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REGULAR ARTICLE

UPPER THERMAL TOLERANCE OF THE WESTERN RIDGED MUSSEL (*GONIDEA ANGULATA*), WESTERN PEARLSHELL (*MARGARITIFERA FALCATA*), AND WESTERN SCULPIN SPECIES (*COTTUS* SPP.)

Rachael Valeria[1]*, Alexa Maine[2], Christine Parent[3], and James J. Nagler[3]

[1] *Idaho Department of Fish and Game, Salmon, ID 83467 USA*
[2] *Department of Natural Resources, Confederated Tribes of the Umatilla Indian Reservation, Walla Walla, WA 99362 USA*
[3] *Department of Biological Sciences, University of Idaho, Moscow, ID 83843 USA*

ABSTRACT

Freshwater mussels are ecologically and culturally important and globally have the highest extinction and imperilment rates of any animal. The Western Ridged Mussel (*Gonidea angulata*) and the Western Pearlshell (*Margaritifera falcata*) are experiencing die-offs across their native ranges, and increasing water temperature is a possible contributing factor. To host their larval life stage, *G. angulata* uses the gills of sculpins (*Cottus* spp.) and *M. falcata* uses the gills of salmonids (*Oncorhynchus* spp.). The purpose of this laboratory study was to determine the upper thermal tolerances of *G. angulata* and *M. falcata* and two sculpin species, Margined Sculpin (*Cottus marginatus*) and Paiute Sculpin (*Cottus beldingi*), by using sublethal acute and chronic survival tests. The acute test consisted of increasing the water temperature until critical thermal maximum (CTmax) temperature behaviors were observed. The mean CTmax temperature was 34.1°C for *G. angulata* and 33.2°C for *M. falcata,* and the mean for the two *Cottus* spp. was 28.9°C. Based on the acute test results, *G. angulata* and *M. falcata* have a higher CTmax temperature than their host fish. The chronic test gradually increased the water temperature surrounding the test organism to 2°C below the CTmax temperature for each species. This temperature was maintained for 7 d, during which survival was recorded. All mussels perished, whereas 58.3% of the sculpins survived the chronic test. Survival curves from the chronic test showed that *G. angulata* survived longer and are more tolerant to prolonged elevated temperatures than *M. falcata*. These thermal tolerance tests demonstrate that a thermal separation between mussel and host fish is possible, thus limiting mussel reproduction as climate change leads to warming surface waters. In addition, tolerance to prolonged elevated temperature varies between these western mussel species.

KEY WORDS: bivalve, teleost, climate, sublethal, Unionidae, Cottidae

INTRODUCTION

Increasing water temperatures from climate change are altering freshwater ecosystems. Average water temperatures, extreme summer temperature events, and patterns of drought are projected to increase in the future (IPCC 2022), further threatening populations of freshwater mussels that are already stressed. In the western USA, two mussel species, the Western Ridged Mussel (*Gonidea angulata*) and the Western Pearlshell (*Margaritifera falcata*), have experienced significant declines over the past 2 decades (Xerces Society 2015; Blevins et al. 2020). These mussels historically were an important food source for Columbia Plateau Tribes such as the Confederated Tribes of the Umatilla Indian Reservation (CTUIR). The CTUIR considers freshwater mussels a First Food (foods of significant traditional and cultural importance). First Food designations are closely tied to tribal creation beliefs that, as the foods promised to take care of the Native people, so the Native people have a reciprocal responsibility to take care of the First Foods (CTUIR 2015). Mussel harvest remains a treaty right for the CTUIR, but it is not currently practiced because of declines in freshwater mussel populations, especially in ceded territory in southeastern Washington and northeastern Oregon.

*Corresponding Author: rachael.valeria@idfg.idaho.gov

Historically, *G. angulata* was found in the western USA (California, Nevada, Idaho, Oregon, and Washington) and British Columbia, Canada (Blevins et al. 2020). It is a medium-to-large mussel with a maximum total length of 170 mm (Owyhee River, Oregon; A. Maine, personal communication) and a lifespan of 50–60 yr. *Gonidea angulata* has disappeared from 43% of its historic range (Blevins et al. 2017; Xerces Society and CTUIR 2020), possibly due to industrial pollution, habitat loss, invasive species, loss of host fish, and climate change (Downing et al. 2010; Snook et al. 2019). *Margaritifera falcata* is found in the western USA from California north to British Columbia and southern Alaska and inland to western Montana, Wyoming, and Utah (U.S. Fish and Wildlife Service 2023). It is a medium-to-large mussel with a maximum length of 162 mm (Salmon River, Idaho; Vannote and Minshall 1982) and a lifespan estimated to be 60–100 yr (Bauer 2001; Haag 2012; Maine et al. 2022). *Margaritifera falcata* has experienced a 17% population decline across its range (Blevins et al. 2017; Xerces Society and CTUIR 2020).

Recently, mass die-offs of *G. angulata* and *M. falcata* have been observed in which hundreds to thousands of mussels were killed (Blevins et al. 2020; Richard et al. 2023). At a long-term monitoring site on the Middle Fork John Day River, Oregon, mussel beds previously containing 500 *G. angulata* per square meter were reduced to zero between 2005 and 2007 (A. Maine, unpublished). Many factors have been implicated in the recent declines of *G. angulata* and *M. falcata* populations in the western USA (Blevins et al. 2020), including elevated water temperatures that occur during the summer. Determining the upper thermal limits of *G. angulata* and *M. falcata* is a first step toward understanding how their life histories allow vulnerability to warming waters and is thus key for conservation efforts to sustain these populations.

Like many freshwater mussels, the larvae (glochidia) of both *G. angulata* and *M. falcata* colonize the gills and/or skin of teleost host fishes for part of their life cycle. Studies show that *G. angulata* glochidia use sculpin gills (*Cottus* spp.; Haley et al. 2007; Stanton and MacConnachie 2012; O'Brien et al. 2013) and *M. falcata* use salmonid gills (e.g., Rainbow Trout, *Oncorhynchus mykiss*, and Cutthroat Trout, *Oncorhynchus clarkia*; Murphy 1942; Meyers and Milleman 1977; Karna and Millemann 1978). Summer die-offs of *Cottus* spp. have been observed on the Middle Fork John Day River, Oregon, between 2005 and 2021 (A. Maine, unpublished) that correlated with high water temperatures. The loss of host fishes would prevent successful reproduction of these mussel species.

A species' thermal tolerance is defined by a temperature range, that, when exceeded, can have sublethal (e.g., abnormal behavior, reduced growth, impaired reproduction) or lethal consequences (Paaijmans et al. 2013; Westhoff and Rosenberger 2016; Cereja 2020). Laboratory thermal tolerance studies for fish species use two primary approaches: critical thermal (CT) or incipient lethal temperature (ILT) (Beitinger et al. 2000). The CT method is a dynamic assay, where an individual is subjected to increasing (i.e., CTmaximum [CTmax]) or decreasing (i.e., CTminimum) temperatures until a sublethal endpoint is reached (Cowles and Bogert 1944; Paladino et al. 1980; Beitinger et al. 2000). The ILT method is a static assay, where an individual is suddenly exposed to and then held at a constant temperature for a specified amount of time, or until specific endpoints are reached. The CT method has the following two advantages over the ILT method: (1) there is gradual linear change in temperature over time until the predetermined endpoints occur; and (2) sample sizes can be smaller, which is useful for studies of species that are threatened or have small population sizes (Beitinger et al. 2000; Rodland et al. 2009). Although CT testing is common in fish, few CT studies have been done on invertebrates, including freshwater mussels (Galbraith et al. 2012).

Our first objective was to determine upper thermal tolerances for *G. angulata* and *M. falcata* by using the CTmax method. Our second objective was to determine the upper thermal tolerances for *C. marginatus* and *C. beldingi*, two western *Cottus* spp. whose ranges overlap with *G. angulata*, also using the CTmax method. The fish hosts for *M. falcata* were not tested because CTmax temperature data are available from previous studies for several salmonids (Heath 1963; Becker and Genoway 1979; Currie et al. 1998). Our third objective incorporated the CTmax temperature data for the mussels and fish in a chronic (7-d) thermal test with a survival endpoint to assess prolonged exposure to elevated water temperature.

MATERIALS AND METHODS

Mussel and Fish Collections

Gonidea angulata (61–88 mm total length) were collected in July 2024 via snorkeling in the Salmon River (Table 1). Upon capture, mussels were put in a draw-string net bag that was placed in a shaded, flowing area of the river. Stream temperature at the time of collection was 17°C. Mussels were layered in clean damp cloths in coolers without water and transported on the day of capture to the CTUIR Mussel Propagation Laboratory (Walla Walla, WA, USA). Ice was added to the coolers during transport, approximately every hour as needed, to maintain river water temperature. *Margaritifera falcata* (23–48 mm total length) were similarly collected and transported from the Grande Ronde River, Oregon, in July 2023 (Table 1). Stream temperature at the time of collection was 21.6°C.

Sculpins (*Cottus* spp.) >40 mm total length were collected by electrofishing from streams in the Walla Walla River basin in June and July 2023 (Table 1). Stream temperatures varied between 12 and 18°C during collection. Captured fish were placed in coolers with aerated stream water for transport to the lab. Ice packs added approximately every hour as needed to maintain stable water temperature. Sculpins were identified as Margined Sculpin (*Cottus marginatus*) and Paiute Sculpin (*Cottus beldingi*), based on external morphological traits (Whitney

Table 1. Collection locations, dates, water temperatures, and total counts (N) of mussels and fish used in this study from the Columbia River Drainage.

Species	Waterbody	Elevation (m)	Date(s)	Temperature (°C)	N (Acute)	N (Chronic)
Margaritifera falcata	Grande Ronde River, OR (45.232439, −118.396278)	1,034	July 3, 2023	17	54	43
Gonidea angulata	Salmon River, ID (45.41889, −116.1620000)	1,829	July 11, 2024	22	32	36
Cottus marginatus	South Fork Walla Walla River, OR (45.8576058, −118.2210385)	542	June 12, July 14, 2023	12, 16	11	4
Cottus marginatus	Mill Creek, WA (46.0818599, −118.2519255)	383	June 5, July 13 and 14, 2023	16, 21, 16	17	8
Cottus marginatus	Walla Walla River, OR (45.9455112, −118.3844809)	306	June 15, 2023	16	3	13
Cottus beldingi	South Fork Walla Walla River, OR (45.8576058, −118.2210385)	542	June 12, July 14, 2023	12,16	14	10
Cottus beldingi	Mill Creek, WA (46.0818599, −118.2519255)	383	June 5, July 13 and 14, 2023	16, 21, 16	3	13

and Wydoski 2003; Page and Burr 2011) and geographical location (Young et al. 2022).

General Laboratory Husbandry

In the laboratory, mussels were held in polycarbonate "Cambro" pans (CamWear Cambro Pans, Huntington Beach, CA, USA; 53 × 32 × 15 cm [25.6 L]), with screened sides and a mixed sand–gravel substrate. Each individual mussel was measured and tagged with a unique number on either a homemade paper or vinyl shellfish tag (Floy Tag and Manufacturing, Seattle, WA, USA). The pans were held in a flow-through water bath (600–800 mL/s) supplied with well water at 13°C. The pans were maintained in a room with a 9:15 (light:dark) photoperiod (light period: 0730 to 1600 h). Mussels were fed 2 mL of Shellfish Diet 1800 and 1 mL of Nannochloropsis 3600 (both feeds from Reed Mariculture, Campell, CA, USA) per liter of water once daily (modified from Wang et al. [2021]).

Sculpins were held individually in 1.5-L polycarbonate tanks, with gravel-sized substrate, in an Aquatic Habitats for Accelerated Bioresearch (AHAB) system (Pentair Aquatic Systems, Apopka, FL, USA) with dechlorinated tap water at 13°C and a flow rate of 15 ml/s. The AHAB system is a prefabricated multitank recirculating system used for fish and mussel propagation. The photoperiod used for sculpins was identical to that of the mussels. Sculpins were fed freshly thawed bloodworms (San Fransico Bay Brand, Newark, CA, USA) to satiation every other day (similar to Walsh et al. [1997]). Fish care and experimentation were conducted in accordance with Animal Care and Use Protocol IACUC-2022-63, reviewed and approved by the University of Idaho Animal Care and Use Committee.

Mussel Thermal Experiment Protocols

The CTmax method was used in acute trials for all species. The CTmax temperature was recorded as the temperature at which "locomotory activity becomes disorganized and the animal loses its ability to escape conditions that will promptly lead to its death" (Paladino et al. 1980; Beitinger et al. 2000). Protocols have been established for measuring CTmax temperature for ectothermic vertebrates (Hutchison 1961; Beitinger et al. 2000; Cereja 2020), but few exist for invertebrate organisms, including for mussels (Rodland et al. 2009; Galbraith et al. 2012). The onset of valve gaping (i.e., valves open visibly wider than normal) has been used as a CTmax temperature behavioral endpoint for other freshwater mussel species (Galbraith et al. 2012), and gape duration and frequency were shown to change as water temperature increased (Rodland et al. 2009). To determine CTmax temperature behavioral endpoints for *G. angulata*, a pilot study was conducted on a few individuals where the water temperature was increased until lethality or close to lethality. Based on Galbraith et al. (2012) and the pilot study observations, the CTmax temperature was determined by observing at least two of the following behaviors: extreme valve gaping with parting of mantle tissue edges; no response from foot and no valve closure to probing with a glass stir rod; and rapid valve closure and opening (i.e., water rushing out quickly, "gasping"). These behaviors were consistent observations of upper thermal stress in *G. angulata* and therefore used as the CTmax temperature behavioral endpoints for this mussel. The acute thermal trials were conducted in an apparatus that gradually increased the water temperature (∼2–2.5°C/h; Lee and Rinne 1980; Smale and Rabeni 1995) over a 7- to 8-h period until the CTmax temperature (Fangue et al. 2006) was reached. Mussels were visually checked (and probed when needed) for responses throughout the whole trial period. Before each trial, mussels were acclimated at 13°C for at least 7 d (McClanahan et al. 1986; Galbraith et al. 2012) and food was withheld 24 h before temperature treatment. Mussels were not fed during the trials. Dissolved oxygen levels were monitored with a dissolved oxygen probe (YSI Inc., Yellow Springs, OH, USA, and multiparameter sonde from In-Situ Inc.,

Fort Collins, Colorado, USA) during trials. Mussels were never used in more than one trial.

Three acute thermal trials were conducted with *G. angulata* in July 2024, with 12 mussels per trial (6 treatment and 6 control) for two of the trials and 8 mussels per trial for one trial (4 treatment and 4 control). For the treatment group, individual *G. angulata* were placed into 960-mL plastic containers (Tupperware, Dart Industries Inc., Orlando, FL, USA) filled with water at 13°C and without substrate. The containers were arranged within a Cambro pan, as a water bath, and filled with 13°C water placed on two magnetic stir plates. Two magnetic stir bars were placed in the bottom of the polycarbonate pan to provide water circulation. Each individual mussel container was equipped with a temperature logger (HOBO MX Temp 400, Onset Computer Corporation, Bourne, MA, USA) for continuous temperature recording and air supplied with an air stone. The Cambro pan was equipped with a submersible water heater (1000W PONG-DANG, KD Heater Co. Ltd., Gyeonggi-do, South Korea) connected to an electric temperature controller (Ranco, Grainger Inc., Lake Forest, IL, USA) to achieve a water temperature increase of 2–2.5°C/h. The control treatment consisted of *G. angulata* subjected to the same conditions but maintained at a constant water temperature of 13°C. Once the trial commenced, water temperature in the heat-treated group was increased until the CTmax temperature for each individual mussel was determined. Once the CTmax temperature was recorded for an individual, the mussel was removed from the test apparatus and returned to 13°C. Feeding was resumed the day after the trial, and mussels were monitored for 1 wk posttrial to observe any delayed mortality.

Five acute thermal trials, using 48 mussels, were conducted with *M. falcata* in July and August 2023. Sample size ranged from 6 (3 treatment and 3 control) to 16 mussels (8 treatment and 8 control) per trial. Testing and posttesting conditions were similar to those for *G. angulata*, but with several exceptions. Glass beakers (300 mL) were used for *M. falcata*, and five (four 25-W, one 125-W) submersible aquarium heaters (Hydor, Bassano del Grappa, Vicenza, Italy) were used to achieve the appropriate water temperature increase. To determine CTmax temperature behavioral endpoints for *M. falcata*, a pilot study like the one used for *G. angulata* was conducted. Based on Galbraith et al. (2012) and pilot study observations, the CTmax temperature for *M. falcata* was determined by observing at least two of the following behaviors: extreme valve gaping with parting of mantle tissue edges, no response from foot to probing with a glass stir rod, and no valve closure or discharging of mucus-like substance.

The chronic thermal trials were a survival (lethal) test, measuring the time to death at a temperature 2°C below the CTmax temperature for each species. When designing this experiment, we determined it would be beneficial to start below the CTmax temperature because mortality would likely occur quickly once it was reached. A custom-built chronic testing apparatus that held 48 1.5-L polycarbonate tanks (one mussel per tank) was used for these trials. Half of the tanks were connected to a heated recirculating well-water supply (heat-treated group), and the other half were connected to a chilled recirculating water supply maintained at 13°C (control group). The chronic trials were performed on 38 naïve (not previously tested) *G. angulata* (19 treatment and 19 control) and 42 naïve *M. falcata* (21 treatment and 21 control) in August 2024 and August 2023, respectively. The water in the chronic testing apparatus was heated at the same rate as for the acute trials, but heating was terminated 2°C below the CTmax temperature for each species. This chronic temperature was constantly maintained for 7 d (168 h). Each individual tank was equipped with a temperature logger (HOBO MX Temp 400 or HOBO Pendant Light/Temp, Onset Computer Corporation). Pretrial acclimation conditions were the same as in the acute thermal trials. Mussels were not fed during the chronic thermal trial. Every day during the chronic trial period, the foot or valve opening of the mussel was probed gently with a glass stir rod at 0800 and 2000 h. Mortality was determined by (1) open valves with over extension of the anterior and posterior adductor muscles and (2) no response to probing. When mortality was observed, the mussel and logger were removed from the tank. This trial was conducted once for each species. Dissolved oxygen was measured twice every day in both treatment and control groups.

Sculpin Thermal Experiment Protocols

Four acute thermal trials, using a total of 48 *Cottus* spp., were conducted in June and July 2023. Each trial used 12 sculpins (6 treatment and 6 control). Overall, 16 *C. marginatus* and 8 *C. beldingi* were used in the heat treatments and 15 *C. marginatus* and 9 *C. beldingi* were used in the control groups. Sculpins were acclimated and tested like the mussels, with the following exceptions. The 1.5 L AHAB holding tanks, containing each fish with a layer of gravel-sized substrate, were moved directly into the Cambro pan water bath. The CTmax temperature behavioral endpoints for sculpin were determined by opercular flaring and failure of a fish to right itself within 3 s when it was rolled over on its side or back by using a glass stir rod (i.e., loss of equilibrium). Because sculpins do not consistently undergo violent muscular spasms (Kowalski et al. 1978; Walsh et al. 1997), as noted in other fish species when experiencing thermal stress, this response was not used. Once the CTmax temperature was attained for each individual, the tank and fish were returned to the AHAB system, where the water temperature was allowed to gradually decline to 13°C. Dissolved oxygen levels were monitored, similar to those for the mussels. As with the mussels, sculpins were never used in more than one trial.

The chronic testing apparatus and conditions used for sculpins were like that for the mussels, with a few exceptions. Every day at 0800 and 2000 h, *Cottus* spp. were checked for movement and probed with a glass stir rod if unmoving. If the fish did not move when probed and there was no opercular movement, this was considered mortality, which was the chronic test endpoint. A total of 48 naïve sculpins (25 *C. marginatus* and 23 *C. beldingi*) were

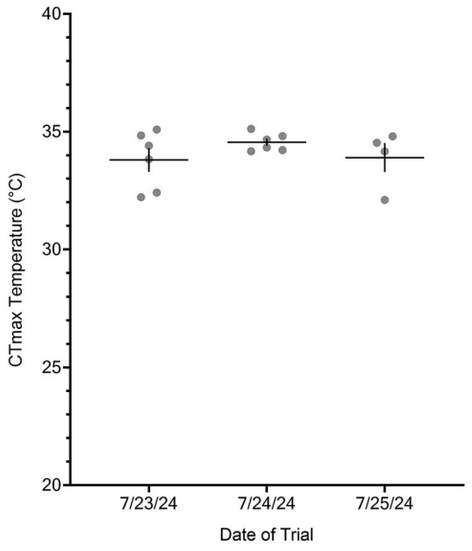

Figure 1. Critical thermal maximum (CTmax) water temperatures for *Gonidea angulata* determined in the acute thermal experiment. Each date represents a separate trial, and each shaded circle represents a single individual. The horizontal lines represent the mean, and the vertical lines represent the SE. The CTmax temperatures did not differ significantly among trials ($F(2, 13) = 0.985$, $P = 0.399$).

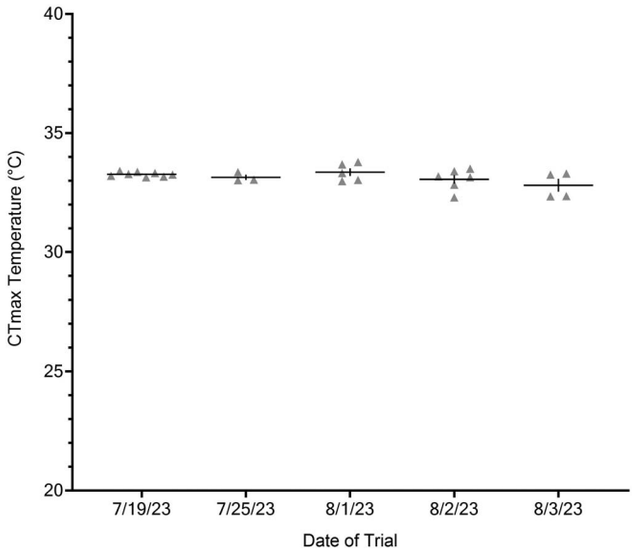

Figure 2. Critical thermal maximum (CTmax) water temperatures for *Margaritifera falcata* determined in the acute thermal experiment. Each date represents a separate trial, and each shaded triangle represents a single individual. The horizontal lines represent the mean, and the vertical lines represent the SE. The CTmax temperatures did not differ significantly among trials ($F(4, 21) = 1.77$, $P = 0.172$).

tested: 24 heat treated (10 *C. marginatus* and 14 *C. beldingi*) and 24 controls (15 *C. marginatus* and 9 *C. beldingi*) in July and August 2023. When mortalities were observed, the fish and temperature logger were removed from the tank. After 7 d, the water temperature was gradually returned to 13°C. During the trial period, and posttrial for 7 d, the fish were observed for signs of distress or mortality. This trial was conducted once, and as with the mussels, the sculpins were not fed during the trial. Dissolved oxygen levels were measured daily.

Statistical Analysis

Differences between the CTmax temperatures across all acute trials for a given species were assessed by one-way analysis of variance (normally distributed data) or Kruskal–Wallis (nonnormally distributed data) tests. Because the CTmax temperatures were not different across trials for each mussel species, a *t*-test was used to compare between species. Statistical significance was set at $\alpha < 0.05$ for all acute trials. The data from the chronic trials for all species were plotted using Kaplan–Meier survival curves (Kaplan and Meier 1958; Rich et al. 2010). We examined differences among survival curves by using the log rank test ($P < 0.05$). All statistical and graphical analyses were performed with Prism 10.6.0 (GraphPad Software, LLC, San Diego, CA, USA).

RESULTS

Acute Thermal Experiments

The mean CTmax temperatures for *G. angulata* ranged from 33.8 to 34.5°C (Fig. 1). The mean CTmax temperatures from the three trials were not significantly different ($F(2,13) = 0.98$, $P = 0.399$) from one another; therefore, a grand mean of 34.1 ± 0.5°C was calculated. The behavioral endpoints used to determine the CTmax temperature for *G. angulata* were consistent across individuals. Mussels extended their foot immediately when placed into the treatment containers. From 26 to 28°C, some mussels retracted their foot and closed their valves completely. *G. angulata* was slow to respond to probing when close to the CTmax temperature. Eventually, all mussels exhibited excessive valve gaping with retraction of mantle tissue and rapid valve closure. No mortalities were observed in any treatment group, and one mortality occurred in the control group. One additional mortality from the control group was observed during the 1-wk posttrial period. In the control group, the mean temperature was 14.6 ± 0.2°C through all trials and had 87.5% survival with a lack of CTmax temperature endpoints. Dissolved oxygen levels were between 87 and 93% saturation throughout the trials.

The mean CTmax temperatures for *M. falcata* in the five trials ranged from 32.8 to 33.4°C (Fig. 2). The CTmax temperatures were found to not differ significantly among trials ($F(4, 21) = 1.77$, $P = 0.172$); therefore, a grand mean of 33.2 ± 0.1°C was calculated. The response of *M. falcata* to increasing temperature during the acute thermal trials was consistent across individuals. Mussels were completely closed at the beginning of the trials, but by 30–31°C displayed foot extension. When reaching the CTmax temperature, *M. falcata* exhibited no response to foot touch by probing and extreme valve gaping with retraction of mantle tissue was observed. Many mussels also discharged a mucus-like substance. No

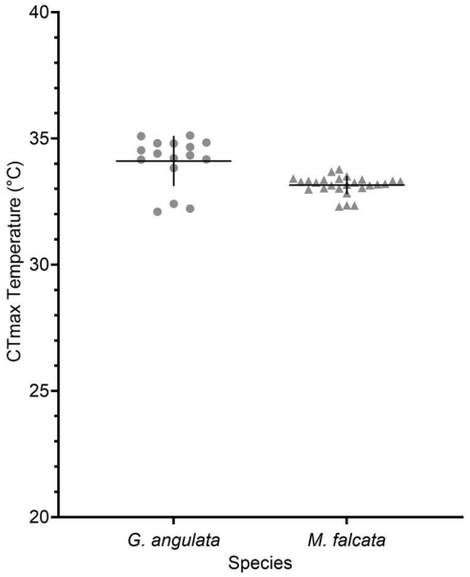

Figure 3. Comparison of critical thermal maximum (CTmax) temperatures of *Gonidea angulata* (gray circles) and *Margaritifera falcata* (gray triangles) from the acute thermal trials. Each point represents an individual mussel. The horizontal lines represent the mean, and the vertical lines represent the standard error. The CTmax temperatures for *G. angulata* and *M. falcata* were significantly different ($F(15, 25) = 7.34$, $P = 0.0017$).

mortalities were observed in either the heat-treated or control groups during the trials or after 1 wk posttrial. The control temperature was 15.10 ± 0.01°C (mean ± SE) during the trials, and both groups had 100% survival with a lack of CTmax temperature endpoints. Dissolved oxygen levels were >80% saturation throughout the trials. The CTmax temperatures for *G. angulata* and *M. falcata* were compared statistically and did differ significantly ($F(15, 25) = 7.34$, $P = 0.0017$; Fig. 3).

The mean CTmax temperature for *Cottus* spp. in the five trials ranged from 28.7 to 29.2°C (Fig. 4). The mean CTmax temperatures did not differ significantly ($F(3, 20) = 1.54$, $P = 0.235$) among trials or between *C. marginatus* and *C. beldingi* ($F(15, 7) = 1.33$, $P = 0.300$; Fig. 5). Therefore, a grand mean of 28.9 ± 0.2°C was calculated. All sculpins in the heat-treated group displayed erratic swimming behavior immediately after the tank was moved into the water bath, but by 3 h (~ 20°C) all fish were calm. Response to the increasing water temperature was notable at 26–27°C. At this temperature, sculpins exhibited more rapid opercular movement and began to push their mouths against the side of the tank and open their mouths widely. Some sculpins swam up and down the tank erratically. The erratic swimming behavior was almost immediately followed by a loss of equilibrium that defined the CTmax temperature endpoint. There was one mortality during the acute thermal experiment, in the heat-treated group, but no mortality occurred in the controls. There was no mortality 1 wk posttrial in either treatment group. The mean control temperature was 15.2 ± 0.1°C. Dissolved oxygen levels were 89–126% saturation throughout the trials.

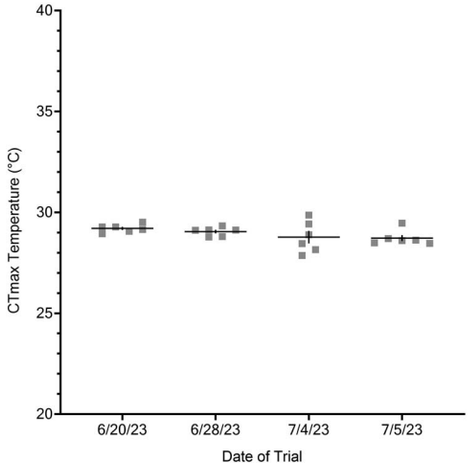

Figure 4. Critical thermal maximum (CTmax) water temperatures for *Cottus* spp. determined in the acute thermal experiment. Each date represents a separate trial, and each shaded square represents a single individual. The horizontal lines represent the mean, and the vertical lines represent the SE. The mean CTmax temperatures did not differ significantly among trials ($F(3, 20) = 1.54$, $P = 0.235$).

Chronic Thermal Experiments

For *G. angulata*, the chronic test temperature used was 32.1°C (2° below the CTmax temperature grand mean of 34.1°C calculated from the acute experiment), attained by 8 hours, and this point was considered the beginning of the trial (hour 0). The survival curve for *G. angulata* showed the first mortality occurred at hour 31 and by hour 139, all mussels were dead (Fig. 6). There was no mortality in the control group during testing or 1 wk posttrial. Dissolved oxygen measurements were 95–113% saturation during the *G. angulata* chronic thermal trial.

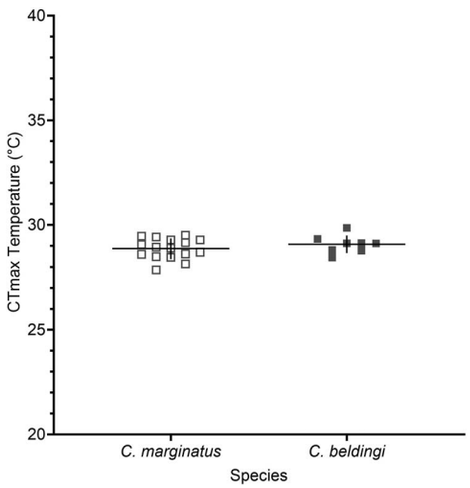

Figure 5. Comparison of the critical thermal maximum (CTmax) temperatures of *Cottus marginatus* (open gray squares) and *Cottus beldingi* (closed gray squares) from the acute thermal trials. Each point represents an individual fish. The horizontal lines represent the mean, and the vertical lines represent the SE. The mean CTmax temperatures did not differ significantly between *C. marginatus* and *C. beldingi* ($F(15, 7) = 1.33$, $P = 0.300$).

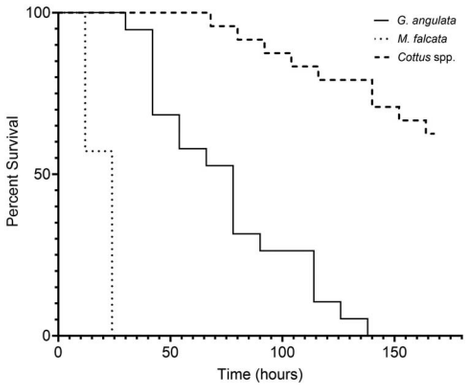

Figure 6. Kaplan–Meir survival curves for *Gonidea angulata* (black), *Margaritifera falcata* (dark gray), and *Cottus* spp. (light gray) from the chronic thermal experiments. Note, the temperature at the beginning (hour 0) of each trial was species specific (2°C below the critical thermal maximum [CTmax] temperature grand mean determined in the acute thermal trials).

For *M. falcata*, the chronic test temperature used was 31.2°C (2° below the CTmax temperature grand mean of 33.2°C calculated from the acute trials), attained by 7 h, and this point was considered the beginning of the trial (hour 0). The survival curve for *M. falcata* had nine mortalities by hour 13 and by hour 25, all remaining *M. falcata* were dead (Fig. 6). There was no mortality in the control group during testing or 1 wk post trial. Dissolved oxygen measurements were 90–111% saturation during the *M. falcata* chronic thermal trial. The survival curves for *G. angulata* and *M. falcata* were significantly different ($P < 0.0001$; Fig. 6).

For *Cottus* spp., the chronic test temperature used was 26.9°C (2° below the CTmax temperature grand mean of 28.9°C calculated from the acute trials), attained by 6 h, and this point was considered the beginning of the trial (hour 0). The survival curve for *Cottus* spp. showed one mortality by hour 69 followed by other additional mortalities, but 14 sculpins (62.5%) survived until the end of the treatment period (hour 168; Fig. 6). The heat-treated group had one mortality an hour after the 7-d trial was concluded. Among the nine mortalities, six were *C. marginatus* and three were *C. beldingi*. There was no mortality in the control group during testing the 1 wk posttrial. Dissolved oxygen measurements were 90–111% saturation during the *Cottus* spp. chronic thermal trial. The *Cottus* spp. survival curve was significantly different from the mussel survival curves ($\chi^2 = 91.04$, df = 2, $P < 0.0001$; Fig. 6).

DISCUSSION

Mussel Thermal Experiments

This study is the first to report the upper thermal limits for two freshwater mussels, *G. angulata* and *M. falcata*, native to the Pacific Northwest. The acute test found similar CTmax temperatures for the two mussel species, with *G. angulata* at 34.1°C and *M. falcata* at 33.2°C. There are a limited number of studies testing upper thermal limits in freshwater mussels (reviewed in Fogelman et al. [2023]). One comparable study used the CTmax method for three species from Pennsylvania: *Alasmidonta varicose* (Brook Floater Mussel), *Elliptio complanate* (Eastern Elliptio Mussel), and *Strophitus undulatus* (Creeper Mussel) (Galbraith et al. 2012). These mussels were acclimated at 15 or 25°C before testing began. The CTmax temperature results were 39.5 and 41.1°C for *A. varicose*, 42.7 and 41.4°C for *E. complanate*, and 40.0 and 42.3°C for *S. undulatus* at each of the acclimation temperatures, respectively. The CTmax temperatures for these three mussel species are all considerably higher than our CTmax temperature results for *G. angulata* and *M. falcata*. Besides the considerable geographic diversity, the differences in CTmax temperatures between these freshwater mussels are likely because they are different species. Another possibility is that Galbraith et al. (2012) used two different acclimation temperatures (15 and 25°C), and there was a clear pattern of an effect of acclimation temperatures on the CTmax temperature. The higher acclimation temperature resulted in a higher CTmax temperature. In other studies, the effects of acclimation temperature on mussels have been mixed (Polhill and Dimock 1996; Pandolfo et al. 2012). However, it is possible that different acclimation temperatures could explain the differences in CTmax temperature for these different mussel species.

We used a 7-d chronic thermal experiment as a survival test. Although substantial mortality was not intended, no mussels of either species survived until the end of the test period. The survival curves were significantly different between the two mussel species with *G. angulata* showing more tolerance to prolonged elevated temperature than *M. falcata*. Several factors may explain these results. As with the acute tests, these are different mussel species. Also, the starting temperature for the chronic tests was 1°C different between the two mussel species; although this difference is small, it cannot be discounted. Finally, the *G. angulata* and *M. falcata* used in this study differed significantly in body size. *M. falcata* (length 23–48 mm) was considerably smaller than *G. angulata* (length 61–88 mm). Smaller mussels would warm more rapidly and have higher mass-specific metabolic rates. The physiological effect(s) of increasing water temperature likely impacted the smaller *M. falcata* more rapidly and resulted in mortality occurring earlier than for *G. angulata* (Spooner and Vaughn 2008; Ismail et al. 2016; Lopez et al. 2022).

Acute thermal testing identified novel behavioral endpoints for *G. angulata* and *M. falcata.* The extreme valve gaping response, as a CTmax temperature behavioral endpoint, has been previously reported (Galbraith et al. 2012) and occurs when adductor muscles relax, mantle edges and siphons part, and the foot is extended or partially extended. In unfavorable water conditions, mussels have been observed lying prone with the valves gaping and foot extended (Allen 1923; Galbraith et al. 2012). In our pilot experiments, most *G. angulata* individuals exhibited foot extension over the duration of the heat treatment, whereas *M. falcata* did not consistently display foot

extension. The extended foot extension time in *G. angulata* may be due to their affinity to burrow deeply into the sediment (Vannote and Minshall 1982; A. Maine, unpublished). Although both mussels in our study showed extreme valve gaping behavior and no response to foot touch, there were slight differences in behavior due to elevated temperature observed between the two species. When nearing their CTmax temperature, many of the *G. angulata* individuals displayed a pattern of slowly gaping wider, bringing in water and then rapidly closing valves, thus pushing out water forcefully. This discharge of water from the mantle chamber has been observed with vigorous probing (Allen 1923), but *G. angulata* displayed this behavior without stimulation. It was not observed in *M. falcata.* However, *M. falcata* consistently discharged a white mucus-like substance when nearing their CTmax temperature that was not consistently observed in *G. angulata.* Some mussel species can develop a mucus plug over mantle tissues to prevent direct exposure to the atmosphere during low water periods (Byrne and McMahon 1994), so mucus shedding could represent a mussel stress response.

Sculpin Thermal Experiments

This study is also the first to report the upper thermal limits for two sculpins, *C. marginatus* and *C. beldingi*, native to the Pacific Northwest. Acute thermal testing for these two *Cottus* spp. showed a CTmax temperature of 28.9°C that is similar to *Cottus* spp. native to the eastern USA. The CTmax temperatures for members of the family Cottidae from the eastern USA varied from 26.3 to 31.56°C: Mottled Sculpin (*Cottus bairdii*) at 30.37°C and 30.9°C, Tallapoosa Sculpin (*Cottus tallapoosae*) 30.32°C, Banded Sculpin (*Cottus carolinae zopherus*) at 31.14 and 31.56°C, Ozark Sculpin (*Cottus hypselurus*) at 28.75°C, Pygmy Sculpin (*Cottus pygmaeus*) at 29.58°C, and Slimy Sculpin subspecies (*Cottus cognatus gracilia*) at 26.3 and 27.3°C (Kowalski et al. 1978; Otto and O'Hara Rice 1977; Walsh et al. 1997). As with mussels, the acclimation temperature is an important pretest variable for fish. The CTmax temperature of many fish species shows a positive correlation with acclimation temperature; thus, when the acclimation temperature is higher, the CTmax temperature tends to increase (Otto and O'Hara Rice 1977; Walsh et al. 1997; Beitinger et al. 2000). All the studies mentioned above from the eastern USA used an acclimation temperature of 15°C, representing a higher than the acclimation temperature that we used (13°C). If we repeated our study and exposed *C. marginatus* and *C. beldingi* to a higher acclimation temperature, we might find a higher CTmax temperature. Our chronic thermal test appears to be the first thermal test subjecting *Cottus* spp. to consistent higher temperatures for long periods of time. Some individual *Cottus* spp. from the chronic trial were able to survive 7 d at 26.9°C, whereas other individuals experienced mortality after hour 69. The survival of individuals *Cottus* spp. after 7 d of consistent high temperatures should be further studied.

Mussels and Host Fish

The upper thermal limits of *G. angulata* and *Cottus* spp. differed in the acute thermal tests. If a host fish is more thermally sensitive than the mussel that parasitizes it, the host fish may be absent when a female mussel releases her glochidia, thus halting mussel reproduction. Based on our acute test results, adult *G. angulata* tolerates higher temperatures than sculpin (CTmax temperature ~4°C warmer). If this occurred under natural environmental conditions, sculpins could be thermally stressed before *G. angulata*. One study found the same scenario with the endangered Dwarf Wedgemussel (*Alasmidonta heterodon*) and its host fishes Tessellated Darters (*Etheostoma olmstedi*) and Slimy Sculpin (*Cottus cognatus*; Galbraith et al. 2020). The CTmax temperature data showed that the fish hosts of *A. heterodon* were more temperature sensitive than the mussel. A similar pattern was evident in a literature review of CTmax temperatures of *M. falcata* salmonid hosts. The average CTmax temperatures of Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkia*; Heath 1963), Coho Salmon (*Oncorhynchus kisutch*; Becker and Genoway 1979; Becker and Wolford 1980) and Rainbow Trout (*Oncorhynchus mykiss*; Strange et al. 1993; Currie et al. 1998; Chen et al. 2015), acclimated to 15°C, were similar between species, ranging from 28.7 to 29.9°C (reviewed in Beitinger et al. [2000]). These fish CTmax temperatures are several degrees lower than the CTmax temperature for *M. falcata.* Other comparisons of thermal tolerance data of mussels and their suspected or known host fishes also showed generally similar or slightly higher thermal tolerance for adult freshwater mussels (Pandolfo et al. 2012). Because *G. angulata* and *M. falcata* are likely host specialists, they are at risk of low or unsuccessful metamorphosis of larval glochidia on their host fishes if those hosts are thermally stressed by elevated water temperature.

In the chronic thermal tests, *G. angulata* and *Cottus* spp. were experimentally held at 2°C below their CTmax temperatures for 7 d, to provide initial data on how mussels and sculpins would react to sustained exposure to elevated water temperatures. It is important to note that maintaining the test organisms at a constant elevated water temperature for a week is a scenario these animals would not likely experience in the wild. Additionally, because test temperatures were 5°C different between mussels and *Cottus* spp., direct comparisons cannot be made. Nevertheless, the *Cottus* spp. survival curve is different from the *G. angulata* survival curve, and some sculpins survived the chronic test while all the mussels perished. Further chronic thermal studies, using the same test temperature for mussels and host fish, would be useful to better compare their long-term thermal tolerance.

In conclusion, this study established the upper thermal limits for two freshwater mussels, *G. angulata* and *M. falcata*, and two sculpins, *C. marginatus* and *C. beldingi*, by using acute and chronic thermal tests. We showed that *G. angulata* and *M. falcata* can withstand acute exposure to higher water temperature than the sculpin species tested. The chronic tests

demonstrate that *G. angulata* survived longer and was more tolerant to prolonged elevated temperature than *M. falcata*. Experimental thermal testing of aquatic organisms is important to inform predictions of their survival as water temperatures increase globally due to climate change (Zhu et al. 2021). These temperature tests on interacting species (rather than on a set of independent species) have the potential to inform how species interactions might be affected by environmental temperature changes. The determination of the upper thermal limits for the mussels and sculpins in this study can be used to determine imperilment risk within the native habitats they occupy. In addition, it will be useful for developing conservation plans for at-risk mussel and fish species and it will provide a reference for future studies on other imperiled species.

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