Impacts of Seasonal Thermal Stress on Energetics of *Popenaias popeii* (Texas hornshell)

by

Evelyn Bates Pieper

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 9, 2023

Keywords: energetics, scope for growth, respirometry, specific dynamic action, Texas hornshell, *Popenaias popeii*

Approved by:

James A. Stoeckel, Chair, Associate Professor, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University
Russell A. Wright, Associate Professor, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University
Hisham A. Abdelrahman, Assistant Professor, Department of Biology, Marine Biology and Environmental Science, Roger Williams University

Abstract

Texas hornshell (*Popenaias popeii*) is a federally endangered mussel occurring in Texas, New Mexico, and Mexico. We examined temperature effects on energetic costs of feeding, and on scope for growth: the net energy balance available for reproduction and growth. Mussels were acclimated to experimental temperatures for ≥ 2 week and then subjected to energetic assays (i.e. respiration rate, clearance rate, and assimilation efficiency). Energetic costs of feeding and digestion were greatest at lowest (16℃) and highest (32℃) temperatures tested, but negligible at intermediate temperatures (20℃). Scope for growth peaked at 28℃ and rapidly fell as temperatures increased from 28 to 32℃. Riverine temperature profiles suggest that the primary growing season is in early summer and early fall, with declining surplus energy in mid-summer. Flow regulations to help minimize unfavorable temperatures during mid-summer may be critical for the long-term survival of this species.

Acknowledgments

I would like to thank the funding agency CEHMM (grant #: G00015494), Charles Randclev and David Berg for their collaboration, and the CEHMM team for their assistance with field work. I would like to thank my advisor, Dr. Jim Stoeckel, for his mentorship, guidance, and kindness through my time at Auburn. He brought me on as a technician, fresh out of undergrad, because he saw potential for more. Only with his support and encouragement through this process was I able to complete my master's degree. Thank you for everything. I would also like to thank my committee members, Dr. Rusty Wright, and Dr. Hisham Abdelrahman, for their support and advise through my thesis writing process. Finally, I would like to thank Gerry Dinkins, the Curator of Malacology at the Univeristy of Tennessee McClung Museum of Natural History, for introducing me to the wonderful world of mussels and making me fall in love with these unique creatures.

I also want to thank all my lab mates, past and current. Thank you to Gabby Elliott and Nicole Tripp for being the best officemates a girl could ask for, your countless hours of encouragement and support were so appreciated. Thank you to Dr. Kaelyn Fogelman, now an assistant professor at Troy University, for your kindness and mentorship through my time at Auburn. Thank you to Hannah Adkins, for her assistance with these experiments and her contribution of her wonderful figures of equipment. Finally, a huge thank you to my good friend and colleague Jessica Radich. You were by my side every step of the way for this research, from fieldwork, to troubleshooting experiments, to figuring out data analysis. I quite literally could not have done this without you and I could not have asked for a better partner in this, thank you for everything.

Finally, I want to thank my friends, family, and loved ones for their constant moral support and believing in me every step of the way.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

THS	Texas hornshell
ESA	Endangered Species Act
SDA	Specific dynamic action
DO	Dissolved oxygen
AFW	Artificial freshwater
RO/DI	Reverse osmosis/dionized
mg O_2/kgWWW/h	Milligram oxygen per kilogram whole wet weight per hour
SFG	Scope for growth
J/gWWW/h	Joules per gram whole wet weight per hour

Chapter 1: Effects of Temperature on Specific Dynamic Action of the Last Remaining Mussel in New Mexico - *Popenaias popeii*

INTRODUCTION

Texas hornshell (THS; *Popenaias popeii)* are a federally listed endangered freshwater mussel with a scattered range in the Rio Grande River drainage basin in Texas and New Mexico (US Fish and Wildlife Service; USFWS, 2018). On February 9, 2018, the USFWS officially listed *P. popeii* as endangered under the Endangered Species Act (ESA) of 1973. A driving factor behind the listing status of this mussel was a dramatic decrease in range continuity. Texas hornshell was historically found throughout the Rio Grande River drainage basin, with its southernmost range in the main river near the Gulf of Mexico, and its northernmost range in the Black River in southwest New Mexico (Karatayev et al., 2018). Currently, it is estimated to exist in only 21% of its presumed range, with one river population being near extirpation (Randklev et al., 2018). Threats to THS include decreased water flow caused by increased agriculture pressure and prolonged droughts, increased salinity, and increasing temperatures, along with habitat degradation and agricultural runoff pollutants (Rangaswami et al., 2023a).

Knowledge of environmental tolerances of this species will greatly aid the development and refinement of conservation strategies. Previous studies have examined thermal tolerances of THS glochidia, juveniles, and presumed fish hosts (Rangaswami et al., 2023a,b), but there has been little to no research examining effects of thermal stress on adult THS.

Energy budgets of ectotherms are strongly affected by temperature. Standard metabolic rate (energy required for basic maintenance of an organism) typically increases with temperature (Allan et al. 2006; Ganser et al. 2015). Energy requirements can be met using a combination of internal energy stores (i.e. glycogen; Mathieu and Lubet, 1993; Isani et al., 1995) and external

energy resources (i.e. food). However, feeding activities themselves incur energetic costs as does digestion of the acquired meal. Energetic costs associated with digestion, absorption, and assimilation of a meal are typically referred to as specific dynamic action (SDA; Jobling 1985; Secor, 2009). Our definition of SDA in the context of this study also includes energetic costs associated with filtration and ingestion of feed. Given that mussels are sessile animals, activity associated with locating and capturing food items is minimal relative to more motile taxa such as fish.

The metabolic costs of feeding have been well documented ever since it was first published in 1789 by Séguin and Lavoisier (Lavoisier and Séguin, 1789). Specific dynamic action may incur substantial metabolic costs for aquatic ectotherms and offset a significant proportion of energy available from ingested food. This phenomenon has been studied in fish for decades (Tander and Beamish, 1979; Jobling and Davies, 1980; Eliason et al., 2007), revealing that SDA may reach twice the resting metabolic rate (Aslop and Wood, 1997; Jourdan-Pineau et al., 2010). Similar to fish, SDA may double the metabolic rate in marine bivalves such as *Perna canaliculus* (Lurman et al., 2016) and *Mytilus edulis* (Thompson and Bayne, 1972). The mechanical costs of feeding have been estimated as representing 18–24% of energy available from ingested food while digestion and assimilation may amount to an additional 6–8% (Bayne and Scullard, 1977; Bayne et al. 1976). Specific dynamic action can be affected by environmental factors such as temperature (Segler et al 2023; Tirsgaard et al 2015). Filtration rates of bivalves are also particularly sensitive to changes in temperature (Bayne et al. 1976).

The ecological significance of SDA in energy budgets of animals is not well understood and has been identified as an area in particular need of additional research (McCue 2006). In the natural environment, organisms may experience thermal stress on a variety of temporal scales

ranging from daily, to seasonal. They may also experience increasing thermal stress across multiple years due to climate change. With the habitat of THS being in the deserts of New Mexico and Texas, the likelihood of their experiencing increased thermal stress due to decreased water flow and increased droughts is significant.

The specific objective of this study was to address the following questions concerning Texas hornshell mussels: (1) how does the relationship between respiration rate and temperature differ between fasted and actively feeding mussels? (2) how do absolute (i.e. feeding – fasted respiration rates) and proportional (i.e. feeding/fasted respiration rate) costs of SDA change with temperature and (3) under a natural, seasonal thermal regime, when is SDA likely to incur the greatest costs for Texas hornshell mussels.

METHODS

Collection and laboratory acclimation

Fifty THS mussels were collected in early October of 2021 from the Black River in Carlsbad, New Mexico under federal collection permit # TE78507C-0. Only 30 of the 50 mussels collected are discussed in this study, the other 20 THS were collected to be used in additional experiments and to account for potential mortalities. On Day 1, we conducted a qualitative search by hand for THS in multiple pools of the Black River and flagged the approximate locations of detected mussels. On Day 2, we collected animals from the three pools that had the highest detection of mussels. Less than 50% of detected mussels were collected from any given pool, for a combined total of 50 mussels across the three pools. Dissolved oxygen (DO), salinity, pH, and temperature were measured *in situ* from each pool. Hardness, alkalinity,

total ammonia nitrogen, and nitrites were measured from 250 mL water samples collected from all three pools and shipped overnight to Auburn, AL.

Mussels and water samples were sandwiched between moist, cotton towels (detergent and softener free) in three hard-sided coolers (≤ 20 mussels/cooler) with 1–2 small ice-packs in each cooler to keep them from overheating while shipping overnight from Carlsbad, NM to the Crustacean and Molluscan Ecology Lab (CAMEL) lab in Auburn, AL. Upon arrival in Auburn, AL, the mussels were measured and weighed and then placed in prepared upwellers containing 5-salt hard AFW (artificial freshwater; Smith et al 1997) to approximate water quality at the collection site. The recipe was as follows: 151 L RO/DI water, 29.07 g sodium bicarbonate, 15.14 g calcium sulfate, 15.14 g calcium chloride, 9.09 g magnesium sulfate, and 1.21 g potassium chloride.

Upwellers were filled with hard AFW and circulated for ≥ 2 weeks prior to the arrival of collected mussels in order to allow for establishment of an active biofilter. Upwellers consisted of an outer insulated container (116 L capacity), within which sat a smaller container (63 cm × 40 cm × 35 cm) with a pump (EcoPlus Eco 264; 1,098 L/h) connected to pull water from the outer water chamber, into the inner chamber (Figure 1.1). Water flowed up and through a suspended mesh-bottom basket containing a layer of pea gravel. The gravel provided surface area for establishment of biofilter bacteria. Upon arrival, mussels were initially held in the gravel baskets but were later transferred to large cups containing pond-bottom sediments to more closely mimic the soft, undercut bank habitat they were collected from.

During the holding period, water quality was monitored weekly. Partial water changes were initiated if water quality parameters fell outside of the ranges presented in Table 1.1. Mussels were held in the lab at room temperature (~21°C) for ≥ 2 weeks to acclimate them to lab

conditions. During this time they were held at a12 hour light cycle and fed a daily ration of 0.2 mL LPB Frozen Shellfish Diet (an algae paste comprised of intact, dead algal cells from Reed Mariculture Inc., Campbell, California) at 2 h increments using an automated feeder (GHL Doser 2.2, GHL USA LLC, Wilmington, North Carolina).

Acclimation to experimental temperatures

To initiate experiments, 30 mussels were randomly assigned to 5 experimental temperatures (16°C, 20°C, 24°C, 28°C, and 32°C), with 6 total mussels/treatment. Two upwellers were assigned to each temperature (10 total upwellers; 3 mussels per upweller). Temperature in each upweller was then increased or decreased at a rate of 1°C per day. Once the experimental temperature was reached, mussels were acclimated to that temperature for ≥ 2 weeks. During this time, mussels were held under the same water quality conditions as described for the initial holding period. Automatic feeder supply rates were adjusted at each temperature to maintain an algal concentration of ~30,000 cells/mL.

Respiration rates of fasted mussels

On Day 0, we filled a respirometry trough (~300 cm × 60 cm × 66 cm) with ~189 L of hard AFW, and brought the water to the appropriate experimental temperature using heaters (Inkbird IRC-306T controllers) or chillers (AquaEuroUSA – 0.07457 kilowatt), and mixed water overnight with a submersible pump.

On the morning of Day 1, the six mussels assigned to the experimental temperature were taken from their holding upweller and cleaned with water and a brush to remove any built-up algae, dirt, or debris from the shell. The mussels were then placed in holding cups in the respirometry trough without food, to begin a 24-h fasting period. We then set up an eight-

chamber optical respirometry system (Loligo® Systems, Viborg, Denmark) in the same respirometry trough. Acrylic respirometry chambers were either 422 mL or 600 mL in volume, based on mussel size. Experimental mussels were then placed in chambers (one mussel per chamber) such that the ratio of chamber volume (mL) to mussel wet mass (g) was approximately 7:1, similar to Haney et al. (2020). Two additional chambers received no mussels and were used as controls to account for background bacterial respiration.

Each chamber was equipped with two pumps, a flush pump and a recirculating pump. Each intermittent respirometry cycle consisted of a flush, wait, and measure period. Recirculating pumps remained on throughout all periods of each cycle and circulated water through a closed loop for each chamber (Figure 1.2). During the flush period, flush pumps were turned on and replenished chambers with ~100% saturation trough water while flushing out metabolic wastes. During the wait and subsequent measurement periods, the flush pumps were turned off and mussels drew down O_2 within each chamber. Dissolved oxygen was continuously measured and recorded through all periods of each cycle.

Measurement periods were adjusted at each temperature such that the DO in any given chamber did not decrease below 80% oxygen saturation (Clark et al., 2016). Flush periods were of a sufficient length that DO within the chamber returned to near 100% saturation for several minutes before the next measurement period was initiated. Once the flush and measurement periods had been set, the AutoResp software (2.3.0; Loligo® Systems, Viborg, Denmark) continued to turn the close and flush pumps on and off at the appropriate times for the remainder of the experiment. During each measurement period, respiration rate (MO_2) was calculated by the AutoResp software using the following formula:

$$\text{RMR (mg } O_2\text{/gWWW/h)} = \frac{V\,([O2]_{t0} - [O2]_{t1})}{t \times BW}$$

Where:

$[O_2]_{t0}$ (mg O_2/L) = DO at time t0

$[O_2]_{t1}$ (mg O_2/L) = DO at time t1

V = chamber volume (L) – volume of mussel (L)

t = time t1 (h) – time t0 (h)

BW = whole mussel body weight (g)

Mussels remained in respiration chambers with no food from the morning of Day 1 through 17:00 on Day 2. Although MO_2 was calculated for each measurement period, we only used MO_2 estimates from 9:00 – 17:00 on Day 2 for analysis. This provided for a 24-h fasting period prior to the first used MO_2 estimate in order to minimize or eliminate any food remaining in the digestive tract. No feces were observed in chambers after the 24-h fasting period. It also allowed the mussels to acclimate to the chambers for ≥12 h overnight (Haney et al. 2020) and allowed for a 2 h adjustment period after the lights came on at 7:00.

Respiration estimates for mussels were corrected for background respiration by calculating MO_2 within each control chamber and subtracting the average control MO_2 from the mussel chamber MO_2 estimates during each measurement cycle.

Respiration rate of feeding mussels

After completing the unfed experiments at 17:00 on Day 2, mussels remained in respiration chambers and sufficient algae was added to trough water to achieve a concentration

of 30,000 cells/mL (± 5,000 cells/mL). Algae concentration was then kept constant at ~30,000 cells/ml using a GHL Doser 2.1 (auto-feeder), set to dose an appropriate amount of algae per hour. Mussels were allowed to feed on algae overnight as flush periods replenished water in chambers. Respirometry on feeding mussels was conducted on Day 3 in the same manner as previously described for the unfed trial with only data from 9:00–18:00 being used for analysis.

Data analysis

Within the 9:00–18:00 time period, we only used data from measurement periods where the mussels were considered to have been open and actively respiring. Any measurement periods for mussel chambers where the relationship between DO and time yielded an $R^2 < 0.9$, as generated by the AutoResp software, was considered a partial or full closure event and that data was not included (Chabot et al. 2020). Similarly, MO_2 from control chamber measurement periods that yielded an $R^2 < 0.9$ were considered to represent negligible background respiration for that period.

To determine the relationship between respiration rate and temperature for fasted mussels, we calculated the mean respiration rate for each individual mussel. The resulting data set was then plotted against temperature and fit with a linear and a quadratic regression using SigmaPlot 13 (Systat Software©, Inc. 2014). The model with the smallest Akaike information criterion (AICc) was then selected as the best fit. This procedure was then repeated for the feeding mussel respiration rates.

Temperature coefficient (Q_{10}) was calculated for MO_2 of fed and unfed mussels based on the following formula (Lampert 1984):

$$Q_{10} = \{MO_2^{t2} / MO_2^{t1}\}^{(10 / [t2 - t1])}$$

where t1 = lower temperature (20°C), t2 = higher temperature (32°C), MO_2^{t1} = MO_2 at lower temperature (20°C), and MO_2^{t2} = MO_2 at higher temperature (32°C).

Because we used the same mussels at a given temperature for fasted and feeding trials, we estimated the absolute SDA for each individual by subtracting the estimated fasting respiration rate from that of the corresponding feeding respiration rate for each mussel. Factorial SDA was estimated as the fed respiration rate divided by the fasted respiration rate.

To estimate seasonal changes in SDA under natural thermal regimes, we obtained 2021 and 2022 temperature data from a logger installed on the Black River, near our collection site by collaborating researchers at Texas A&M University (Rangaswami et al. 2023b). Data was collected daily at 30-minute intervals, allowing for incorporation of diurnal temperature swings. Within each year, we selected the dates where temperatures fell within the temperature range (16°C – 32°C) tested in this study. We then applied linear regression (for unfed MO_2) and quadratic regression (for fed MO_2) to estimate fed and unfed respiration rates on each date based on temperature.

RESULTS

Mussel length ranged from 73 – 106.7 mm and whole wet weight ranged from 44.5 – 110.1 g. Water quality parameters from the Black River at the collection site were as follows: hardness > 500 mg/L $CaCO_3$; alkalinity = 160 – 250 mg/L $CaCO_3$; DO = 7.2 mg/L, 81.4% saturation; salinity = 0.81 ppt; and pH = 9.09; temperature = 21.25°C. The hard AFW recipe used to fill the laboratory upwellers exhibited water quality parameters of ~ 8.35 pH, total hardness ~ 197.5 mg/L $CaCO_3$, and total alkalinity ~ 120 mg/L $CaCO_3$. Water quality parameters seldom fell outside of the acceptable levels that triggered a water change (Table 1.1). No mussel

deaths were observed during the holding period prior to conduction experiments or during experiments.

The relationship between respiration rate and temperature was best described by a linear regression for fasted mussels ($\hat{y} = 0.4117x - 3.126$, $R^2 = 0.65$, $p < 0.0001$; Figure 1.3A). Conversely, when those same mussels were actively feeding, the relationship was best described by a quadratic regression ($\hat{y} = 18.5994 - 1.5317x + 0.0426x^2$, $R^2 = 0.65$, $p < 0.0001$; Figure 1.3B) with respiration rates remaining fairly stable between 16 and 20℃ and increasing rapidly from 20 to 32°C. The Q_{10} was estimated to be 1.45 for unfed mussels and 2.65 for fed mussels across a temperature range of 20–32℃.

The relationship between SDA, calculated as an absolute or factorial value, and temperature followed a concave unimodal pattern with greatest SDA estimates observed at either end of the temperature range tested (Figure 1.4A, B). At 16°C, mean respiration rate was 2.1 mg O_2/kgWWW/h higher for feeding mussels compared to fasted mussels. This represented a 66% increase in respiration rate. At 32°C, mean respiration rate was 2.9 mg O_2/kgWWW/h higher for feeding mussels compared to fasted mussels. This represented a 28% increase in respiration rate.

Seasonal patterns in temperature showed an expected pattern of rising temperatures in the spring, and declining temperatures in the fall with maximum summer temperatures peaking between 30 and 33℃ in both years (Fig. 5). In late spring and fall, there was little difference between estimated unfed and fed respiration rates. However, during the summer months, feeding was estimated to incur substantial metabolic costs at daily maximum temperatures. At daily minimum temperatures, respiration rates estimated for fed mussels showed a strong degree of overlap with that of unfed mussels (Figure 1.5).

DISCUSSION

Freshwater unionid mussels are at particular risk of thermal stress due to their sessile nature and limited ability to move to thermal refuges – although some species may temporarily burrow down into the sediments to reach cooler temperatures (Gough et al. 2012). Thermal stress is of particular importance for low discharge rivers such as the Black River, New Mexico where temperatures frequently reach or exceed 30°C, and in regions where temperatures are predicted to increase over time due to climate change (Milly et al. 2008; U.S. Bureau of Reclamation, 2011). Increased temperatures lead to increases in energy required by mussels for basic maintenance, with maintenance costs peaking in the hot summer months. This potentially reduces the amount of surplus energy (more than that required for maintenance) that is available for vital processes such as growth and reproduction (Ganser et al. 2015). Similar to previous studies (Haney et al. 2020), Texas hornshell mussels from the Black River showed a strong increase in resting metabolic rate with increasing temperature, indicating an increase in energy required for basic maintenance.

In addition to energy required for basic maintenance, SDA — energetic costs associated with feeding and digestion — also tend to increase with water temperature for fish (Tirsgaard et al 2015). Unlike fish, energetic costs associated with SDA have rarely, if ever, been measured and reported for freshwater unionid mussels. In this study, we found that respiration rates of feeding mussels responded to temperature differently than that of fasting mussels — reflecting the temperature-dependent effects of SDA. As temperatures warmed from 20–32°C, the magnitude of the increase in respiration for feeding mussels was substantially higher than that of fasting mussels; shown by the higher Q_{10} for feeding THS as compared to fasting mussels. Thus, SDA appears to be more sensitive to temperature than energetic costs of basic maintenance. A

relatively low effect of temperature on respiration rates of fasting mussels has also been reported for the marine mussel *Mytilus californianus* (i.e. Q_{10} = 1.20; 13–22℃; Bayne et al. 1976) suggesting that this may be a common feature across distantly related bivalve taxa.

Metabolic costs of SDA appeared to be less severe for the freshwater THS compared to some marine bivalve taxa. In *Mytilus spp*., SDA may double the metabolic rate (Lurman et al., 2013; Thompson and Bayne, 1972). However, costs of SDA were considerably lower for THS across a range of temperatures. At moderate temperatures (i.e. 20℃), THS did not appear to incur any metabolic costs of SDA. Estimated SDA increased as temperatures rose from 16 – 32℃ and also as temperatures cooled from 16–32℃, but the mean factorial SDA rose to only 66% at 16℃ and 28% at 32℃. In *Mytilus spp*., the mechanical costs of feeding can represent 18–24% of the energy available from ingested food, with costs of digestion and assimilation representing an additional 6–8% (Bayne and Scullard, 1977; Bayne et al. 1976). Because SDA costs appear to be substantially lower for THS than for *Mytilus spp*., SDA likely requires a lower proportion of available energy from ingested food, but this is something that should be investigated in future studies.

Although costs of SDA were less for freshwater THS than has been previously estimated for marine bivalves, proportional increases in respiration of 66 and 28% for actively feeding mussels at colder and warmer temperatures may still incur substantial energetic stress on mussels during different times of the year. Even though the proportional increase in respiration was greater at cool temperatures, the absolute difference in respiration rate between fed and unfed mussels was actually higher at warm temperatures. This translated to only relatively minor increases in estimated respiration rates of feeding mussels during spring in the Black River, but major increases during the hot summer months. Even as THS require more and more energy to

meet their basic maintenance needs during the hot summer months, the costs of obtaining energy from their environment (i.e. feeding and digestion) are increasing at an even faster rate. The hotter the summer, the higher the energetic cost to these mussels. Temperatures approaching 32°C are seen regularly in the Black River during summer. As the climate continues to change, river water temperatures are estimated to increase on average by 1.2°C by 2100 (van Vliet et al. 2013). Additional effects of reduced water flow from increased agriculture withdrawal and drought frequency, make it likely that mussels will see an increase in extreme temperature events, significantly increasing metabolic stress.

This study expands our knowledge of costs of feeding for aquatic ectotherms, with the added benefit of quantifying these vital physiological parameters for a federally endangered mussel facing current and future thermal stress in its remaining natural habitat. Additional studies are needed to examine changes in SDA at even higher temperatures in order to predict the impacts of further increases in temperature due to climate change and reduced flow resulting from water demand and drought. Similarly, studies examining the impact of increasing temperature on energetic intake and energy budgets are needed. Do mussels keep pace with higher energetic costs of feeding by simply ingesting/digesting more food? Or will they be running an energetic deficit, unable to ingest and digest enough feed to keep up with higher energetic costs? These questions are addressed in the following chapter regarding scope for growth for THS mussels.

References

Allan, E.L., Froneman, P.W., and Hodgson, A.N. 2006. Effects of temperature and salinity on the standard metabolic rate (SMR) of the caridean shrimp *Palaemon peringueyi*. Journal of Experimental Marine Biology and Ecology, 337 (1), 103-108.

Augspurger, T., Keller, A.E., Black, M.C., Cope, W.G. and Dwyer, F.J., 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. Environmental Toxicology and Chemistry: An International Journal, 22(11), 2569-2575.

Bayne, B.L., Bayne, C.J., Carefoot, T.C., and Thompson, R.J., 1976. The Physiological Ecology of *Mytilus californianus* Conrad. Oecologia, 22, 211-228.

Bayne, B.L. and Scullard, C., 1977. An apparent specific dynamic action in Mytilus edulis L. Journal of the Marine Biological Association of the United Kingdom, 57(2), 371-378.

Boyd, C.E., 2014. Nitrite toxicity affected by species susceptibility, environmental conditions. Glob Aquac Advocate, 17, 34-36.

Chabot, D., Zhang, Y., and Farrell, A.P., 2020. Valid oxygen uptake measurements: using high r^2 values with good intentions can bias upward the determination of standard metabolic rate. Fish Biology, 98, 1206-1216.

Clark, T.D., Sandblom, E., and Jutfelt, F., 2016. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. The Journal of experimental Biology, 216, 2771-2782.

Eliason, E.J., Higgs, D.A. and Farrell, A.P., 2007. Effect of isoenergetic diets with different protein and lipid content on the growth performance and heat increment of rainbow trout. Aquaculture, 272(1-4), 723-736.

Ganser, A.M., Newton, T.J. and Haro, R.J., 2015. Effects of elevated water temperature on physiological responses in adult freshwater mussels. Freshwater Biology, 60, 1705-1716.

Gough, H.M., Gascho Landis, A.M., and Stoeckel, J.A., 2012. Behaviour and physiology are linked in the responses of freshwater mussels to drought. Freshwater Biology, 57(11), 2356-2366.

Haney, A., Abdelrahman, H. and Stoeckel, J.A., 2020. Effects of thermal and hypoxic stress on respiratory patterns of three unionid species: implications for management and conservation. Hydrobiologia, *847*, 787-802.

Isani, G., Cattani, O., Zurzolo, M., Pagnucco, C. and Cortesi, P., 1995. Energy metabolism of the mussel, Mytilus galloprovincialis, during long-term anoxia. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 110(1), pp.103-113.

Jobling, M. and Davies, P.S., 1980. Effects of feeding on metabolic rate, and the specific dynamic action in plaice, Pleuronectes platessa L. Journal of Fish Biology, 16(6), 629-638.

Jobling, M. Growth In: Fish energetics: new perspectives, pp 213-230. Edited By P. Tytler and P. Calow. Baltimore: Johns Hopkins Press 1985.

Jourdan-Pineau, H., Dupont-Prinet, A., Claireaux, G., and McKenzie, D.J., 2010. An investigation of metabolic prioritization in the European Sea Bass, *Dicentrarcus labrax*. Physiological and Biochemical Zoology, 83 (1), 68-77.

Karatayev, A.Y., Burlakova, L.E., Miller, T.D. and Perrelli, M.F., 2018. Reconstructing historical range and population size of an endangered mollusc: long-term decline of Popenaias popeii in the Rio Grande, Texas. Hydrobiologia, 810, 333-349.

Lampert, W., 1984. The measurement of respiration. A manual on methods for the assessment of secondary productivity in fresh waters, *17*, 413-468.

Lavoisier, A.L. and Séguin, A., 1789. Premier mémoire sur la respiration des animaux. Mémoires de l'Académie des Sciences, 566.

Lurman, G.J., Hilton, Z. and Ragg, N.L., 2013. Energetics of byssus attachment and feeding in the green-lipped mussel Perna canaliculus. The Biological Bulletin, 224(2), 79-88.

Mathieu, M. and Lubet, P., 1993. Storage tissue metabolism and reproduction in marine bivalves—a brief review. Invertebrate Reproduction & Development, 23(2-3), 123-129.

McCue, M.D., 2006. Specific dynamic action: a century of investigation. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 144(4), 381-394.

Milly, P.C., Betancourt, J., Falkenmark, M., Hirsch, R.M., Kundzewicz, Z.W., Lettenmaier, D.P. and Stouffer, R.J., 2008. Stationarity is dead: Whither water management?. Science, 319(5863), 573-574.

Randklev, C.R., Miller, T., Hart, M., Morton, J., Johnson, N.A., Skow, K., Inoue, K., Tsakiris, E.T., Oetker, S., Smith, R. and Robertson, C., 2018. A semi-arid river in distress: Contributing factors and recovery solutions for three imperiled freshwater mussels (Family Unionidae) endemic to the Rio Grande basin in North America. Science of the Total Environment, 631, 733-744.

Rangaswami, X.L., Goldsmith, A.M., Khan, J.M., Robertson, C.R., Lopez, R.R. and Randklev, C.R., 2023a. Thermal tolerances of Popenaias popeii (Texas hornshell) and its host fish from the Rio Grande Basin, Texas. Scientific Reports, 13(1), p.4603.

Rangaswami, X.L., Kiser, A.H., Ramey, M., Stoeckel, J.A., Berg, D.J., Lopez, R.R., and Randklev, C.R., 2023b. Thermal tolerance exceedances of an endangered unionid mussel in the

Rio Grande Basin, with implications for river management. Aquatic Conservation: Marine and Freshwater Ecosystems, 1-14.

Secor, S.M., 2009. Specific dynamic action: a review of the postprandial metabolic response. Journal of Comparative Physiology B, 179, 1-56.

Segler, P., Vanselow, K.H., Schlachter, M., Hasler, M., and Schulz, C. 2023. SDA coefficient is temperature dependent in rainbow trout (*Oncorhynchus mukiss,* Walbaum 1792) in a practical approach using group respirometry. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 265, 110832.

Smith, M.E., Lazorchak, J.M., Herrin, L.E., Brewer-Swartz, S. and Thoeny, W.T., 1997. A reformulated, reconstituted water for testing the freshwater amphipod, Hyalella azteca. Environmental Toxicology and Chemistry: An International Journal, 16(6), 1229-1233.

Tandler, A. and Beamish, F.W.H., 1979. Mechanical and biochemical components of apparent specific dynamic action in largemouth bass, Micropterm salmoides Lacéepède. Journal of Fish Biology, 14(4), 343-350.

Thompson, R.J., and Bayne, B.L., 1972. Active metabolism associated with feeding in the mussel *Mytilus edulis L.* Journal of Experimental Marine Biology, 9, 111-124.

Tirsgaard, B., Svendsen, J.C. & Steffensen, J.F. 2015. Effects of temperature on specific dynamic action in Atlantic cod *Gadus morhua*. Fish Physiology and Biochemistry, 41, 41–50.

U.S. Bureau of Reclamation (USBR). 2011. Reclamation managing water in the West: Interim Report No. 1, Colorado River Basin water supply and demand study, status report. U.S Department of the Interior, U.S. Bureau of Reclamation, Denver, Colorado, USA.

U.S. Fish and Wildlife Service (USFWS). 2018. Endangered and threatened wildlife and plants; endangered species status for Texas hornshell. Federal Register 83, 5720-5735.

van Vliet, M.T.H., Franssen, W.H.P., Yearsley, J.R., Ludwig, F., Haddeland, I., Lettenmaier, D.P., and Kabat, P., 2013. Global river discharge and water temperature under climate change. Global Environmental Change, 2 (2), 450-464.

Table 1.1: Acceptable ranges of upweller water quality parameters (Augspurger et al., 2003; Boyd, 2014)

Water quality parameter	**pH**	**Alkalinity (mg/L $CaCO_3$)**	**Hardness (mg/L $CaCO_3$)**	**Ammonia (mg/L TAN)**	**Nitrite (mg/L N)**
Lower limit	7.0	20	50	-	-
Upper limit	8.5	-	-	0.3	0.5

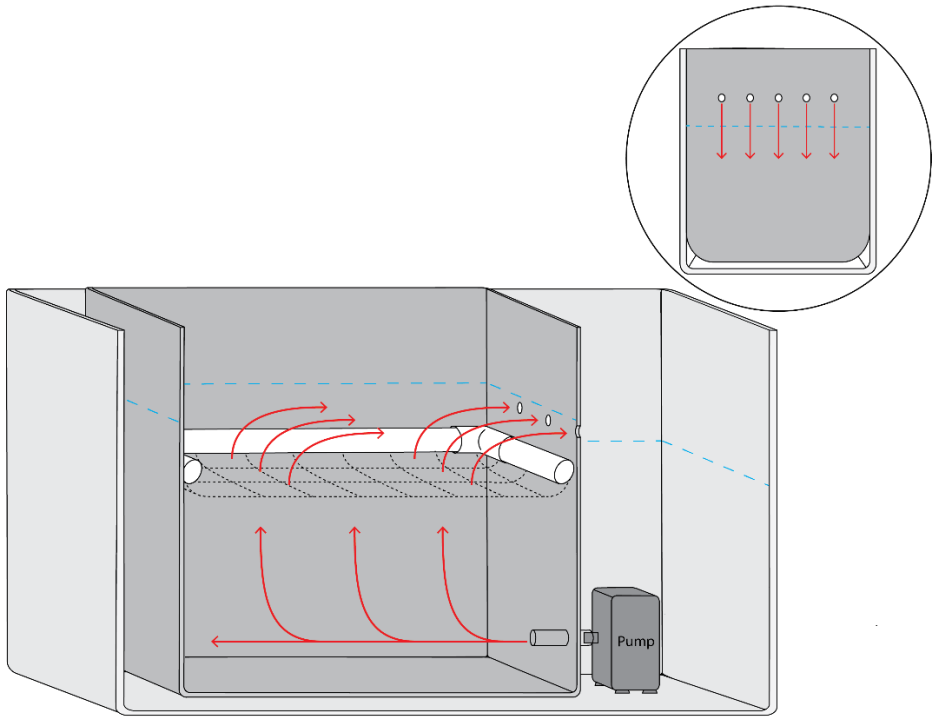

Figure 1.1: Diagram of upweller systems. A submersible pump was placed under the water line (blue dotted line) and pushed water from the outer insulated tub into the bottom of an inner chamber, through a gravel basket (mesh pattern), and back out through holes at the top of the inner chamber. Diagram credit to Hannah Adkins 2023.

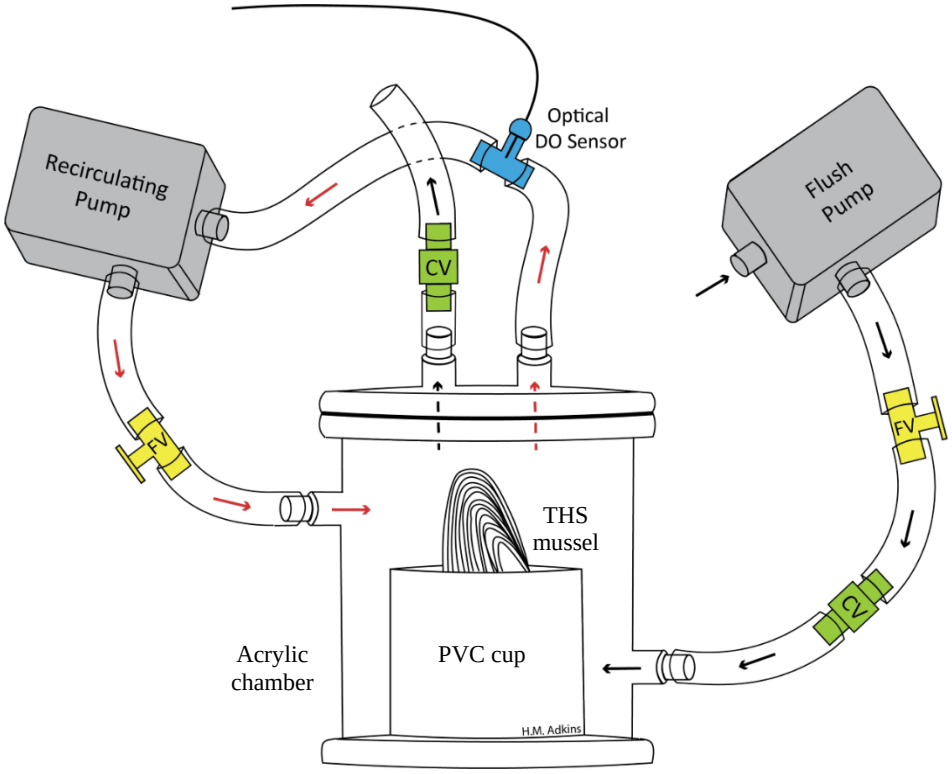

Figure 1.2: Diagram of intermittent respirometry system adapted from Haney et al. (2020) where THS were placed inside a PCV cup in an acrylic chamber, attached to a flush pump to pump fresh, ambient trough water through the chambers during flush cycle, and a recirculating pump to circulate water through the chamber during measure cycles. It also includes the optical DO sensor that measures oxygen levels in water leaving the chamber, check valves (CV) to control the direction of water flow, and flow valves (FV) to control the rate of flow into the chamber. Arrows indicate direction of water flow (red = recirculating water, black = flushing water). Diagram credit to Hannah Adkins 2023.

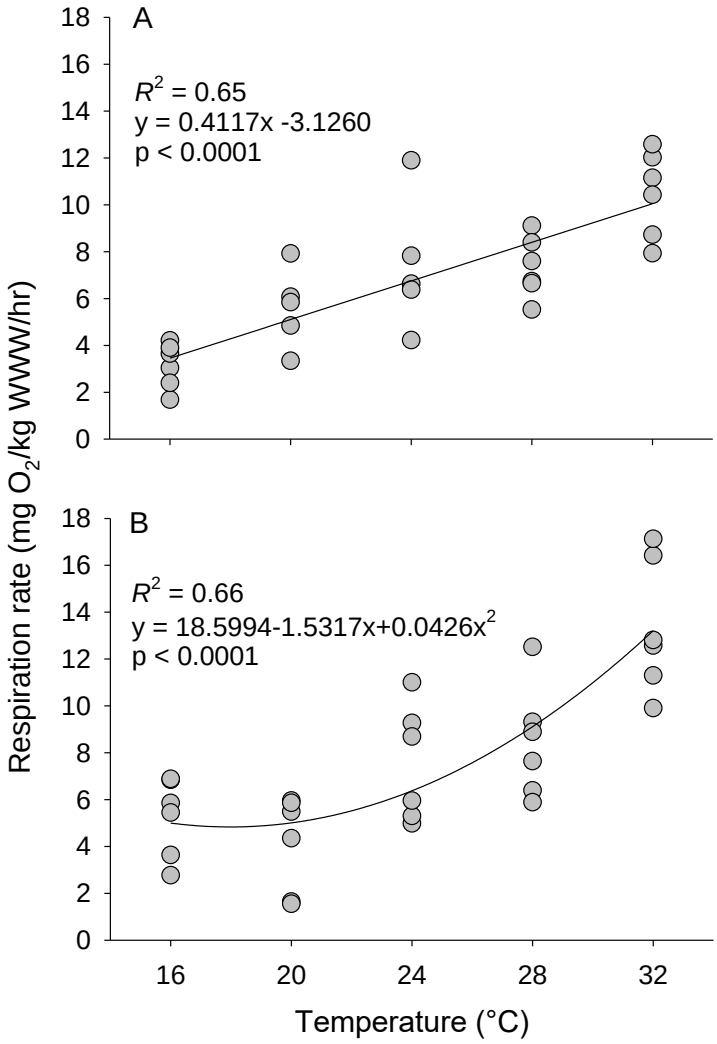

Figure 1.3. Relationships between respiration rate and temperature for A) fasting and B) feeding mussels. Each data point represents an individual mussel. The same individuals were used for unfed and fed trials at the same respective temperatures.

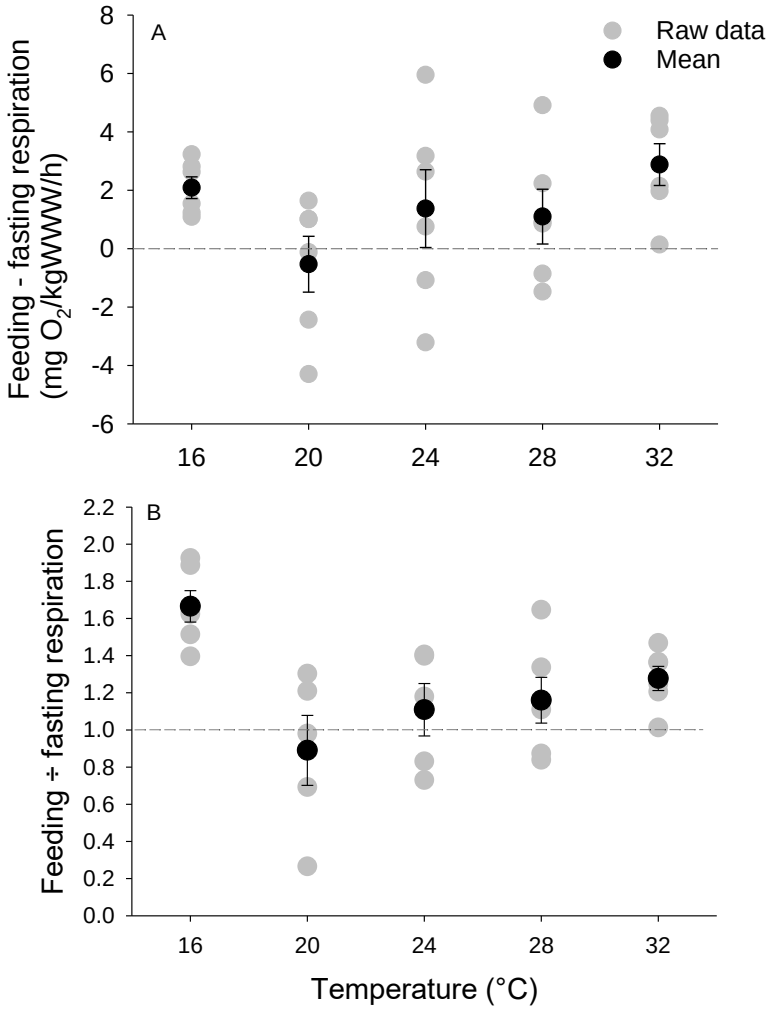

Figure 1.4. Estimates of specific dynamic action calculated as A) difference between feeding and fasting mussel respiration rates (i.e. absolute SDA) and B) feeding divided by fasting respiration rates (factorial SDA). Each grey circle point represents SDA of an individual mussel. Error bars represent standard errors. Dotted line represents no difference between feeding and fasting respiration rates.

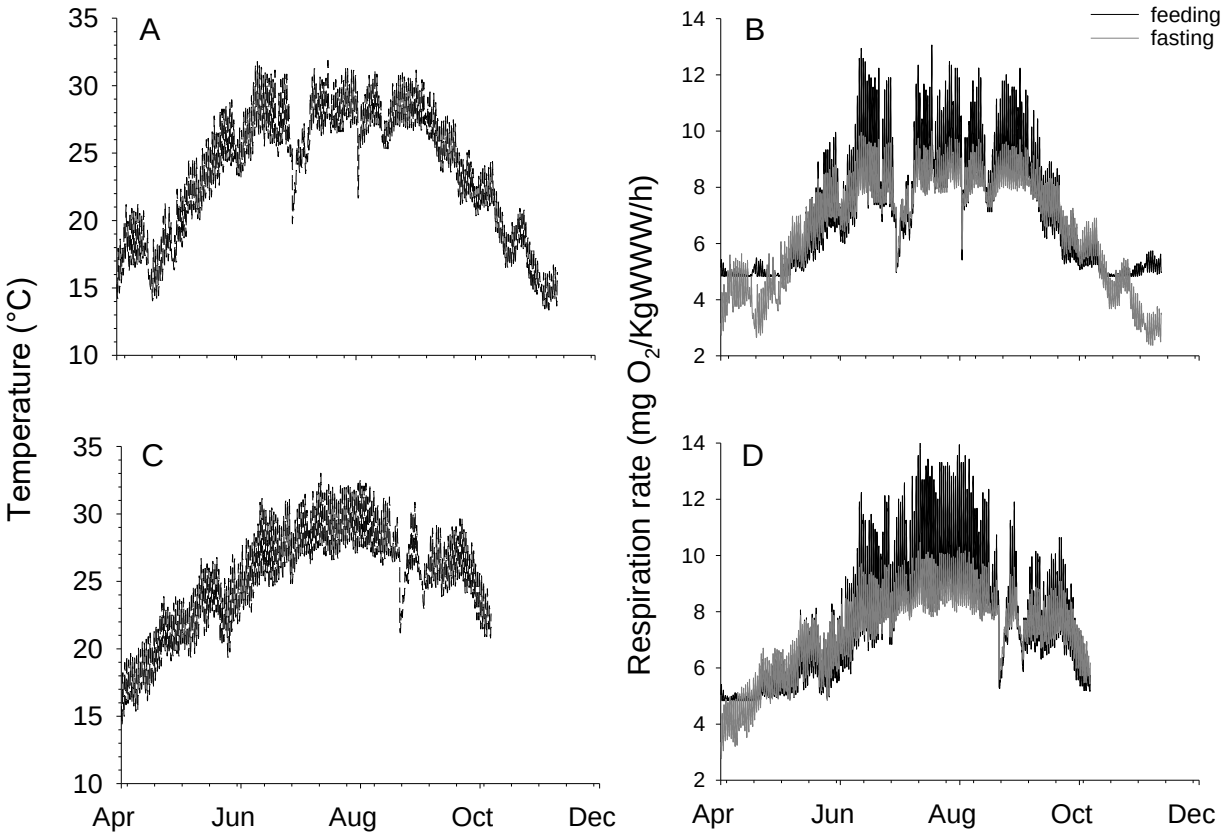

Figure 1.5. Seasonal patterns in Black River water temperatures in A) 2021 and C) 2022. Seasonal patterns in estimated fasting and feeding respiration rates of Texas hornshell as predicted from water temperatures in the Black River in B) 2021 and D) 2022.

Chapter 2: Effects of Temperature on Scope for Growth of *Popenaias popeii*

INTRODUCTION

Predicting effects of increasing temperatures on the health of aquatic organisms is receiving increasing attention in light of climate change, increasing demand for water withdrawal, and alteration of natural flow regimes (Xenopoulos et al., 2005; Martins et al., 2017). Water temperatures are already increasing in many rivers and streams in the United States (Kaushal et al., 2010; Isaak et al., 2012). In the southwestern U.S., annual mean precipitation is very likely to decrease and temperatures are predicted to increase (Intergovernmental Panel on Climate Change, 2023). States such as New Mexico with relatively little rainfall and relatively few surface water systems are at particular risk of flow reductions and rising temperatures — putting their already limited aquatic fauna at risk (Center of Excellence for Hazardous Materials Managment, 2017; Cayan et al., 2010).

One approach to examining sublethal effects of thermal stress on aquatic organisms is to construct energy budgets. Energy budgets of ectotherms are strongly affected by temperature. One type of energy budget that is frequently used is scope for growth (SFG) — amount of energy available to an organism for both reproduction and growth after basic maintenance costs have been met. This approach has been used for assessing multiple stressors on a wide variety of aquatic ectotherms including (*Gammarus pullex* - Naylor et al., 1989; *Perna viridis* – Wang et al., 2011; *Placopecten magellanicus* – MacDonald & Thompson, 1986). It requires the measurement of ingestion and absorption efficiency to estimate energy moving into an organism and respiration rates to estimate energy expended by an organism (Widdows and Staff, 2006; Luck and Ackerman, 2021).

Unionid mussels provide important ecological services and are considered ecosystem engineers — they clear the aquatic systems and play a key role in nutrient cycling (Vaughn et al., 2008; Haag, 2012; Vaughn, 2018). They are also an optimal organism for measuring SFG due to their sessile nature. Unlike fish or other more mobile animals, mussels do not require quantification of energetic costs of motor movements to locate and find prey. Respiration rates can be directly measured while mussels are actively filter-feeding. Given this mode of feeding, we can quantify their food intake by simply measuring the mussel's clearance rate (the volume of water cleared entirely of particles per unit time). The ability to directly measure energetic intake while estimating energetic expenditure allows for a relatively easy estimation of SFG relative to other taxa such as fish.

Texas hornshell (THS; *Popenaias popeii)* are a federally listed endangered freshwater mussel with a scattered range in the Rio Grande River drainage basin in Texas and New Mexico (USFWS, 2018). On February 9, 2018, the U.S. Fish and Wildlife Service officially listed *P. popeii* as endangered under the Endangered Species Act (ESA). A driving factor behind the listing status of this mussel was a dramatic decrease in range continuity. THS was historically found throughout the Rio Grande River drainage basin, with its southernmost range in the main river near the Gulf of Mexico, and its northernmost range in the Black River in southwest New Mexico (Karatayev et al., 2018). Currently, it is estimated to exist in only 21% of its presumed range, with one river population being near extirpation (Randklev et al., 2018). Threats to THS include decreased water flow caused by increased agriculture pressure and prolonged droughts, increased salinity, and increasing temperatures, along with habitat degradation and agricultural runoff pollutants (Rangaswami et al., 2023).

Knowledge regarding environmental tolerances are required for continued development and refinement of conservation strategies for THS. Previous studies have examined thermal tolerances of THS glochidia, juveniles and presumed fish hosts in the Devils River, Texas and found evidence for stream temperatures exceeding LT05/50 values for those life stages (Rangaswami et al., 2023). However, there has been little to no published research examining effects of thermal stress on adult THS and whether temperatures in their remaining habitat regularly exceed thresholds above which sublethal and lethal effects are likely to occur.

In this study, we therefore address the following questions: 1) How does temperature affect physiological rates and SFG of THS and 2) What times of year (if any) are THS at particular risk of sublethal thermal stress due to water temperature?

METHODS

Collection & Lab acclimation

Fifty THS mussels were collected in early October of 2021 from the Black River in Carlsbad, New Mexico under federal collection permit #TE78507C-0. On Day 1 we conducted a qualitative search for THS in multiple pools of the Black River, flagging the approximate locations of detected mussels. On Day 2 we collected animals from the three pools that had the highest numbers of detected mussels. Less than 50% of detected mussels were collected from any given pool, for a combined total of 50 mussels across the three pools. Multiple water quality measurements were measured *in situ* from each pool and recorded at the time of collection: dissolved oxygen (DO), salinity, pH, temperature, and atmospheric pressure.

Mussels were sandwiched between moist, cotton towels (detergent and softener free) in three hard-sided coolers (≤ 20 mussels/cooler) with 1–2 small ice-packs in each cooler to keep

them from overheating while shipping. Two, replicate water samples (250 mL each) were collected from each of the three collection sites and placed in coolers for subsequent hardness/alkalinity analysis and estimation of shipping temperature at the Auburn lab. The coolers with the mussels and water samples were shipped overnight from Carlsbad, NM to the CAMEL lab in Auburn, AL. Upon arriving in Auburn, AL the mussels were placed in prepared upwellers containing 5-salt Hard AFW (artificial freshwater; Smith et al., 1997) to approximate water quality at the collection site.

Upwellers were filled with hard AFW and circulated for ≥ 2 weeks prior to the arrival of experimental mussels in order to allow for establishment of an active biofilter. Upwellers consisted of an outer insulated container (116 L capacity), within which sat a smaller container (63cm x 40cm x 35cm) with a pump (EcoPlus Eco 264, 1098 L/h) connected to pull water from the outer water chamber, into the inner chamber. Water flowed up and through a suspended mesh-bottom basket containing a layer of pea gravel. The gravel provided surface area for establishment of biofilter bacteria. Upon arrival, mussels were initially held in the gravel baskets but were later transferred to large cups containing pond-bottom sediments to more closely mimic the soft, undercut bank habitat they were collected from.

During the holding period, water quality was monitored weekly. Partial water changes were initiated if water quality parameters fell outside of the ranges presented in Table 2.1. Mussels were held in the lab at room temperature (~21°C) for ≥ 2 weeks prior to acclimating them to experimental temperatures. During this time they were held at a 12 hour light cycle and fed a daily ration of 0.2 ml LPB Diet (Reed Mariculture Inc.: Campbell, California) at 2 h increments using an automated feeder (GHL Doser 2.2, GHL USA LLC, Wilmington, North Carolina).

Acclimation to experimental temperatures

To initiate experiments, 6 mussels were assigned to each of 5 experimental temperatures (16°C, 20°C, 24°C, 28°C, and 32°C). Two upwellers were assigned to each temperature (10 total upwellers) and oysters assigned to a given temperature were divided between the two upwellers. Temperature in each upweller was then increased or decreased at a rate of 1°C per day. Once the experimental temperature was reached, oysters were acclimated to that temperature for ≥ 2 weeks. During this time, mussels were held under the same conditions and feeding regime as described for the initial holding period.

General Scope for Growth Assay Design

Scope for Growth methodology was adapted from Widdows and Staff (2006), using temperature as the stressor. After acclimation to laboratory conditions for >2 weeks, thirty mussels were randomly assigned to one of five temperatures (16°C, 20°C, 24°C, 28°C, 32°C), with six mussels per temperature. All mussels were acclimated to their assigned temperature for at least 2 weeks prior to conducting SFG assays. Each individual was tested at only one temperature, but each individual was subjected to multiple SFG assays at that temperature. During acclimation to their assigned temperature, and throughout all subsequent SFG assays, mussels were fed at a nominal concentration of 30,000 cells/ml (± 5,000 cells/mL) using automated feeders (GHL Doser 2.2, GHL USA LLC, Wilmington, North Carolina).

To determine the relationship between SFG and temperature of acclimated mussels, on Day 1 we measured respiration rates of mussels assigned to each temperature in order to assess respiratory energy expenditure. Day 2 consisted of measuring clearance rates of the same mussels at that temperature to assess energy consumed or ingested. At the end of Day Two, feces

were collected to assess food absorption efficiency. SFG was calculated for each individual mussel using the formula:

$$SFG = A - R$$

Where

A = absorbed food energy (J/gWWW/h) = $C \times FAE$

where

C = Energy consumed or ingested (J/gWWW/h)

FAE = Food absorption efficiency

R = Respiratory energy expenditure = $MO_2 \times 14.06$

Where

MO_2 = mass-specific respiration rate (mg O_2/gWWW/h)

14.06 = factor to convert oxygen consumption to Joules (Gnaiger 1983)

Methodology and calculations for A, C, FAE, and R are described below.

Respiratory Energy Expenditure (R)

Respiration rate of actively feeding mussels was measured using intermittent respirometry, as described in Chapter 1. Mussels were in their assigned respiration chambers with algal concentration maintained at 30,000 cells/mL (± 5,000 cells/mL) on by 17:00 on Day 0, allowing for overnight acclimation to the respiration chambers. Day 1 of the SFG assay consisted of running intermittent respirometry from 9:00 – 17:00 at the algal concentration of 30,000 cells/mL (± 5,000 cells/mL).

Absorbed Food Energy Measurements (A, C, FAE)

At the termination of the respiration assays, mussels were removed from the chambers and transferred to filtration cups. Filtration cups were 1L food-grade plastic cups that had been notched to hold 750 mL and allow for water pumped into each cup to overflow back into the experimental trough after reaching capacity (Figure 2.1). Each filtration trial included ten filter cups – six cups held one mussel each whereas the remaining four cups contained no mussels and served as controls to correct for background algal settling rates. During all filtration trials, algal concentrations in the experimental trough were maintained at 30,000 cells/mL (± 5,000 cells/mL) using an automated feeder as described previously. Algal concentrations were monitored by measuring absorbance and converting to cell concentration (cells/mL) using a previously calculated calibration curve between absobance and cells/mL (Figure 2.2).

Mussels were allowed to acclimate to filtration cups overnight. During the acclimation period, water was continually pumped into each cup to maintain a constant algal concentration. Filtration trials were initiated at 9 AM the following morning (Day 2) at which time all pumps were turned off, eliminating water flow into the cups. Two replicate 5 mL initial samples were collected from each cup, and algal concentration was determined via spectrophotometry. Mussels were allowed to filter undisturbed for 30 – 90 minutes, with lower temperatures requiring longer filtration periods. Algal concentrations were not allowed to decrease below 60% of the initial concentration, to avoid skewing filtration rate based on dramatically different algal concentrations. At the end of that time, two replicate 5 mL water samples were collected from all cups and algal cell concentration was again determined via spectrophotometry. Pumps were then turned back on for a minimum of 15 minutes before initiating the next filtration measurement to

ensure algae levels had returned to ambient trough algae concentration (30,000 ± 5,000 cells/mL).

Filtration rates were measured repeatedly in this fashion from 09:00 – 17:00, with 4 to 7 estimates for each mussel at their assigned temperature. At 17:00 water flow to the cups was turned off for the final time. Mussels were removed from cups and feces collected with a pipette, and placed on pre-weighted glass fiber filters. Pseudofeces production was minimal and care was taken when retrieving feces to avoid any psuedofeces in the cups. We collected as much feces as we could from each individual mussel. Feces filters were then dried at 105°C overnight in a drying oven (VWR 1320 Drying Oven), and dry weight was recorded. Feces filters were then combusted at 550°C for 1 hour in a muffle furnace (Thermolyne F62735, ThermoFisher Scientific), and ashed weight was recorded. Ash-free dry mass was calculated as dry weight minus ashed weight and corrected for any changes in filter blanks that had gone through the same process with no feces. Cumulative mass of feces produced by the mussel was not calculated. Only the ratio of ash-free : dry mass of feces was used in SFG calculations. If the dry mass of feces on a given filter was < 0.001 g, we considered the sample to be too small for reliable estimation of ash-free : dry mass ratios, as our scale had a precision of 0.0001g, and did not include that data in SFG calculations for that individual. Instead, we used the mean ash-free : dry mass ratios calculated from feces samples of sufficient size collected from other individuals at that temperature.

At 17:00 we also collected 6 replicate samples of ~0.8 L trough water to determine the ash-free : dry mass ratios of suspended algae. Water was vacuum filtered through 1-micron glass fiber filters to retain algae. Algal filters were then processed in the same manner as the feces

filters. Concentration of ash-free dry mass (mg/L) was calculated by dividing ash-free dry mass by the volume (L) of water filtered.

Absorbed Food Energy Data Analysis

Clearance rate (L/gWWW/h) was calculated for each mussel during each filtration measurement period (4 – 7 measurement periods per mussel) using the following equation (Coughlan, 1969):

$$Vol \times \log_e ac_1 - \log_e ac_2 / t$$

Where

Vol = volume of the filtration chamber in L

ac_1 = initial algal cell concentration

ac_2 = final algal cell concentration

t = time interval in h

Energy consumed (C) was calculated as the maximum clearance rate observed for a given mussel × the initial concentration of ash-free dry mass of algae (mg/L) for that clearance rate estimate × 23 J/mg ash-free dry dry mass of algae (Slobodkin and Richman, 1961, Widdows et al., 1979).

Food absorption efficiency (FAE) was calculated as:

$$(F - E) / [F (1 - E)]$$

Where

F = ash-free dry weight : dry weight ratio of algae

E = ash-free dry weight : dry weight ratio of feces

Energy absorbed (A) was then calculated as C × FAE for each individual mussel.

Data analysis was completed using both SigmaPlot 13.0 (Systat Software, Inc. 2014©) and R Statistical Software (v4.3.0; R Core Team 2023). Regressions between clearance rate, energy ingested, absorption efficiency, respiration rate, and temperature were calculated using SigmaPlot 13. Regressions tested included linear, quadratic, and sigmoidal regressions. Best-fit regressions were identified as having the lowest AICc score. The relationship between SFG and temperature was determined using a smoothing spline via the "npreg" package (Helwig, 2022) in R.

To estimate seasonal shifts in SFG based on natural thermal regimes, we obtained daily temperature data from temperature loggers in the Black River near the mussel collection sites. Scope for growth at each temperature measurement was estimated using the previously described smoothing spline formula relating SFG to temperature. In this manner, SFG was estimated during periods in 2021 and 2022 when temperatures fell within our tested range of 16 – 32°C.

RESULTS

Water samples collected from the Black River had a hardness of > 500 mg/L $CaCO_3$, and alkalinity ranging from 160 – 250 mg/L CaCO3. *In situ* DO = 7.2 mg/L (81.4% saturation), salinity = 0.81 ppt, pH = 9.09, temperature = 21.25°C, and atmospheric pressure = 599.5 mmHg. The hard AFW recipe used to fill the laboratory upwellers exhibited water quality parameters of ~ 8.35 pH, total hardness ~ 197.5 mg/L $CaCO_3$, and total alkalinity ~ 120 mg/L $CaCO_3$. Water quality parameters seldom fell outside of the acceptable levels that triggered a water change (Table 2.1). Mussel length ranged from 73 – 106.7 mm and whole wet weight ranged from 44.5

– 110.1 g. No mussel deaths were observed during the holding period prior to conducting experiments or during experiments.

The relationship between clearance rate (L/gWWW/h) and temperature was best fit with a quadratic regression, with clearance rate increasing rapidly as temperatures increased beyond 24°C ($\hat{y} = 0.0251 - 0.0026x + 0.00007x^2$, $R^2 = 0.78$, $p < 0.0001$; Figure 2.3). The energy ingestion rate (J/gWWW/h) did not follow this pattern but was best fit by a sigmoidal, four-parameter curve:

($f = 0.033 + \frac{0.1644}{1+e^{-\left(\frac{x-24.7}{0.885}\right)}}$, $R^2 = 0.76$, $p < 0.0001$; Figure 2.4).

Energy ingestion rate was fairly stable from 16 – 24°C, increased rapidly from 24–28°C, and then leveled off between 28°C and 32°C. The proportion of ingested energy that was absorbed by mussels did not remain constant with rising temperatures, but rather, was best fit by a quadratic regression ($f = -0.2826 + 0.0873x - 0.0017x^2$, $R^2 = 0.33$, $p = 0.0062$; Figure 2.5). Absorption efficiency initially increased as temperatures rose above 16°C, peaking between 24–28°C and subsequently declining as temperatures increased further. Multiplying absorption efficiency by energy ingested resulted in a sigmoidal curve for energy absorbed that was similar in shape but lower than that for energy ingested (Figures 2.4, 2.5).

($f = 0.023 + \frac{0.136}{1+e^{-\left(\frac{x-24.134}{0.176}\right)}}$, $R^2 = 0.76$, $p < 0.0001$; Figure 2.6). The relationship between energetic costs of actively feeding mussels, as represented by respiration (J/gWWW/h), and temperature was best fit by a quadratic curve ($\hat{y} = 0.262 - 0.022x + 0.000599x^2$, $R^2 = 0.66$, $p < 0.0001$; Figure 2.7). Similar to clearance rate, energy expenditures increased rapidly as temperatures increased above 24°C. Scope for growth remained stable from 16°C – 24°C, then

rose sharply, peaking at 28°C and subsequently declining sharply as temperatures increased from 28 to 32°C. Scope for growth estimates were negative below 24°C, becoming positive between 24 and 28°C, and dipping back down to negative values at 32°C (Figure 2.8).

Water temperatures in the Black River generally fell within the range promoting a positive SFG late May through late October in both 2021 and 2022. However, mid-summer temperatures were warmer in 2022, frequently exceeding the upper threshold (31°C) above which SFG declined once again to negative values. Water temperatures in Spring and Fall were generally below the thermal threshold for a positive SFG, especially in 2021 (Figure 2.9 A, B). Daily maximum temperatures generally resulted in a maximum daily SFG from April through June and from October through November. This pattern reversed between June and October with daily maximum temperatures frequently resulting in a minimum daily SFG, particularly in 2022 (Figure 2.9 C, D).

DISCUSSION

In this study, our first goal was to determine the effects of temperature on SFG and how various physiological rates interacted to drive this pattern. We found that temperature had a significant, but non-linear effect on mussel SFG, due to diverging patterns of various physiological rates as temperature rose from 16 – 32°C. This resulted in three apparent thermal phases. In the first phase (16–24°C), SFG was negative, and relatively stable with a slight increase as temperatures approached 24°C. Because clearance rates did not begin to increase until ~24°C and absorption efficiency did not peak until ~24°C, the energy absorbed by mussels remained less than the amount of energy expenditures represented by respiratory costs throughout this temperature range. Respiratory costs, measured on actively feeding mussels, represented basic maintenance plus specific dynamic action (SDA: energy expended on food

acquisition, digestion, and assimilation). The finding that energetic costs were higher than energy gains within the range of 16–24°C, and thus not conducive for growth of adult THS, is supported by empirical observations from previous studies using juveniles of different species in laboratory and natural settings. In a previous lab study, growth of juvenile mussels (*E. brevidens*, *E. capsaeformis*, and *L. fasciola*) was positive but significantly lower at 20 and 22°C compared to ≥ 24°C (Carey et al., 2013). Field studies examining the relationship between temperature and growth of *L. cardium* in Kentucky streams showed minimal growth of juvenile *L. cardium* at 20–22°C but temperature effects were likely compounded with additional, unidentified stressors. When defaunated streams were removed from the dataset, zero growth was predicted at 17.8°C (Haag et al., 2019).

In the second phase, SFG was positively related to temperature, increasing sharply and becoming positive between 24 and 28°C. During this phase, even though energetic costs were increasing with temperature, clearance rate was also increasing rapidly and absorption efficiency peaked. This resulted in energy absorption rates exceeding respiratory costs, and a peak in SFG at ~28°C. The rise in SFG suggests that temperatures ~28°C would support optimal growth for SFG. This pattern is also supported by empirical observations from previous laboratory and field studies. Maximum growth rates of juvenile mussels (*E. brevidens*, *E. capsaeformis*, and *L. fasciola*) fed a commercial algal formula in downweller buckets were observed at 26–28°C (Carey et al., 2013). Field studies showed a weak but positive relationship between growth of juvenile *C. cardium* from ~22.5 to 25, which corresponds to the temperature range where SFG of adult THS began slowly increasing towards positive values (Figure 2.9B, Haag et al., 2019).

In the third phase, SFG was negatively related to temperature, decreasing sharply back down to negative values as temperatures increased from 28 to 32°C. During this phase, clearance

rate and respiratory costs continued to increase. Because clearance rate represents the volume of water cleared of algal particles per unit time (Coughlan, 1969), and algal concentration remained constant, an increase in clearance rate could have resulted in a sufficient increase in energy intake to offset the increased respiratory costs. However, energy ingested and absorbed did not increase, but instead leveled off at high temperatures. This was due to a reduction in absorption efficiency coupled with reduced food quality (i.e. organic content per cell) at high temperatures. Because we used non-living algal cells in our study, the reduction in organic content was likely due to increased decomposition rates at high temperatures. Unionid mussels in lotic and lentic systems may derive a substantial portion of their food resources from detrital coarse and fine particulate matter (CPOM and FPOM) in addition to suspended particulate organic matter (Fogelman et al., 2022, 2023). The importance of food quality in driving the decline of SFG at high temperatures points out the importance of considering the relationship between temperature and quality of benthic detritus and phytoplankton as food resource pools. Phytoplankton community composition, size, and resource allocation (Schabhüttl et al., 2013; Toseland et al., 2013; Zohary et al., 2021) can all change with temperature, resulting in potential changes to food quality for freshwater mussels.

In the Black River, seasonal temperatures frequently fall outside of the thermal bounds for positive SFG found in this study. As a consequence, THS can regularly experience zero or negative SFG during most months of the year. Temperatures promoting positive SFG primarily occurred from late May through early October, indicating that this is when maximum investment in growth and reproduction occurs. Previous studies have shown that gonadal activity is lowest in October and November, and females have been observed as gravid from March through August (Smith et al., 2003). However, during mid-summer, water temperatures can frequently

reach temperatures of 32°C – resulting in SFG declining back to negative values. Thus in years that exhibit warmer than normal temperatures, mid-summer temperatures become too hot to facilitate optimal growth and reproduction, dividing the optimal growing season into two smaller periods (late spring/early summer and late summer/early fall). In 2022 (assuming Jan. –Apr. and Nov. –Dec. were too cold to promote positive SFG), there were only ~2 months of the year (June and Sept.) when THS had an SFG above 0.02. Given the March – August gravidity of females, if summer months are too warm, the THS females would likely be running at a deficit of energy during their gravidity.

Different mussel species and/or mussels from different latitudes may exhibit different SFG patterns than found in the current study. Adult male *L. siliqoidea* collected from the Thames River near Innerkip, Canada (43.215N, -80.692W) exhibited a lower optimal SFG (20℃) under conditions of moderate water velocity and low turbidity (Luck and Ackerman, 2021). In contrast, our mussels were collected from the Black River in New Mexico, U.S.A. (Lat Long coordinates not provided due to this being a federally listed mussel). Additional studies examining the relationship between latitude and optimal SFG temperature are needed to determine whether SFG changes in a predictable fashion between northern and southern North American unionid mussel populations and subpopulations.

In our study, physiological rates were measured at a constant temperature for individuals acclimated to that temperature. Additional studies are needed to determine whether acute temperature changes have similar effects on SFG as temperature changes to which they have had sufficient time to acclimate. In natural systems, mussels experience daily changes in temperature as waters typically warm from morning to daily maximum in late afternoon. If mussel SFG response to acute temperature changes follows the same pattern as for acclimated temperatures

(i.e. this study), daily increases are likely to be beneficial in the Spring and Fall as temperatures rise closer to the optimum 28℃. However, in the summer months, these increases are likely detrimental if daily maxima exceed 28℃ and SFG begins to decline. Sensitivity to daily increases in temperature would increase the importance of monitoring flow and temperature during the summer months. Even if daily average temperatures were within an optimal temperature range, daily maximum temperatures could cause SFG to plunge toward or below zero on a daily basis

In the current study, estimated SFG was often negative. It is possible that we underestimated SFG in the natural environment because we only measured energy intake from filter feeding, not accounting for the possibility of pedal feeding. Multiple mussel species from Texas rivers derive an average of 51% of their diet from coarse particulate organic matter of benthic origin, suggesting that the importance of pedal feeding may be overlooked (Gatenby et al., 1996; Nichols et al., 2005; Fogelman et al., 2022; Fogelman et al., 2023). Texas Hornshell mussels used in the current study were collected from a highly organic soft sediment in undercut banks in the Black River; the availability of benthic organic energy may be significant for THS and unaccounted for in our SFG calculations.

Due to climate change, global river water temperatures are estimated to increase on average by 1.2°C by 2100 (van Vliet et al., 2013). This increase in combination with reduced water flow from predicted increases in water withdrawal and droughts (CEHMM, 2017; Cayan et al., 2010, Zektser et al., 2005), will likely mean that THS will see an increase in these more extreme temperatures, significantly affecting physiological rates, and potentially decreasing the energy available for growth and reproduction.

Texas hornshell are already experiencing thermal stress in the Black River. If summer temperatures continue to increase, their ability to grow and reproduce may decline. Information regarding the relationship between temperature and SFS will help managers of the Black River to implement effective flow regime regulations. If flow regimes are managed to minimize the impacts of droughts, isolated pools warming in the hot summer months, and other factors that increase the frequency of thermal stress, the federally endangered THS have a better chance of survival and possibly even recovery in the decades to come.

References

Augspurger, T., Keller, A.E., Black, M.C., Cope, W.G. and Dwyer, F.J., 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. Environmental Toxicology and Chemistry: An International Journal, 22(11), 2569-2575.

Boyd, C.E., 2014. Nitrite toxicity affected by species susceptibility, environmental conditions. Glob Aquac Advocate, 17, 34-36.

Carey, C.S., Jones, J.W., Hallerman, E.M. and Butler, R.S., 2013. Determining optimum temperature for growth and survival of laboratory-propagated juvenile freshwater mussels. North American Journal of Aquaculture, 75(4), 532-542.

Cayan, D.R., Das, T., Pierce, D.W., Barnett, T.P., Tyree, M. and Gershunov, A., 2010. Future dryness in the southwest US and the hydrology of the early 21st century drought. Proceedings of the National Academy of Sciences, 107(50), 21271-21276.

Center of Excellence (CEHMM), US Fish and Wildlife Service, US Bureau of Land Management. 2017. Candidate Conservation Agreement for the Texas Hornshell (*Popenaias popeii*) and other Covered Species. 136 pages.

Coughlan, J., 1969. The estimation of filtering rate from the clearance of suspensions. Marine biology, 2(4), pp.356-358.

Fogelman, K.J., Stoeckel, J.A., Abdelrahman, H.A., and Helms, B.S., 2022. The relative importance of suspended versus benthic food resources to freshwater mussels in central Texas, U.S.A. Freshwater Biology, 67, 1063-1078.

Fogelman, K.J., Stoeckel, J.A., Miller, J.M. and Helms, B.S., 2023. Feeding ecology of three freshwater mussel species (Family: Unionidae) in a North American lentic system. Hydrobiologia, 850(2), 385-397.

Gatenby, C.M., Neves, R.J. and Parker, B.C., 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. Journal of the North American Benthological Society, 15(4), 597-609.

Haag, W.R., 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press.

Haag, W.R., Culp, J.J., McGregor, M.A., Bringolf, R. and Stoeckel, J.A., 2019. Growth and survival of juvenile freshwater mussels in streams: Implications for understanding enigmatic mussel declines. Freshwater Science, 38(4), 753-770.

Helwig N.E., 2022. npreg: Nonparametric Regression via Smoothing Splines. R package version 1.0-9, URL: https://CRAN.R-project.org/package=npreg.

IPCC. 2023. Synthesis report of the IPCC Sixth Assessment Report (AR6). IPCC, Geneva, Switzerland, 86 pages.

Isaak, D.J., Wollrab, S., Horan, D. and Chandler, G., 2012. Climate change effects on stream and river temperatures across the northwest US from 1980–2009 and implications for salmonid fishes. Climatic change, 113, 499-524.

Karatayev, A.Y., Burlakova, L.E., Miller, T.D. and Perrelli, M.F., 2018. Reconstructing historical range and population size of an endangered mollusc: long-term decline of Popenaias popeii in the Rio Grande, Texas. Hydrobiologia, 810, 333-349.

Kaushal, S.S., Likens, G.E., Jaworski, N.A., Pace, M.L., Sides, A.M., Seekell, D., Secor D.H., and Wingate, R.L., 2010. Rising stream and river temperatures in the United States. Frontiers in Ecology and the Environment 8: 461–466.

Luck, K., and Ackerman, J.D., 2021. Threats to freshwater mussels: The interactions of water temperature velocity and total suspended solids on ecophysiology and growth, Science of the Total Environment, 821, 153101.

MacDonald, B.A. and Thompson, R.J., 1986. Influence of temperature and food availability on the ecological energetics of the giant scallop Placopecten magellanicus: III. Physiological ecology, the gametogenic cycle and scope for growth. Marine Biology, 93, 37-48.

Martins, R.T., Rezende, R.D.S., Gonçalves Júnior, J.F., Lopes, A., Piedade, M.T.F., Cavalcante, H.D.L. and Hamada, N., 2017. Effects of increasing temperature and, CO2 on quality of litter, shredders, and microorganisms in Amazonian aquatic systems. Plos One, 12(11), p.e0188791.

Naylor, C., Maltby, L. and Calow, P., 1989. Scope for growth in Gammarus pulex, a freshwater benthic detritivore. Hydrobiologia, 188, 517-523.

Nichols, S.J., Silverman, H., Dietz, T.H., Lynn, J.W. and Garling, D.L., 2005. Pathways of food uptake in native (Unionidae) and introduced (Corbiculidae and Dreissenidae) freshwater bivalves. Journal of Great Lakes Research, 31(1), 87-96.

R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org.

Randklev, C.R., Miller, T., Hart, M., Morton, J., Johnson, N.A., Skow, K., Inoue, K., Tsakiris, E.T., Oetker, S., Smith, R. and Robertson, C., 2018. A semi-arid river in distress: Contributing factors and recovery solutions for three imperiled freshwater mussels (Family Unionidae) endemic to the Rio Grande basin in North America. Science of the Total Environment, 631, 733-744.

Rangaswami, X.L., Goldsmith, A.M., Khan, J.M., Robertson, C.R., Lopez, R.R. and Randklev, C.R., 2023. Thermal tolerances of Popenaias popeii (Texas hornshell) and its host fish from the Rio Grande Basin, Texas. Scientific Reports, 13(1), 4603.

Schabhüttl, S., Hingsamer, P., Weigelhofer, G., Hein, T., Weigert, A. and Striebel, M., 2013. Temperature and species richness effects in phytoplankton communities. Oecologia, 171, 527-536.

Slobodkin, L.B. and Richman, S., 1961. Calories/gm. in species of animals. Nature, 191(4785), 299.

Smith, M.E., Lazorchak, J.M., Herrin, L.E., Brewer-Swartz, S. and Thoeny, W.T., 1997. A reformulated, reconstituted water for testing the freshwater amphipod, Hyalella azteca. Environmental Toxicology and Chemistry: An International Journal, 16(6), 1229-1233.

Smith, D.G., Lang, B.K. and Gordon, M.E., 2003. Gametogenetic cycle, reproductive anatomy, and larval morphology of Popenaias popeii (Unionoida) from the Black River, New Mexico. The Southwestern Naturalist, 333-340.

Toseland, A.D.S.J., Daines, S.J., Clark, J.R., Kirkham, A., Strauss, J., Uhlig, C., Lenton, T.M., Valentin, K., Pearson, G.A., Moulton, V. and Mock, T., 2013. The impact of temperature on marine phytoplankton resource allocation and metabolism. Nature Climate Change, 3(11), 979-984.

U.S. Fish and Wildlife Service (USFWS). 2018. Endangered and threatened wildlife and plants; endangered species status for Texas hornshell. Federal Register 83, 5720-5735.van Vliet, M.T.H., Franssen, W.H.P., Yearsley, J.R., Ludwig, F., Haddeland, I., Lettenmaier, D.P., and Kabat, P., 2013. Global river discharge and water temperature under climate change. Global Environmental Change, 2 (2), 450-464.

van Vliet, M.T., Franssen, W.H., Yearsley, J.R., Ludwig, F., Haddeland, I., Lettenmaier, D.P. and Kabat, P., 2013. Global river discharge and water temperature under climate change. Global Environmental Change, 23(2), 450-464.

Vaughn, C.C., Nichols, S.J., and Spooner, D.E., 2008. Community and foodweb ecology of freshwater mussels. Journal of North American Benthological Society, 27(2), 409-423.

Vaughn, C.C., 2018. Ecosystem services provided by freshwater mussels. Hydrobiologia, 810, 15 – 27.

Wang, Y., Hu, M., Wong, W.H., Shin, P.K. and Cheung, S.G., 2011. The combined effects of oxygen availability and salinity on physiological responses and scope for growth in the green-lipped mussel Perna viridis. Marine pollution bulletin, 63(5-12), 255-261.

Widdows, J., Moore, M.N., Lowe, D.M. and Salkeld, P.N., 1979. Some effects of a dinoflagellate bloom (Gyrodinium aureolum) on the mussel, Mytilus edulis. Journal of the Marine Biological Association of the United Kingdom, 59(2), 522-524.

Widdows, J., and Staff, F., 2006. Biological effects of contaminants: Measurements of scope for growth in mussels. ICES Techniques in Marine Environmental Sciences, 40.

Xenopoulos, M. A., Lodge, D.M., Alcamo, J., Märker, M., Schulze, K., and Van Vuuren, D.P., 2005. Scenarios of freshwater fish extinctions from climate change and water withdrawal. Global Change Biology 11: 1557–1564.

Zektser, S., Loáiciga, H.A. and Wolf, J.T., 2005. Environmental impacts of groundwater overdraft: selected case studies in the southwestern United States. Environmental Geology, 47, 396-404.

Zohary, T., Flaim, G. and Sommer, U., 2021. Temperature and the size of freshwater phytoplankton. Hydrobiologia, 848, 143-155.

Table 2.1. Acceptable ranges of upweller water quality parameters (Augspurger et al., 2003; Boyd, 2014)

Water quality parameter	**pH**	**Alkalinity (mg/L $CaCO_3$)**	**Hardness (mg/L $CaCO_3$)**	**Ammonia (mg/L TAN)**	**Nitrite (mg/L N)**
Lower limit	7.0	20	50	-	-
Upper limit	8.5	-	-	0.3	0.5

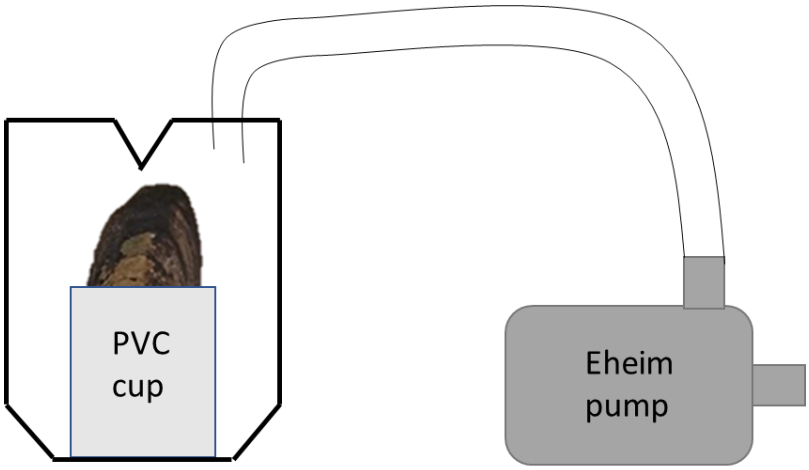

Figure 2.1. Diagram of filtration set-up, including a 750 mL chamber, a PVC cup for positioning mussel, and a recirculating pump to flush fresh water into the cup during flush periods. The V-notch at the top of the cup allows excess water to flow out of the chamber when the flush pump is turned on.

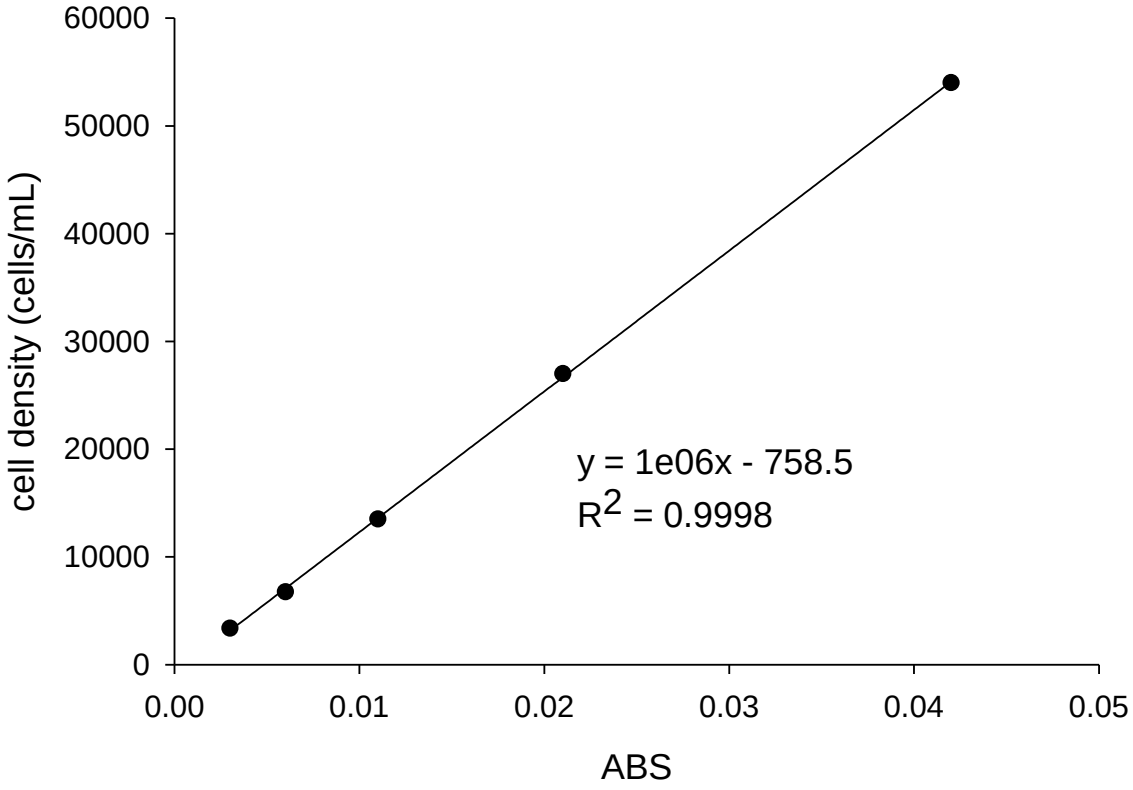

Figure 2.2: Calibration curve of cells/mL vs. absorbance at 450 nm.

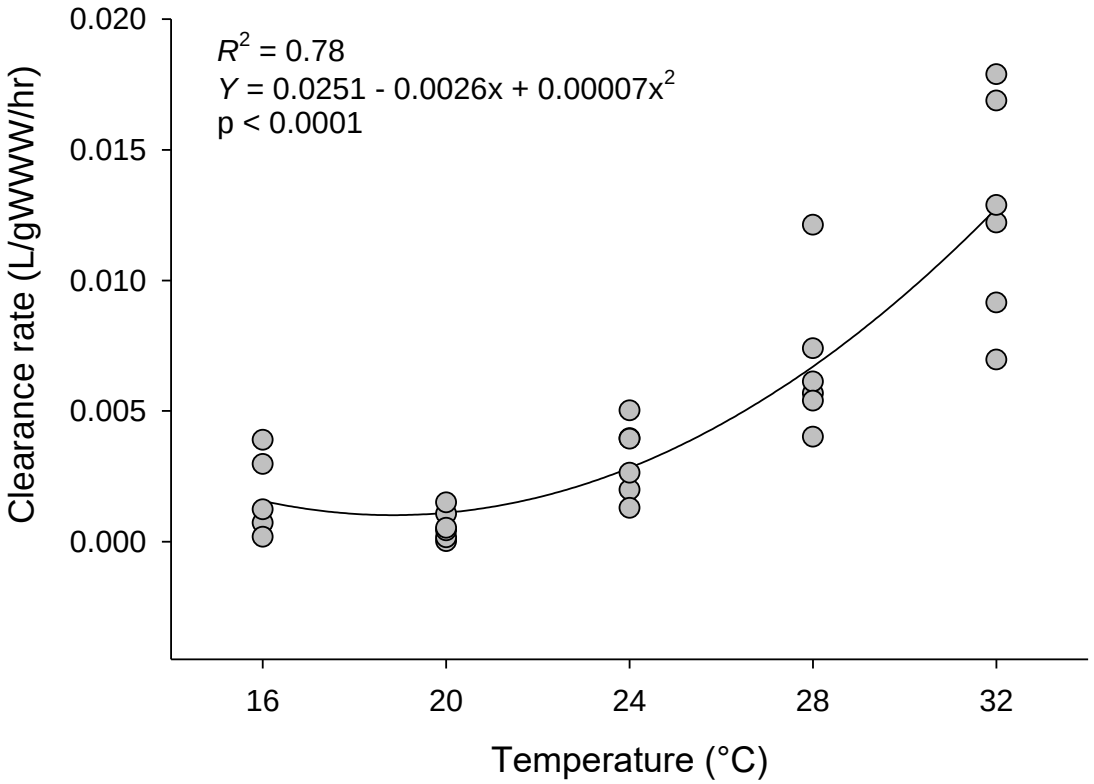

Figure 2.3: Relationship between maximum observed clearance rate and temperature. Each grey dot represents an individual mussel.

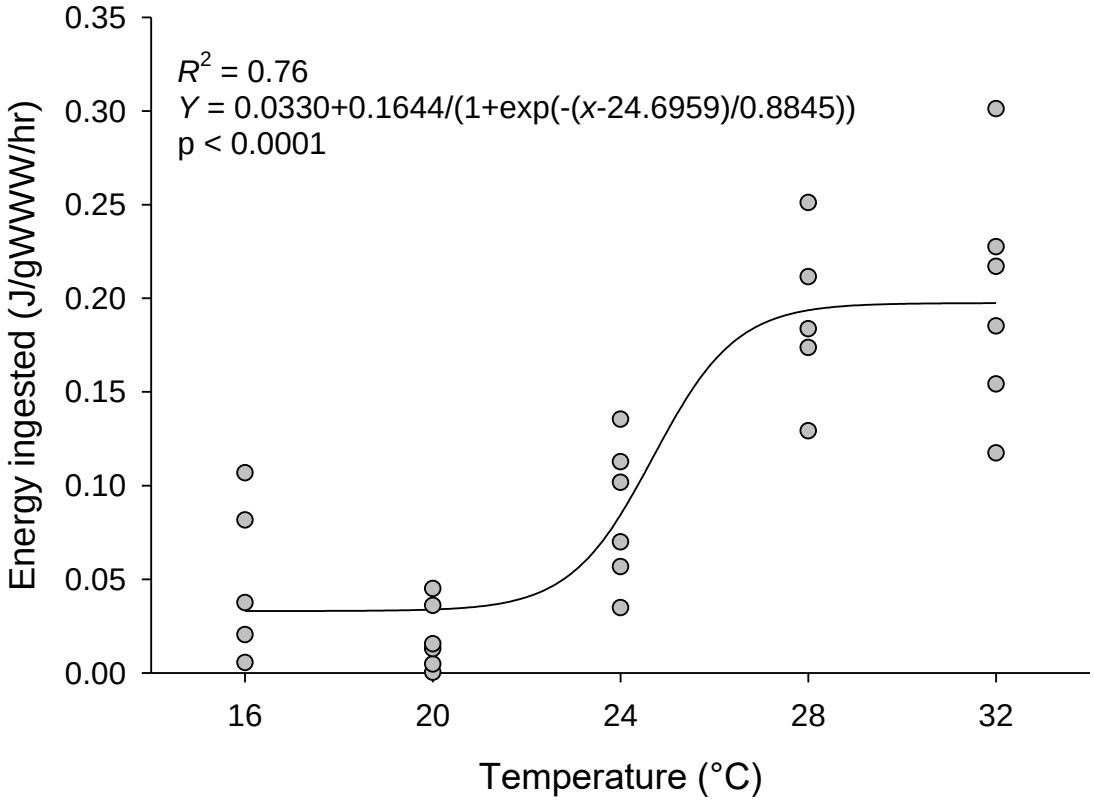

Figure 2.4: Relationship between energy ingested and temperature. Each grey dot represents an individual mussel.

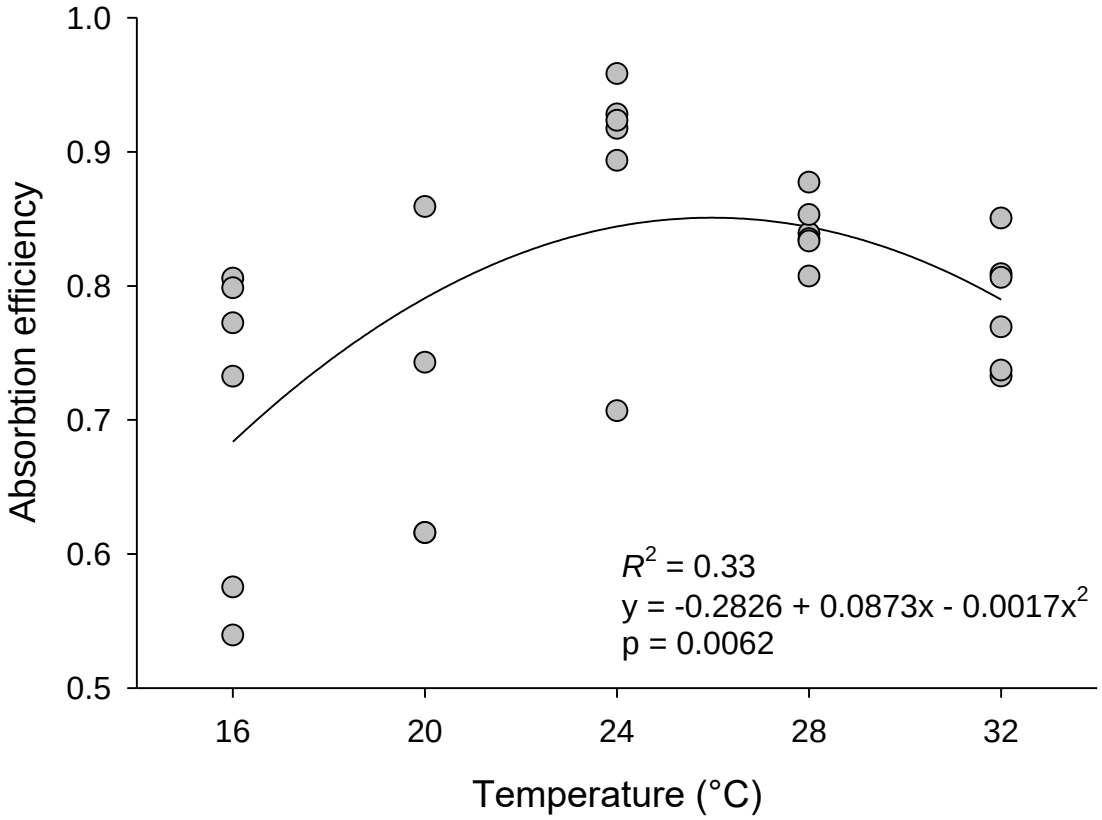

Figure 2.5: Relation between absorption efficiency and temperature. Each grey dot represents an individual mussel.

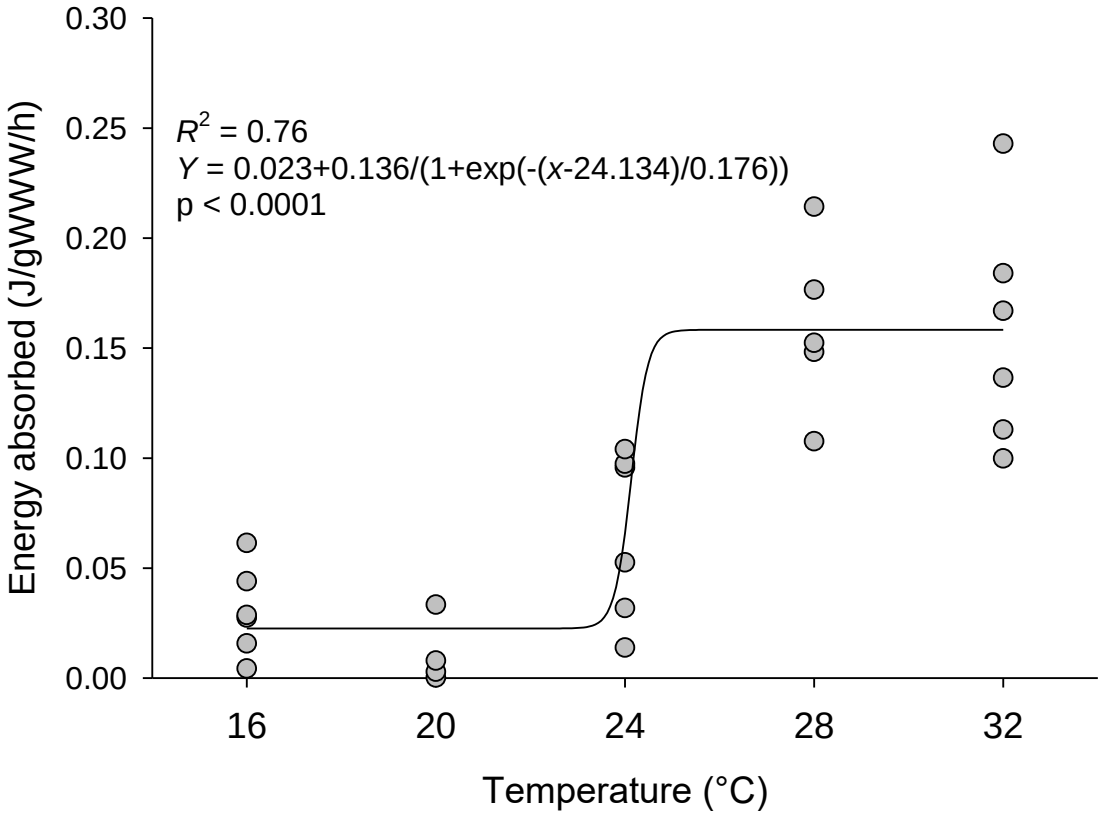

Figure 2.6. Relationship between energy absorbed and temperature. Each grey dot represents an individual mussel.

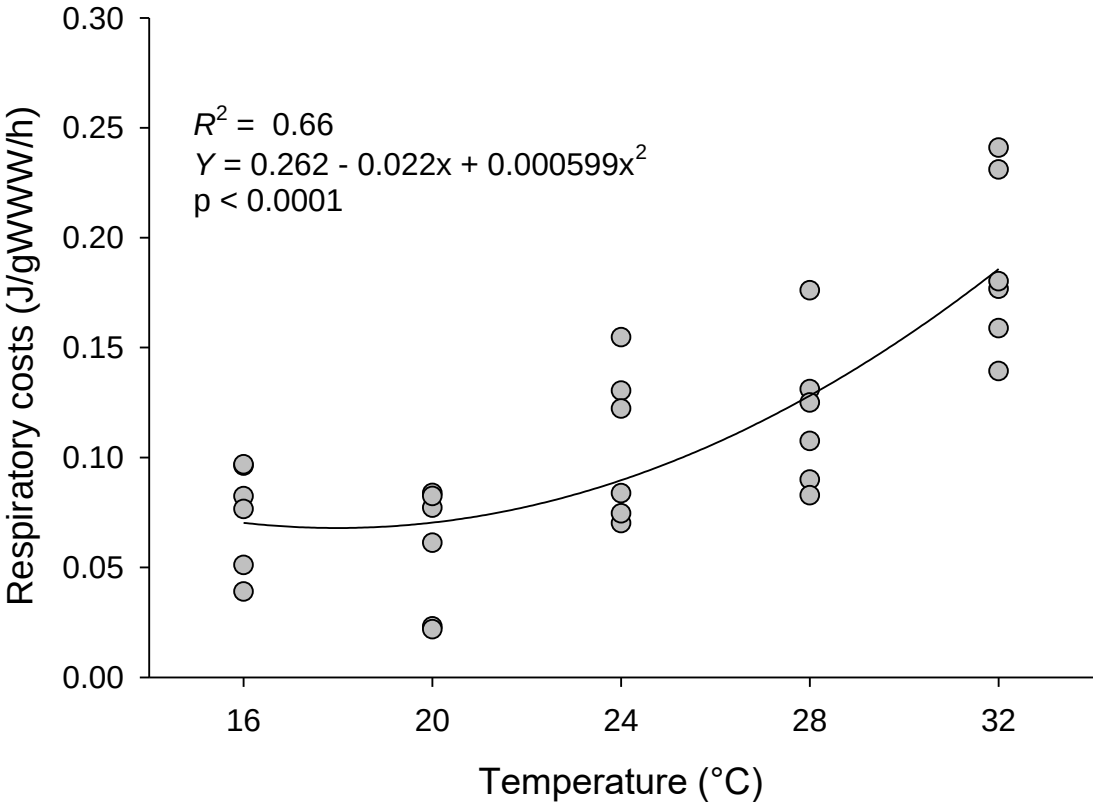

Figure 2.7. Relationship between respiratory costs and temperature. Each grey dot represents an individual mussel.

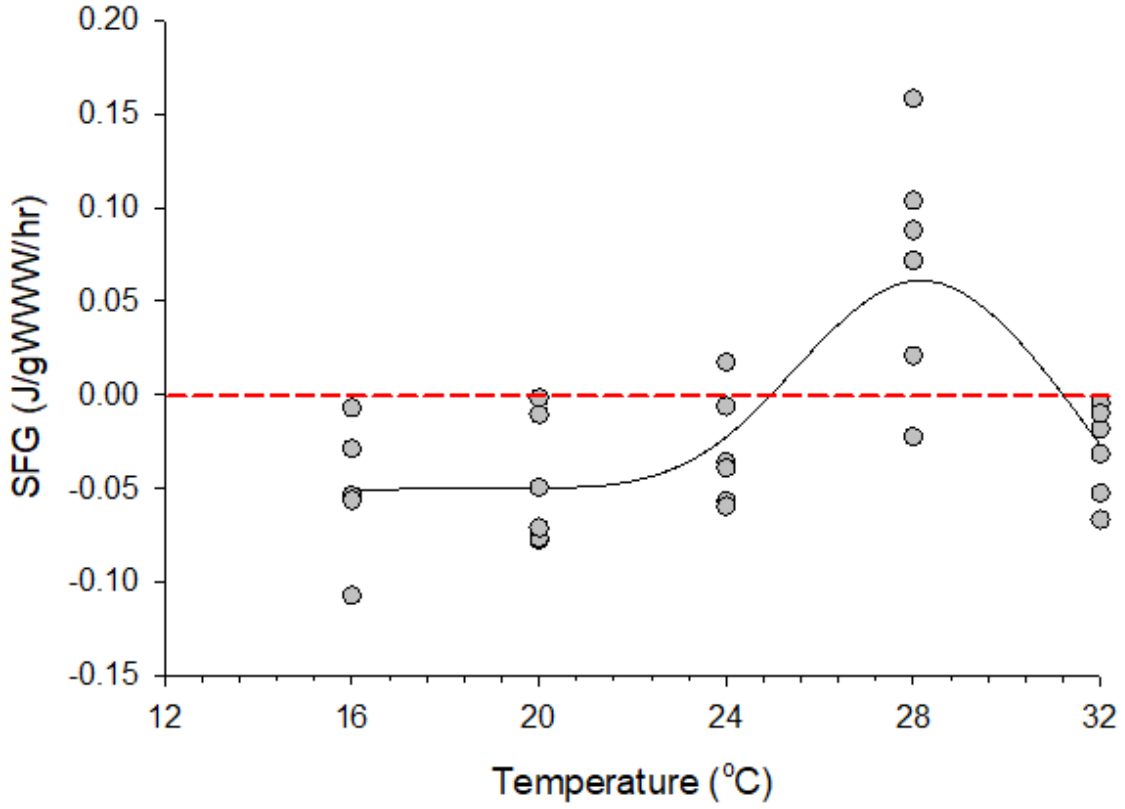

Figure 2.8. Relationship between scope for growth (SFG) and temperature as described by a smoothing spline. Each grey dot represents an individual mussel. Red dotted line indicates the threshold between positive and negative scope for growth.

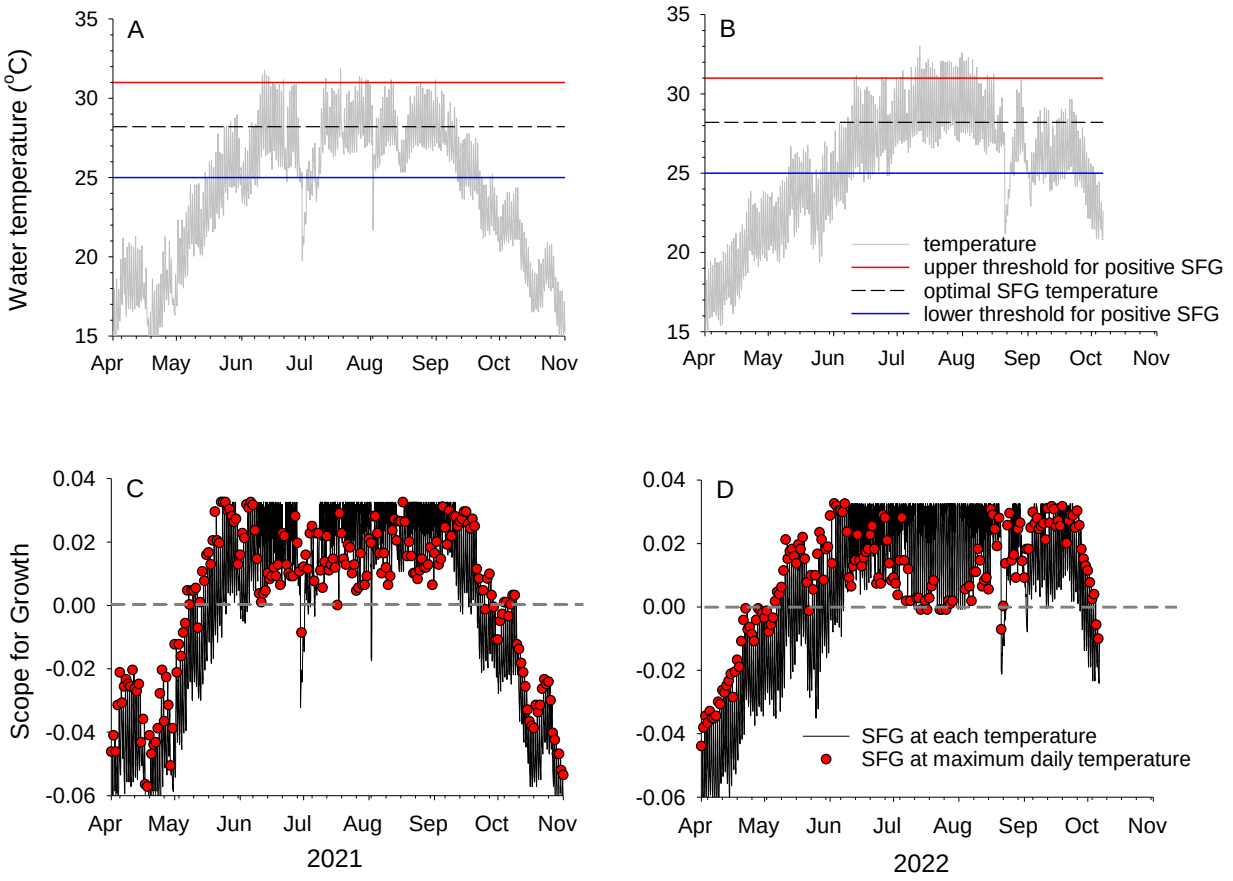

Figure 2.9. Water temperature in the Black River near the collection site in A) 2021 and B) 2022 with horizontal lines showing thresholds for positive and optimal scope for growth. Water temperatures were recorded every 30 minutes. Scope for growth estimated from water temperatures in C) 2021 and D) 2022 where the black line shows SFG estimates for each water temperature recorded and the red dots show SFG estimates at the maximum daily temperatures. Horizontal grey dotted line indicates the threshold between positive and negative scope for growth.

Appendix

Table 1: Regressions tested in Chapter 1 and 2. The highlighted rows indicate the regressions chosen based on lowest AICc.

Chapter/Figure	Parameter measured	Regression type	AICc	R^2
Ch. 1	Unfed Respiration Rate	Quadratic	40.8720	0.6526
Ch. 1/Figure 1.1A	Unfed Respiration Rate	Linear	38.1382	0.6525
Ch. 1	Fed Respiration Rate	Linear	62.3406	0.5707
Ch. 1/Figure 1.1B	Fed Respiration Rate	Quadratic	58.1299	0.6588
Ch. 2/Figure 2.3	Clearance Rate	Quadratic	-342.5522	0.7833
Ch. 2	Energy Ingested	3-parameter sigmoidal	-139.9251	0.3573
Ch. 2/Figure 2.4	Energy Ingested	4-parameter sigmoidal	-165.8604	0.7581
Ch. 2	Absorbtion effeiciency	4-parameter sigmoidal	-126.1260	0.3626
Ch. 2/Figure 2.5	Absorbtion efficiency	Quadratic	-127.8731	0.3337
Ch. 2	Energy absorbed	3-parameter sigmoidal	-168.8871	0.7252
Ch. 2/Figure 2.6	Energy absorbed	4-parameter sigmoidal	-169.899	0.763
Ch. 2	Respiration Rate (J/gww/h)	Linear	-193.5247	0.5707
Ch. 2/Figure 2.7	Respiration Rate (J/gww/h)	Quadratic	-197.735	0.659