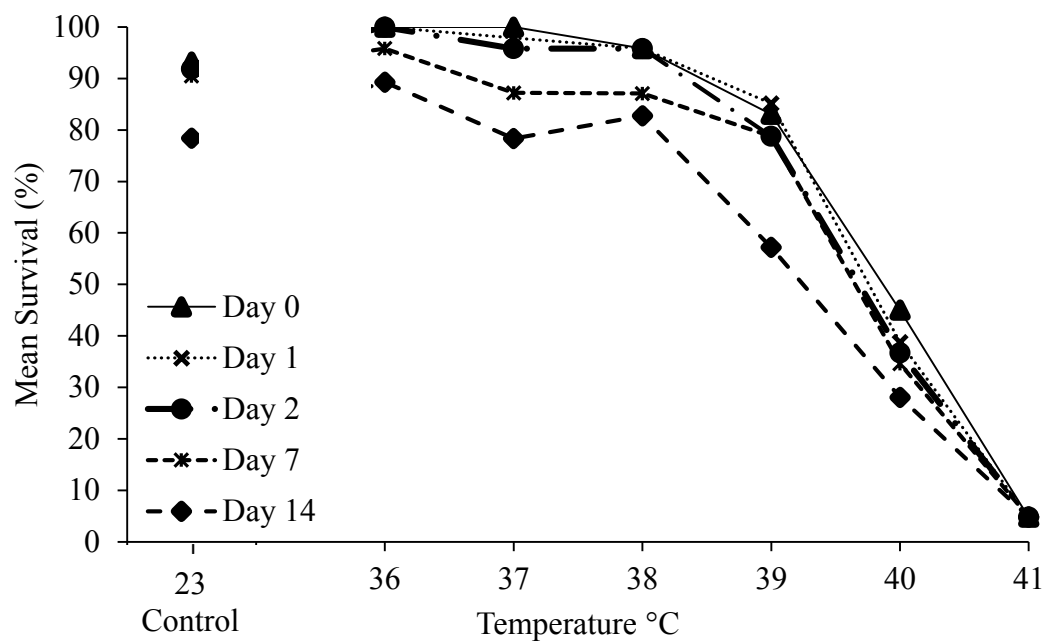


Figure 6. Washboard from Upper Mississippi River (0-2 week old) acclimated to 23°C. Each point represents the mean survival of 13 - 25 individuals in 3 test groups. Symbols indicate the day of observation following the temperature exposure.

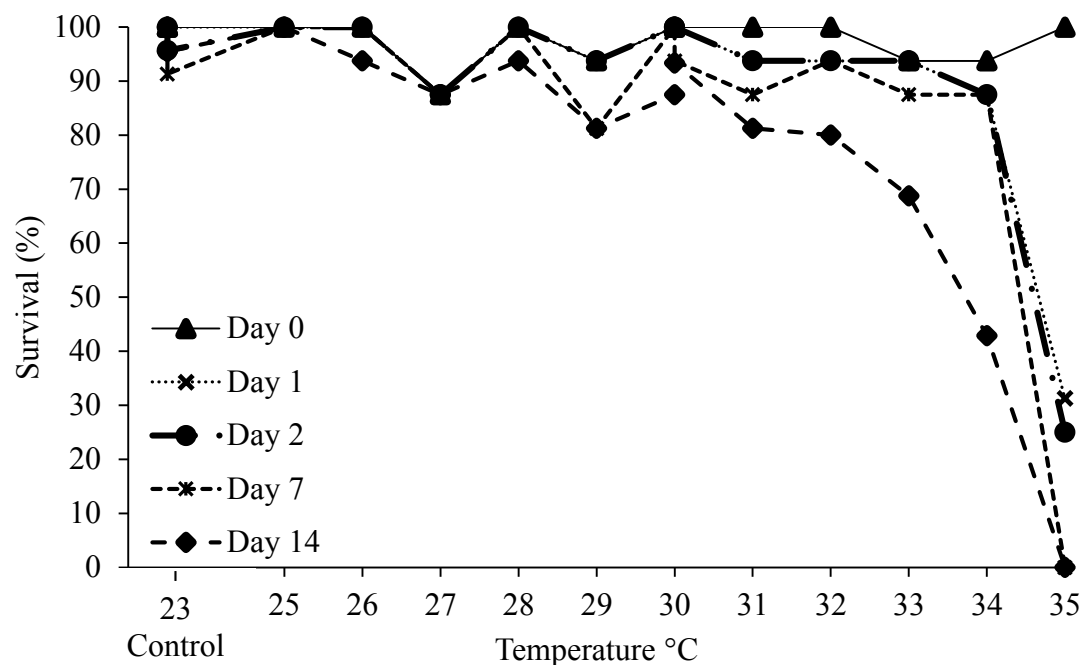


Figure 7. Western Pearlshell from Upper Columbia River (< 48 hours old) acclimated to 23°C tested in thermal cyler. Each point represents the mean survival of 13 - 23 individuals in 1 test group, tests overlapped at 30°C which has 2 test groups. Symbols indicate the day of observation following the temperature exposure.

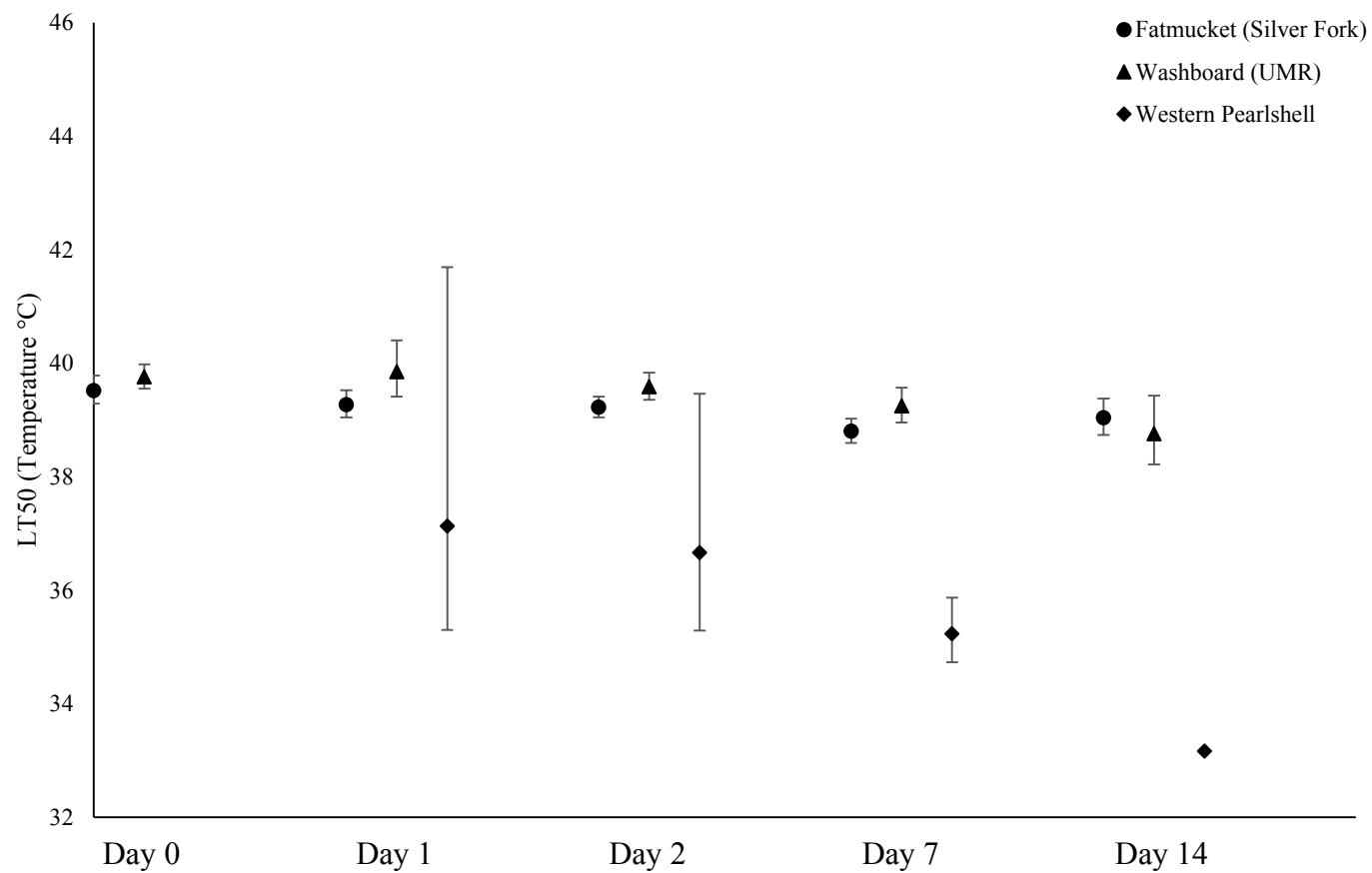


Figure 8. LT₅₀ and 95% confidence intervals of < 3 week old Fatmucket, Washboard, and Western Pearlshell.

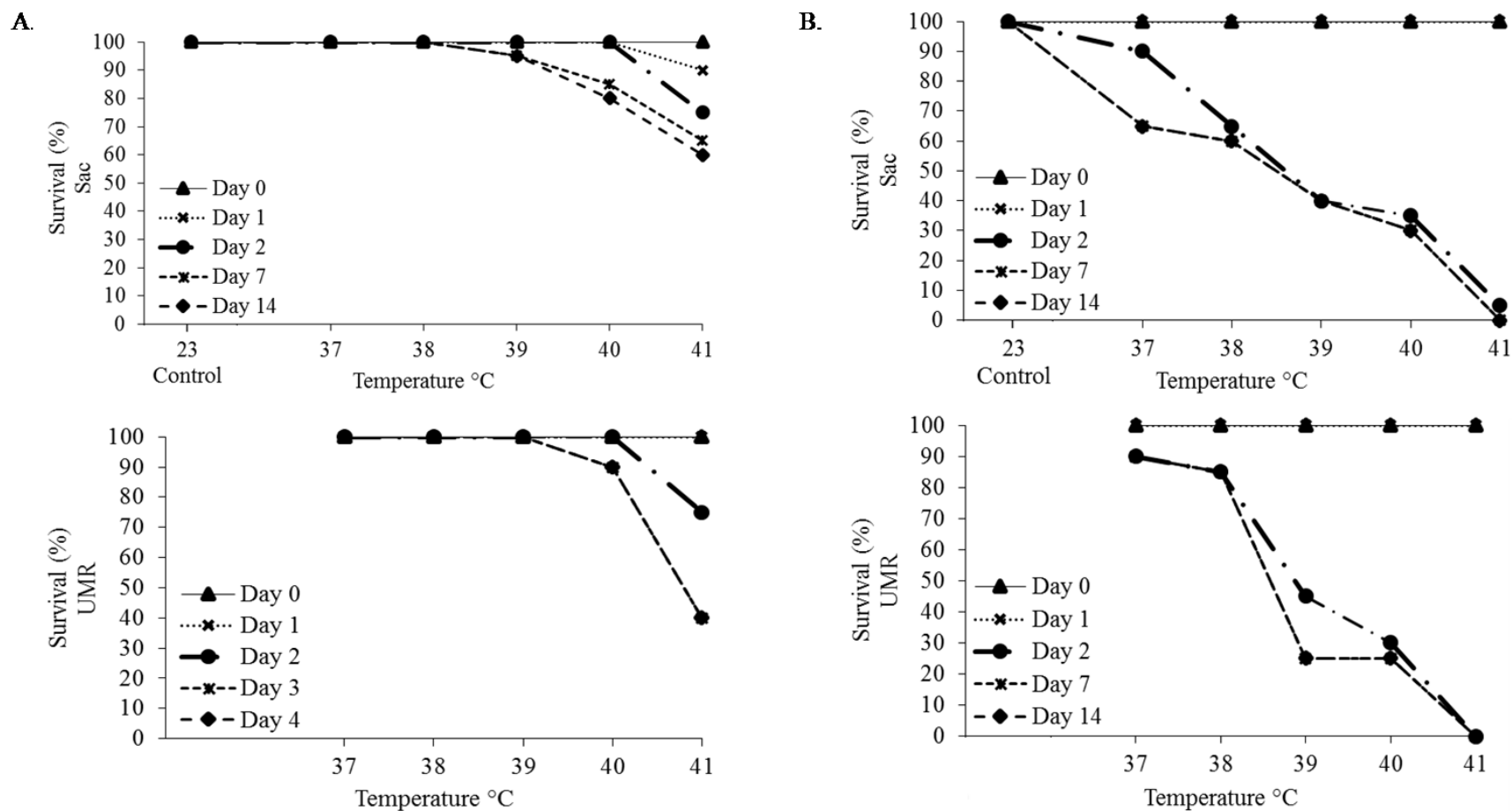


Figure 9. Washboard, Sac River and UMR, acclimated to 23°C. Panel A – Immersed, B – Emerged. Each point represents the mean survival of 20 individuals in 1 test group, 5 individuals in the control group (Sac River population only). Symbols indicate the day of observation following the temperature exposure in water bath.

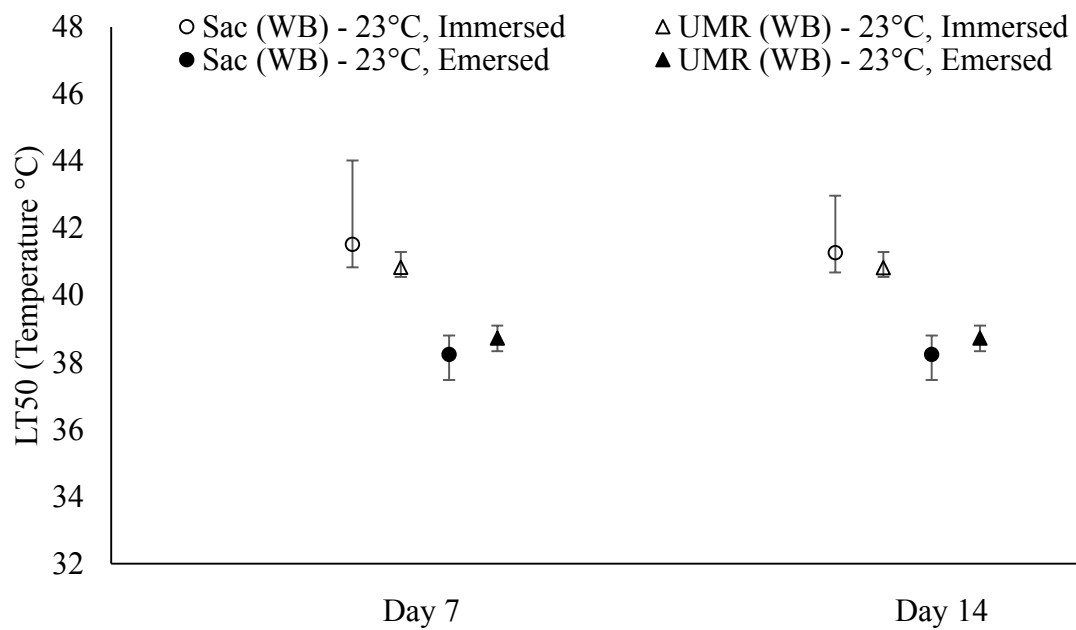


Figure 10. LT₅₀ and 95% confidence intervals of 1–2 year old Washboard, Sac and UMR in immersed and emersed conditions, acclimated to 23°C.

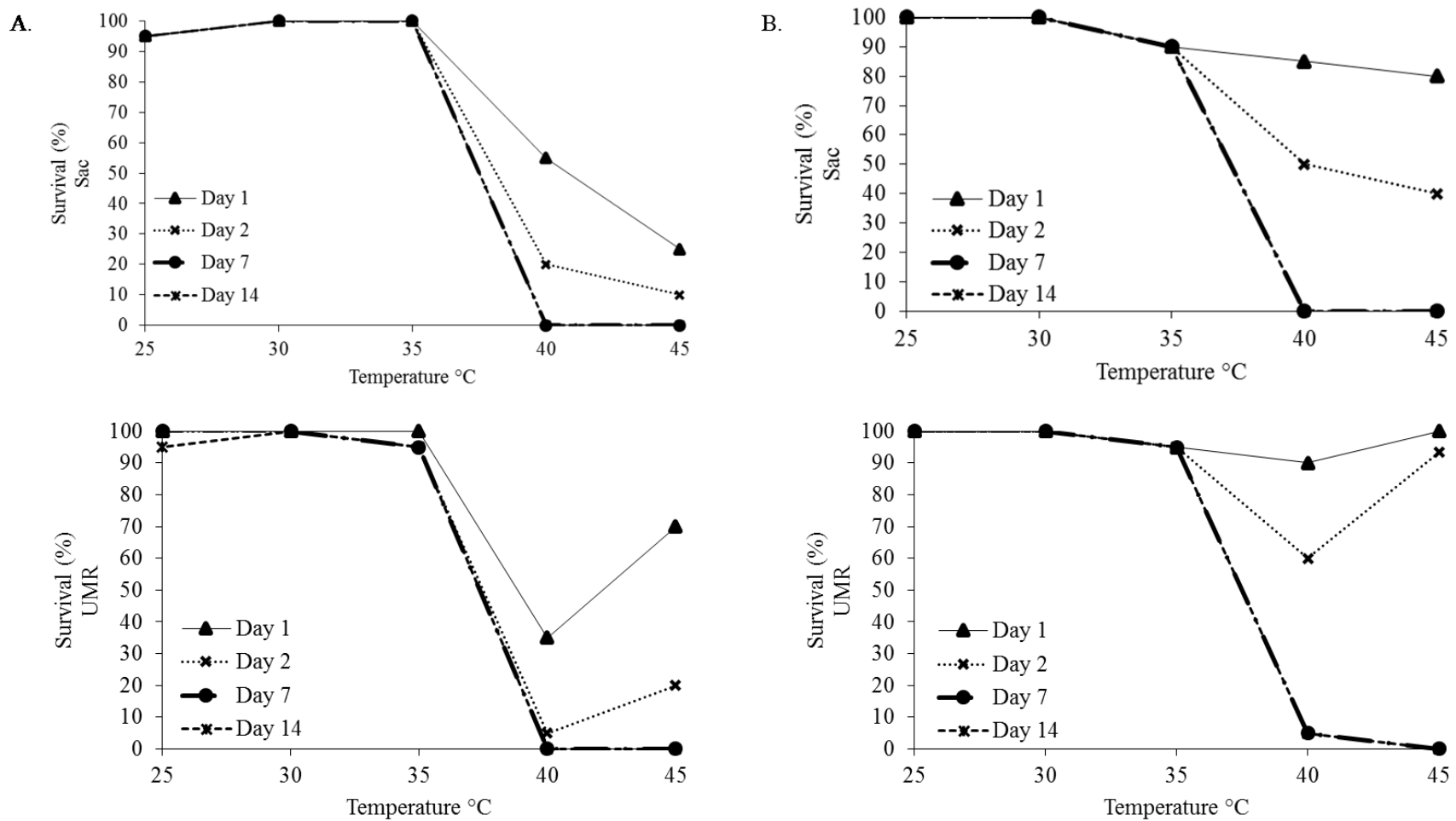


Figure 11. Washboard, Sac River and UMR, (1- 2 year old) acclimatized to 7°C. Panel A – Immersed, B – Emersed. Each point represents the mean survival of 20 individuals in 1 test group. Symbols indicate the day of observation following the temperature exposure in water bath.

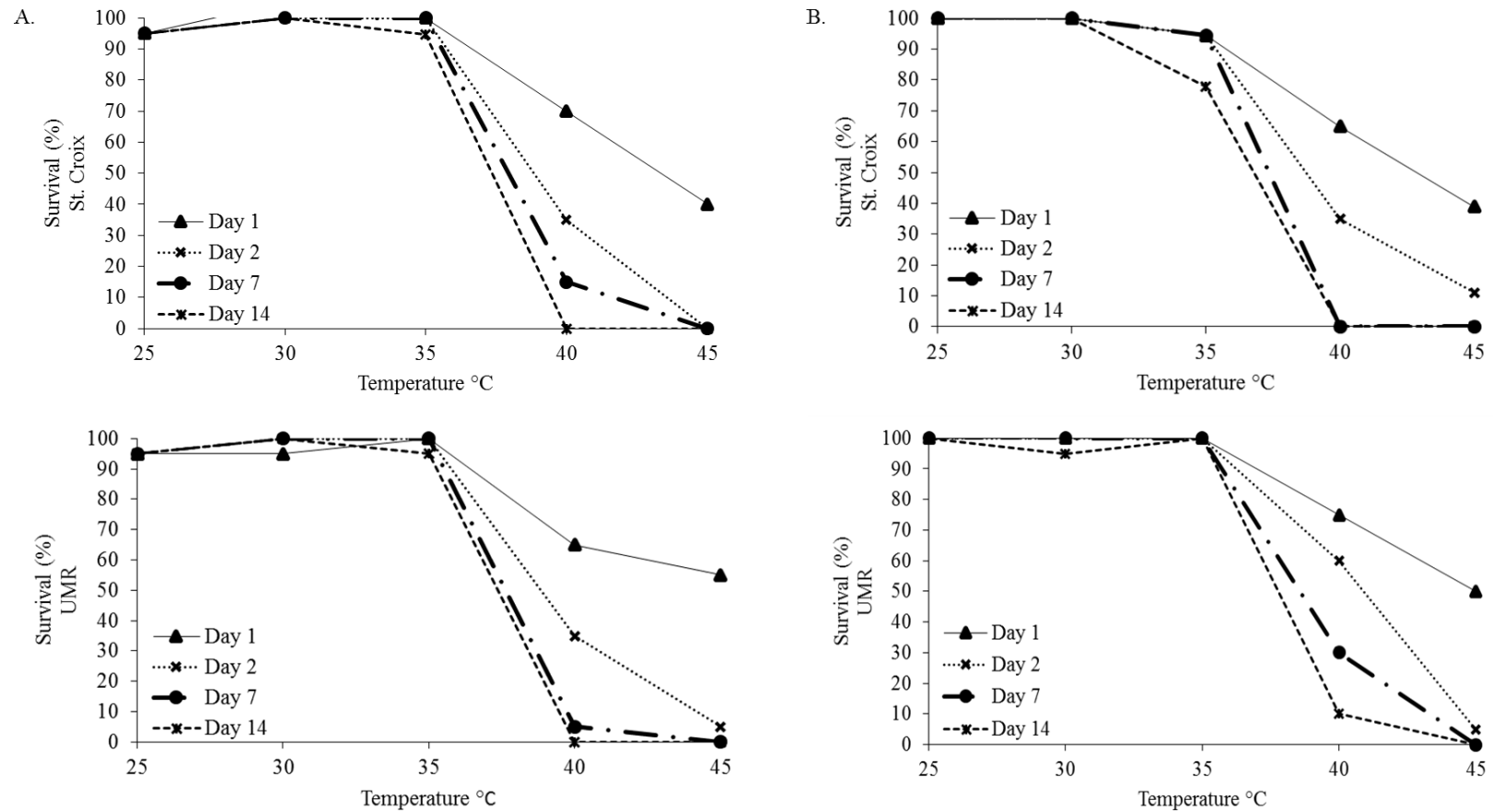


Figure 12. Fatmucket, St. Croix and UMR, (1- 2 year old) acclimated to 7°C. Panel A – Immersed, B – Emersed. Each point represents the mean survival of 18 - 20 individuals in 1 test group. Symbols indicate the day of observation following the temperature exposure in water bath.

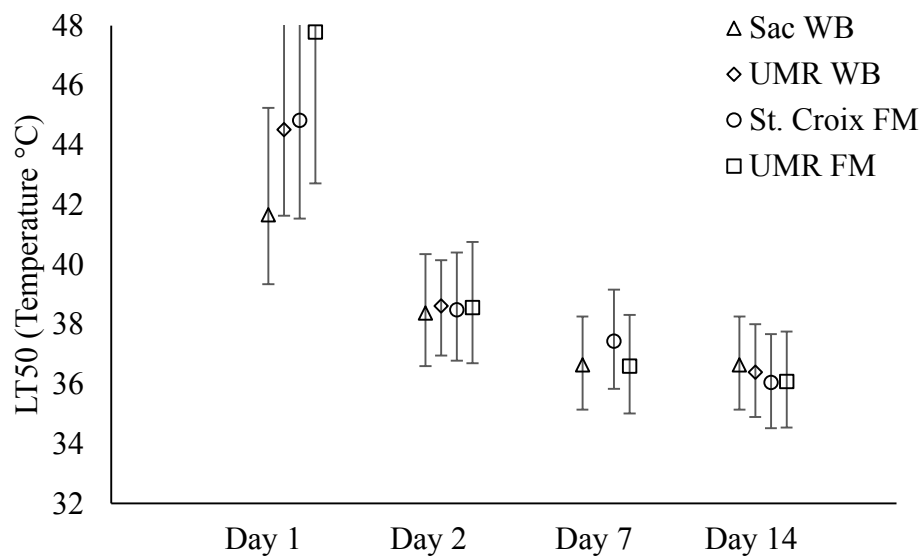


Figure 13. LT₅₀ and 95% confidence intervals of 1–2 year old Washboard, Sac and UMR, and Fatmucket, St. Croix and UMR, in immersed conditions, acclimated to 7°C. LT₅₀ could not be calculated for UMR WB – Day 7.

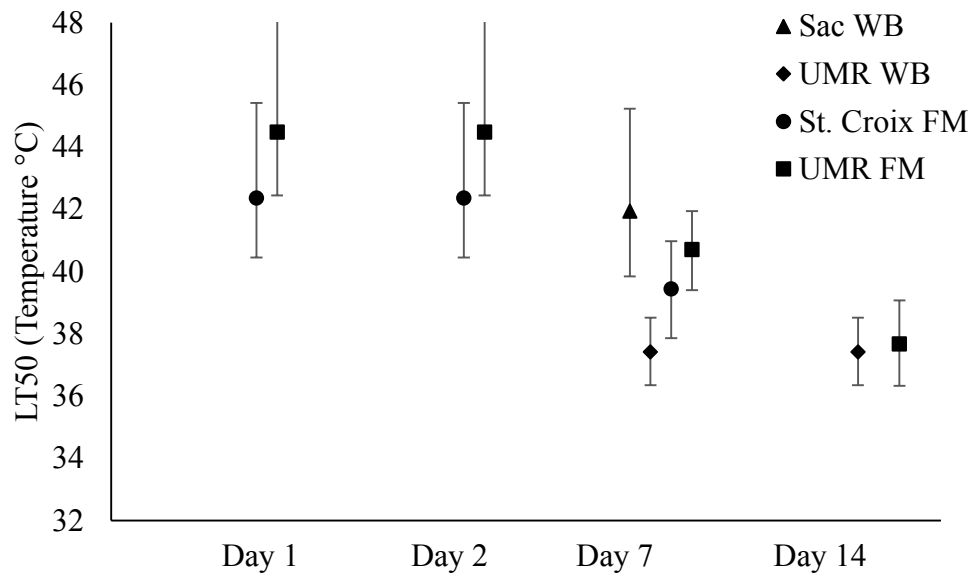


Figure 14. LT₅₀ and 95% confidence intervals of 1–2 year old Washboard, Sac and UMR, and Fatmucket, St. Croix and UMR, in emersed conditions, acclimated to 7°C. LT₅₀s could not be calculated for: Sac WB - Days 1, 7, and 14; UMR WB – Days 1 and 2; and St. Croix FM – Day 14.

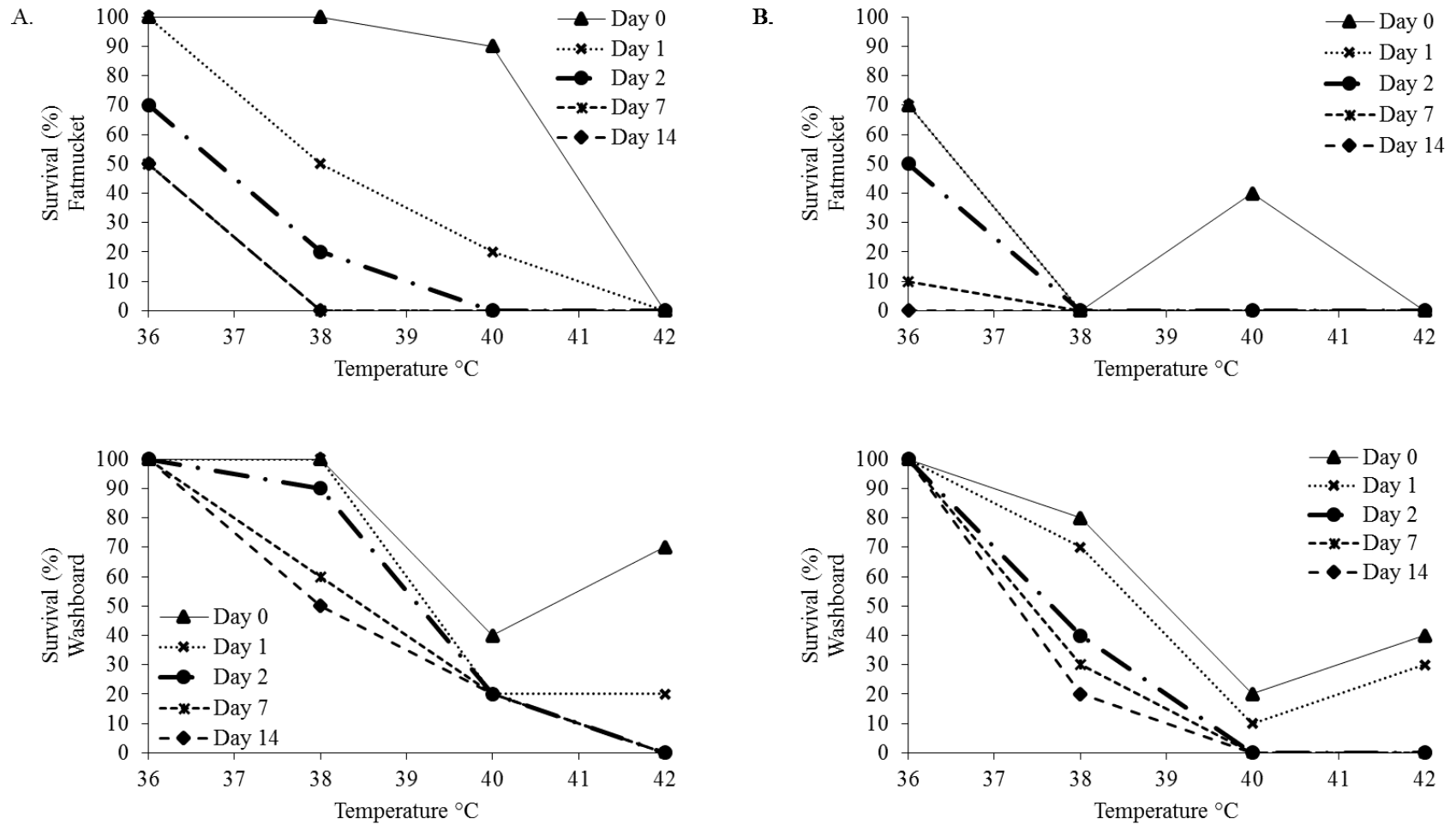


Figure 15. Fatmucket, St. Croix, and Washboard, UMR - Immersed, (2 - 3 year old) acclimated to 23°C. Panel A – Immersed, B – Emerged. Each point represents the mean survival of 10 individuals in 1 test group. Symbols indicate the day of observation following the temperature exposure in water bath.

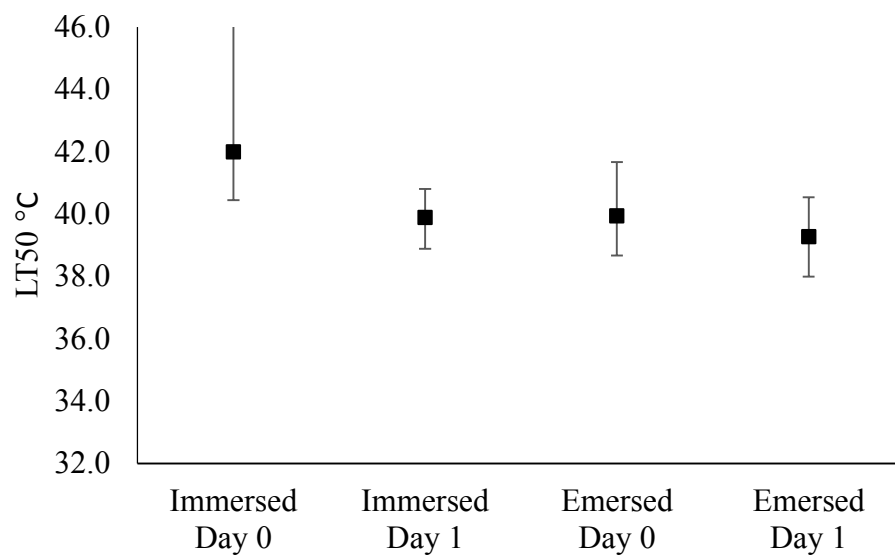


Figure 16. LT₅₀ and 95% confidence intervals of 2–3 year old Washboard, UMR, in immersed and emersed conditions, acclimated to 23°C. LT₅₀s could not be calculated for Days 2, 7, and 14.

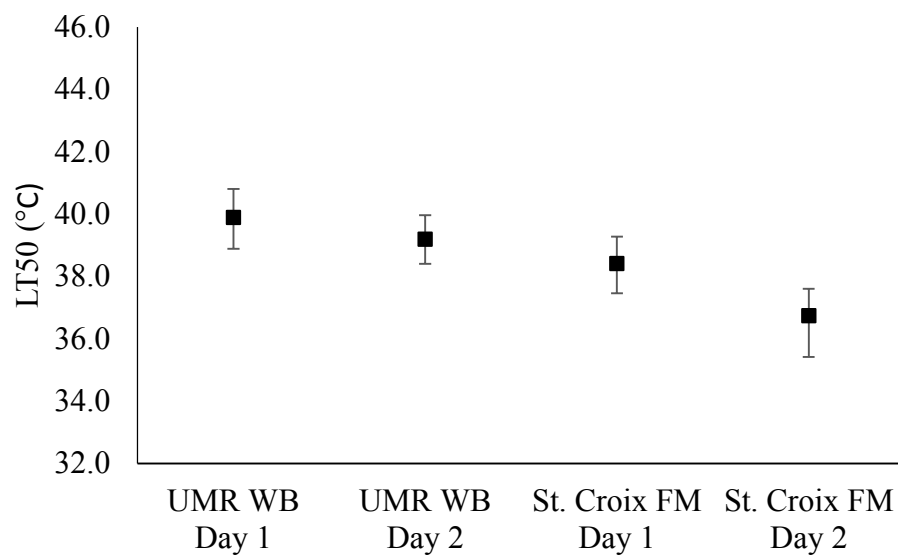


Figure 17. LT₅₀ and 95% confidence intervals of 2–3 year old Washboard, UMR, and Fatmucket, St. Croix, in immersed conditions, acclimated to 23°C. LT₅₀s could not be calculated for Days 0, 7, and 14.

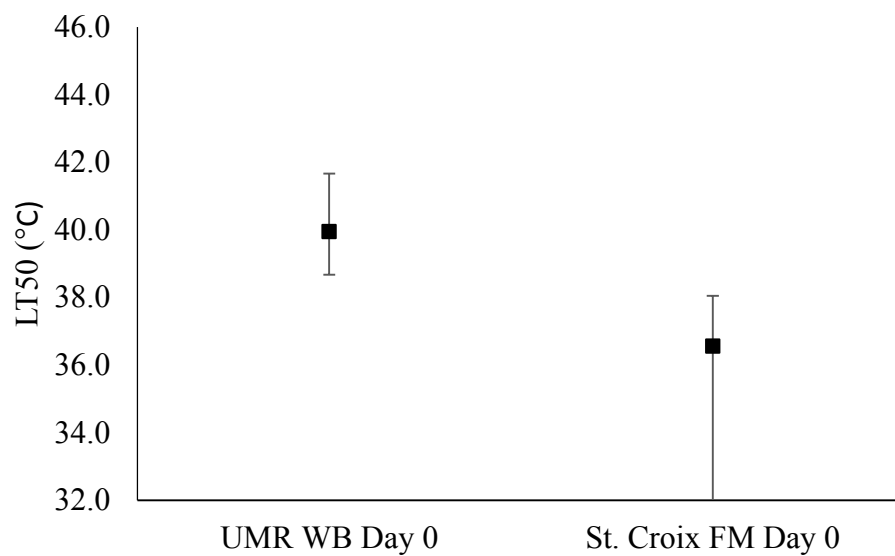


Figure 18. LT₅₀ and 95% confidence intervals of 2-3 year old Washboard, UMR, and Fatmucket, St. Croix, in emersed conditions, acclimated to 23°C. LT₅₀s could not be calculated for Days 1, 2, 7, and 14.

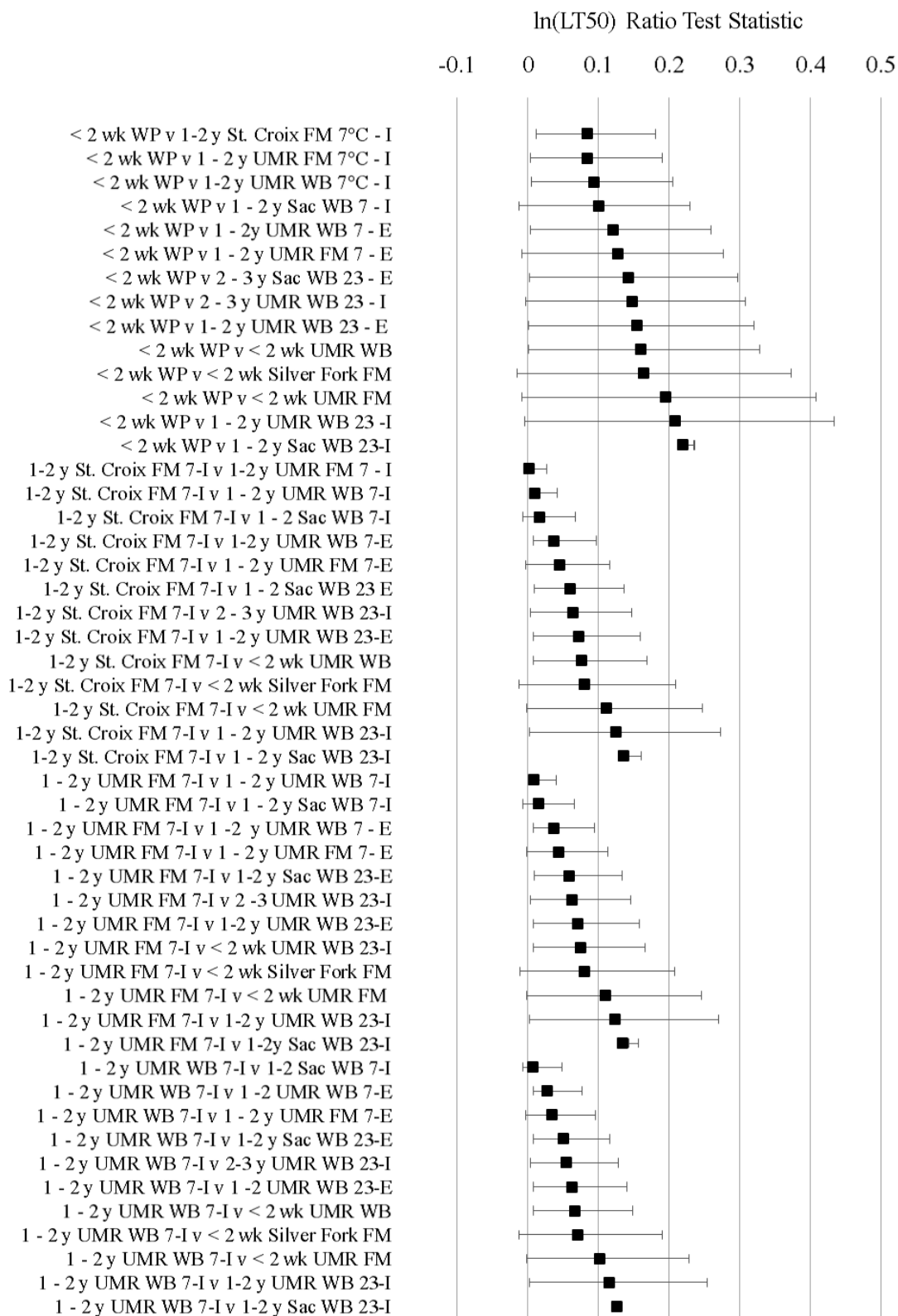


Figure 19a. – ln(LT₅₀) Ratio Test Confidence Intervals. The LT₅₀s are not statistically different when the confidence interval includes 0.

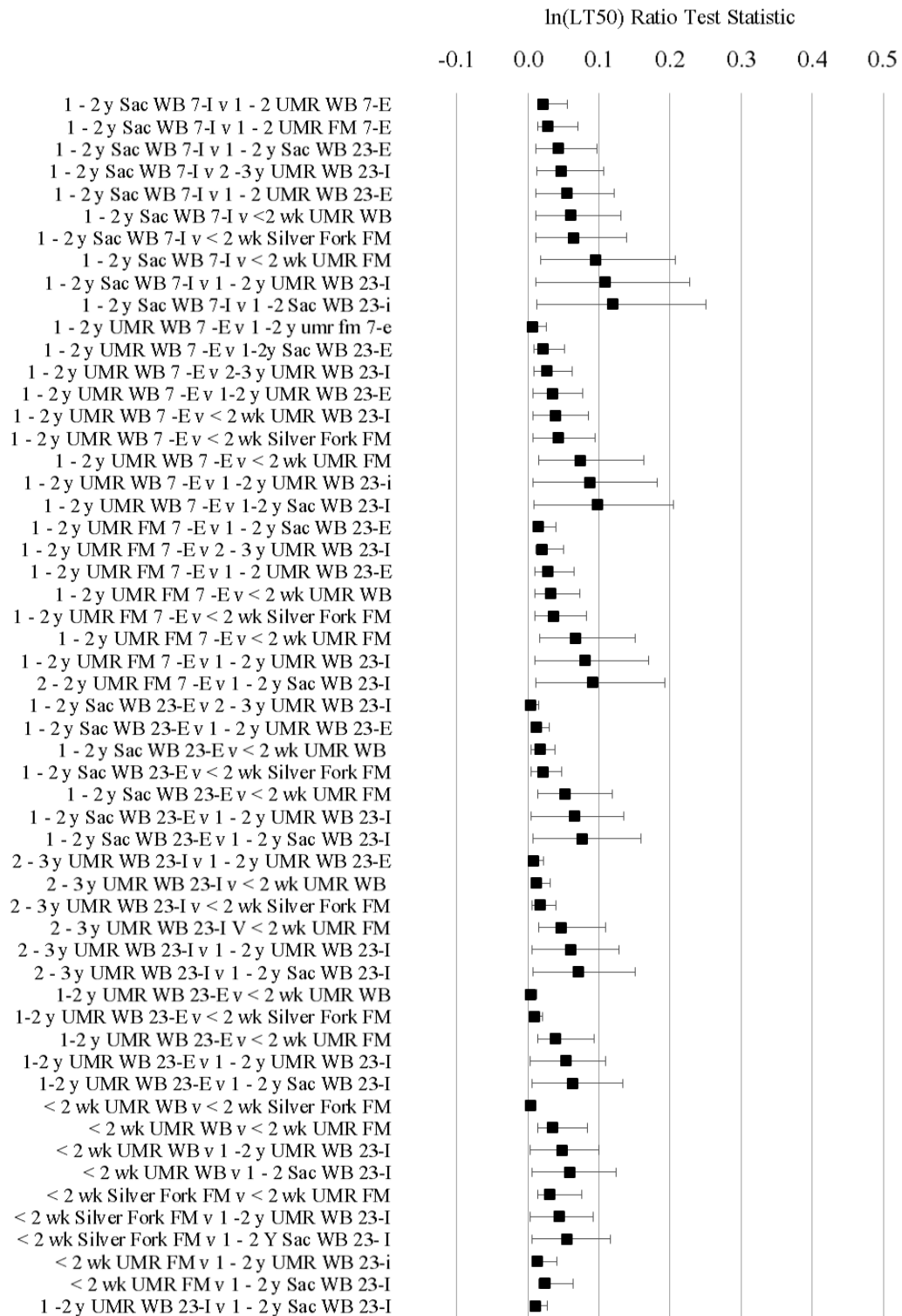


Figure 19b. ln(LT₅₀) Ratio Test Confidence Intervals. The LT₅₀s are not statistically different when the confidence interval includes 0.