Feeding ecology and lethal thermal tolerances among selected North American freshwater mussels (Order: Unionida)

by

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Abstract

Sixty-five percent of North American unionid mussel species are considered imperiled. Knowledge gaps persist in unionid feeding ecology and lethal thermal tolerance limits which could aid in the conservation and management of these imperiled species. To address these knowledge gaps two studies were conducted to quantify stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) isotopic signatures for mussels in lotic systems (five mussel species across three seasons and four rivers in central Texas, USA) and a lentic system (three mussel species in Gantt Lake, Alabama, USA). In Texas rivers, overall, C derived from coarse particulate organic matter (CPOM; bulk detrital leaf packs) contributed, on average, 51% to the mussel diet and C derived from suspended particulate organic matter (SPOM) contributed, on average, 41% to the mussel diet and in Gantt Lake limnetic benthic fine particulate organic matter (FPOM) contributed, on average, 99% to the mussel diet. Mussels in both the lotic and lentic systems showed evidence of undergoing self-catabolism and relying on internal nutrient stores as a mechanism for coping with stress (poor food quality; and temperature and emersion for lotic and lentic systems, respectively). Together these data suggest the C in mussel tissues in both system types were primarily of benthic origins, but lotic mussels also received secondary contributions from suspended materials. A third study performed a quantitative fatty acid analysis on five species of mussels from four Texas lotic systems to investigate the relative contribution of putative food resources. Mussels across seasons and drainages had 55% of their fatty acid composition in common, but we observed variation in fatty acid profiles and ecologically relevant groupings of fatty acids across drainages. Algal-derived, source-specific fatty acids comprised 7–10% of fatty acid profiles, while bacterial-derived fatty acids comprised <1% of profiles, indicating that algae are the primary source of dietary fatty acids across drainages sampled. Finally, a systematic

literature review of North American unionids was conducted identifying lethal tolerance estimates for only 28 of 302 species in the order Unionida. The mean acute median lethal temperatures were 32.8°C for glochidia (19 species), 35.0°C for juveniles (13 species), and 36.3°C for adults (4 species).

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List of Abbreviations

$\delta^{13}C$	Carbon stable isotope, $^{13}C/^{12}C$
$\delta^{15}N$	Nitrogen stable isotope, $^{15}C/^{14}C$
ALA	α-linolenic acid; 18:3n-6
ANOSIM	Analysis of similarity
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARA	arachidonic acid; 20:4n-6
C:N	Stoichiometric carbon to nitrogen ratio
CPOM	Coarse particulate organic matter
CTM	Critical thermal maximum
DHA	docosahexanoic acid; 22:6n-3
EFA	Essential fatty acid
EPA	eicosapentaenoic acid; 20:5n-3
FA	Fatty acid
FAME	Fatty acid methyl ester
FPOM	Fine particulate matter
GC-MS	Gas chromatography-mass spectroscopy
GF/F	Glass fiber filter
HSD	Tukey's Honestly Significant Differences post-hoc test
IPCC	Intergovernmental Panel on Climate Change
LC50	The concentration of a toxicant expected to cause mortality in 50% of exposed individuals in a specified time

LIN	linoleic acid; 18:2n-6
LT	Lethal temperature
LT05	The temperature expected to cause mortality in 5% of exposed individuals in a specified time
LT50	The temperature expected to cause mortality in 50% of exposed individuals in a specified time
MixSIAR	R package for Bayesian mixed modeling analysis of stable isotopes
MUFA	Monounsaturated fatty acid
NMDS	Non-metric multidimensional scaling
PUFA	Polyunsaturated fatty acid
SAFA	Saturated fatty acid
SPOM	Suspended particulate organic matter
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
VPDB	Vienna Pee Dee Belemite

CHAPTER 1
INTRODUCTION

1.1 Historical contributions of mussel decline

North American unionid mussels are experiencing significant imperilment with 65% of species listed as threatened, endangered, or vulnerable (Haag & Williams, 2014). The declines in mussel diversity, distribution, and abundance are due to an array of anthropogenic sources. Population declines of freshwater mussels in North America began in the early 1900s with commercial harvest for the button and pearl industries. By the 1920s, beds across North America were strongly reduced. With populations depleted, musselers would move to different locations, allowing residual populations to recover (Haag, 2012). Historic habitat alteration through impoundments and channelization altered flow, temperature, and oxygenation in aquatic systems and led to the first wave of extinction of North American mussels from the 1920s to the 1980s (Vaughn et al., 2008; Strayer & Dudgeon, 2010; Haag, 2012). Habitat alteration, coupled with widespread instances of pollution throughout the early and mid-1900s, attributed to the first recorded North American mussel extinctions. During this period in North America, there are many documented incidences of industrial and municipal waste being disposed of directly into lakes and rivers (Strayer & Dudgeon, 2010).

The impacts from these drivers of decline were initially reduced in the 1970s with the 1972 passage of the Clean Waters Act and the 1973 passage of the Endangered Species Act. Waterways in the United States began to improve as a result of this legislation; and imperiled mussel species began to receive protections (Strayer & Dudgeon, 2010; Haag, 2012). Despite efforts to reverse or lessen these impacts, river landscapes across North America were changed greatly, and the current imperilment and extinction rates for freshwater mussels are one of the

greatest of any group of organisms in North America (Williams et al., 1993; Strayer & Dudgeon, 2010; Haag, 2012; Haag & Williams, 2014).

1.2 Role of mussels in aquatic ecosystems

Mussels perform critical functions in aquatic systems serving as keystone species and ecosystem engineers, thus the decline or extirpation of mussel populations can have large-scale implications on the function and ecology of these systems. The increased awareness of their ecological function and their imperiled status has driven greater concern for mussel conservation. Mussels can comprise 50 to 90% of total benthic biomass and are capable of filtering immense volumes of water when present in dense assemblages (Haag, 2012; Vaughn et al., 2008). This filtering activity can reduce suspended particulate organic matter concentrations (Vaughn et al., 2008; Christian et al., 2004) which also provides benefits for humans through purification of water (Vaughn, 2018). Mussel presence and activity benefits sympatric organisms in aquatic systems. Mussel shells provide physical habitat for other organisms (Vaughn et al., 2008) and improve habitat for other benthic organisms through bioturbating the sediment with their burrowing activities. This bioturbation increases oxygen, water, and nutrients present (Howard & Cuffey, 2006; Spooner & Vaughn, 2006). The nutrient and energy transfer that mussels facilitate through their feeding and burrowing activity links the benthic and pelagic interfaces of aquatic systems and ultimately drives production across all trophic levels (Spooner & Vaughn, 2006; Vaughn et al., 2008; Howard & Cuffey, 2006). Additionally, mussel burrowing behavior is capable of stabilizing streambed substratum, as streambed substrates remain stable during flood events when dense mussel beds are present (Vaughn et al., 2008; Gangloff & Feminella, 2007; Strayer et al., 2004).

1.3 Research problem

Despite widespread unionid decline and the degradation of aquatic systems in general, many knowledge gaps remain regarding the life history and environmental tolerance of freshwater mussels. Much remains unknown regarding mussels' primary food resources, biological mechanisms for feeding, ontogenetic changes in feeding patterns, and patterns associated with variations in their feeding ecology (Yeager et al., 1994; Strayer, 2008; Galbraith et al., 2009; Newton et al., 2013; Weber et al., 2017). Mussels have historically been classified as filter-feeders, but existing research suggests that their known dietary constituents are derived from both pelagic and benthic sources, and the use of these food resources may also vary depending on habitat and food availability (Yeager et al., 1994; Raikow & Hamilton, 2001; Christian et al., 2004; Nichols et al., 2005; Vaughn et al., 2008; Newton et al., 2013; Weber et al., 2017; Vaughn, 2018). Mussels are also epibenthic organisms, which allows them to access both pelagic and benthic food resources. Mussel diets are comprised of phytoplankton, protozoans, detritus, bacteria, and dissolved organic carbon (Strayer, 2008; Haag, 2012). Previous studies using stable isotope analysis, fatty acid analysis, and biochemical markers have elucidated the more prevalent components of basal food resources that mussels use for food. Bacteria (Nichols & Garling, 2000; Christian et al., 2004), algae, and phytoplankton (Raikow & Hamilton, 2001; Newton et al., 2013; Weber et al., 2017) have been identified as important contributors to the freshwater mussel diet.

While individual components of the mussel diet have become clearer, what they are actually assimilating from bulk food resources is poorly understood. Although mussels are largely considered filter-feeders, burrowing habits and associated pedal feeding (sweeping their ciliated foot through sediments) allow access to benthic food sources such as sediment-based

organisms and detritus (Raikow & Hamilton, 2001; Nichols et al., 2005). What is poorly understood is the extent to which mussels utilize benthic versus suspended food resources, the relative importance of specific dietary components, and how feeding relationships vary taxonomically, geographically, or temporally (Vaughn et al., 2008; Strayer, 2008; Haag, 2012). While evidence shows that juveniles can consume benthic organic matter (Yeager et al., 1994, Gatenby et al., 1996, 1997) the extent to which adult mussels utilize non-suspended food sources through deposit or pedal feeding is not fully understood. Knowledge gaps in the existing literature reveal the need to investigate food sources for mussels, which can inform whether environmental conditions are suitable for critical physiological processes such as growth and reproduction; and inform the role of mussels in aquatic ecosystems through their linkage of benthic and pelagic interfaces.

Habitat degradation, particularly thermal pollution, has resulted in freshwater mussels being exposed to multiple stressors (Strayer & Dudgeon, 2010; Ganser et al., 2013). Understanding organismal thermal tolerance is of increasing importance in the face of ongoing climate change, with surface waters predicted to continue increasing in temperature (IPCC, 2021). Unionid mussels are aquatic ectotherms, and these organisms are particularly vulnerable to changes in environmental temperatures because temperatures are a critical factor regulating many physiological processes (Fry, 1947; Westhoff & Rosenberger, 2016). Conservation and restoration of imperiled fauna are dependent upon knowledge of upper lethal temperature limits, but this information is surprisingly scarce despite the imperilment of unionids.

To evaluate thermal tolerance of North American unionids, a literature review could be conducted to determine the status of our knowledge. Investigating existing thermal tolerance trends across life stages and taxa can allow for extrapolation of existing data for congeners

within life stages, which may be necessary for managers and conservationists due to the paucity of thermal tolerance data. Once the existing data has been synthesized, patterns within the framework of ecological and climate change implications can be identified. Finally, knowledge gaps can be revealed and recommendations for future research based on anticipated surface water warming trends can be made.

1.4 Techniques for assessing mussel feeding

A highly efficient method of investigating mussel feeding ecology is the analysis of naturally occurring stable isotopes. This approach is effective in inferring both ultimate energy sources and trophic position of organisms and it has been used to elucidate components of the mussel diet (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Christian et al., 2004; Nichols et al., 2005; Gustafson et al., 2007; Vuorio et al., 2007; Newton et al., 2013; Weber et al., 2017). The use of carbon stable isotope ratios (13C/12C, or $\delta^{13}C$) and nitrogen stable isotope ratios (15N/14N, or $\delta^{15}N$) of consumer tissue is an effective method of analyzing food webs because consumers reflect the isotopic signatures of their food sources after accounting for fractionation that occurs through physiological processes (DeNiro & Epstein, 1978, 1981; Minagawa & Wada, 1984; Peterson & Fry, 1987; Cabana & Rasmussen 1996; Post, 2002). The putative basal energy source of an organism can be determined through carbon stable isotope ratios, and trophic position can be determined through nitrogen stable isotope signatures (Cabana & Rasmussen, 1996; Peterson & Fry, 1987; Post, 2002). Although the $\delta^{13}C$ signatures of many primary producers vary, the stable C isotope ratios of consumers are similar to that of their food, reflecting the ultimate carbon source (DeNiro & Epstein, 1978). However, the N pools of animals are enriched with ^{15}N relative to their food and this enrichment is on average +3.4‰, i.e. 3.4‰ difference in trophic levels (DeNiro & Epstein, 1981; Minagawa & Wada, 1984).

Stable isotope analysis is an effective tool for mapping aquatic food webs but this technique has limitations when analyzing bulk food compartments and differentiating between dietary carbon sources (Newton et al., 2013). To date, isotopic analyses have identified food resource compartments, but have not been able to definitively isolate specific components of mussel diets (Nichols & Garling, 2000; Christian et al., 2004; Newton et al., 2013; Weber et al., 2017). Quantitative fatty acid (FA) analysis is a technique that can identify food sources and can aid in mapping aquatic food webs when coupled with stable isotope analysis (Dalsgaard et al., 2003; Kelly & Scheibling, 2012; Newton et al., 2013; Fritts et al., 2018). Most animals cannot synthesize polyunsaturated fatty acids (PUFAs) *de novo*, and PUFAs are necessary for many critical physiological processes, making certain PUFAs essential fatty acids (EFAs; Ahlgren et al., 2009). The three most important PUFAs in vertebrates, and likely in invertebrates, are eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6). Eicosapentaenoic acid, DHA, and ARA must be obtained from a consumer's diet or the precursor FAs.

There are several FA metrics in addition to EFAs that can be used to distinguish between bacteria and plants (including algal basal food resources as well as food resources of autochthonous vs allochthonous origins). Total PUFAs (n-3 and n-6 FAs) are higher in algal than in terrestrial or cyanobacterial sources (Ahlgren et al., 2009) which could be useful when evaluating allochthonous or autochthonous food source origins. Short-chain monounsaturated FAs (MUFAs) and saturated FAs (SAFAs) are characteristic biomarkers for bacteria in terrestrial soil and benthic aquatic habitats (Cavigelli et al., 1995; Newton et al., 2013). Total EFAs, derived from algae in aquatic systems, and total FAs can provide insight into the contribution of algal versus bacterial food sources to the consumer diet. Both the absolute concentrations and the

dietary proportions between n-3 (EPA and DHA) and n-6 PUFAs [ARA, α-linolenic acid (ALA; 18:3n-6), and linoleic acid (LIN; 18:2n-6;)] are important indicators of food quality (Arts et al., 2009). The n-3:n-6 ratio of aquatic zoobenthos can inform food quality as taxa that feed on decomposing particles have lower n-3:n-6 ratios compared to taxa feeding on fresh algae in littoral or lotic habitats (Sargent et al., 1995; Arts et al., 2009). Generally, in aquatic food webs, the n-3:n-6 ratio is >1 while the ratio is <1 in terrestrial food webs (Arts et al., 2009).

1.5 Purpose of study

The purpose of this study is to examine the primary food sources of mussels in a lotic systems in central Texas and a lentic system in Alabama and the lethal thermal tolerance limits of North American unionid mussels to further inform conservation efforts. Understanding their feeding ecology and thermal tolerance limits is necessary to further understand their role in ecosystem processes and the causes of their decline, to inform management decisions and priorities, and make predictions on the future impacts of abiotic stressors. To address knowledge gaps in unionid feeding ecology, I performed three original research projects. First, I evaluated food resources of five species of unionids across four rivers and three seasons in central Texas using stable isotope analysis. The objectives of this study were to: (1) determine whether primary diet components are stable or vary across seasons, sites, and species, (2) compare the relative importance of benthic and suspended carbon sources to gain insight into feeding mechanisms (deposit versus suspension feeding), and (3) evaluate relationships between isotopic signatures and adult body sizes. Second, I evaluated food resources of three species of unionid mussels in Gantt Lake, Alabama and the objectives of this study were to: (1) determine whether primary diet components vary across species; (2) compare the relative importance of benthic and suspended carbon sources to gain insight into feeding mechanisms (deposit versus suspension

feeding); (3) evaluate relationships between isotopic signatures and body sizes; and (4) compare isotopic signatures between recently-immersed and emersed (8 weeks) individuals. The final study evaluated feeding ecology of unionids investigated five species of unionids across four rivers and three seasons in central Texas using fatty acid analysis to (1) quantify the fatty acid profiles of freshwater mussels; (2) evaluate ecological drivers of fatty acid variation including season, drainage, and species; and (3) determine if fatty acid profiles provide insight into the specific components with the basal food compartments previously determined with stable isotope studies. Finally, to investigate the knowledge gaps for lethal thermal tolerance limits of unionid mussels in North America, I conducted a systematic literature review to (1) summarize lethal thermal tolerance data for unionids by life stage and taxonomy; (2) discuss ecological and climate change implications of existing lethal tolerance data; and (3) identify needs for future research.

1.6 References

Ahlgren, G., T. Vrede, and W. Goedkoop. 2009. Fatty Acid Ratios in Freshwater Fish, Zooplankton and Zoobenthos – Are There Specific Optima? Pages 147–178 *in* M. Kainz, M. T. Brett, and M. T. Arts (editors). Lipids in Aquatic Ecosystems. Springer, New York, NY.

Arts, M. T., M. T. Brett, and M. j. Kainz. 2009. Lipids in Aquatic Ecosysytems. Springer, New York, NY.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America 93:10844–10847.

Cavigelli, M. A., G. P. Robertson, and M. J. Klug. 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170:99–113.

Christian, A. D., B. N. Smith, D. J. Berg, J. C. Smoot, and R. H. Findlay. 2004. Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca:Unionidae) in 2 small Ohio streams. Journal of the North American Benthological Society 23:101–113.

Dalsgaard, J., M. St John, G. Kattner, D. Müller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46:225–340.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341–351.

Fritts, A. K., B. C. Knights, T. D. Lafrancois, J. M. Vallazza, L. A. Bartsch, M. R. Bartsch, W. B. Richardson, S. W. Bailey, R. M. Kreiling, and B. N. Karns. 2018. Evaluating potential effects of bigheaded carps on fatty acid profiles of multiple trophic levels in large rivers of the Midwest, USA. Food Webs 16:e00095.

Fry, F. E. J. 1947. Effects of the environment on animal activity. Publications of the Ontario Fisheries Research Laboratory 68:1-62.

Galbraith, H. S., S. E. Frazier, B. Allison, and C. C. Vaughn. 2009. Comparison of gill surface morphology across a guild of suspension-feeding unionid bivalves. Journal of Molluscan Studies 75:103–107.

Gangloff, M. M., and J. W. Feminella. 2007. Stream channel geomorphology influences mussel abundance in southern Appalachian streams, U.S.A. Freshwater Biology 52:64–74.

Ganser, A., T. Newton, and R. Haro. 2013. The effects of elevated water temperature on native juvenile mussels: Implications for climate change. Freshwater Science 32:1168–1177.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1996. Influence of Sediment and Algal Food on Cultured Juvenile Freshwater Mussels. Journal of the North American Benthological Society 15:597–609.

Gatenby, C., B. Parker, and R. J. Neves. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae) reared on algal diets and sediment. American Malacological Bulletin 14:57–66.

Gustafson, L., W. Showers, T. Kwak, J. Levine, and M. Stoskopf. 2007. Temporal and spatial variability in stable isotope compositions of a freshwater mussel: Implications for biomonitoring and ecological studies. Oecologia 152:140–150.

Haag, W. R. 2012. North American Freshwater Mussels. Cambridge University Press, New York, NY.

Haag, W. R., and J. D. Williams. 2014. Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. Hydrobiologia 735:45–60.

Howard, J. K., and K. M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460–474.

IPCC. 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press.

Kelly, J. R., and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446:1–22.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. Geochimica et Cosmochimica Acta 48:1135–1140.

Newton, T. J., C. C. Vaughn, D. E. Spooner, J. Nichols, and M. T. Arts. 2013. Profiles of biochemical tracers in unionid mussels across a broad geographical range. Journal of Shellfish Research 32:497–507.

Nichols, S. J., and D. Garling. 2000. Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. Canadian Journal of Zoology 78:871–882.

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

Peterson, B. J., and B. J. Fry. 1987. STABLE ISOTOPES IN ECOSYSTEM STUDIES.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703–718.

Raikow, D. F., and S. K. Hamilton. 2001. Bivalve diets in a midwestern U.S. stream: A stable isotope enrichment study. Limnology and Oceanography 46:514–522.

Sargent, J. R., J. G. Bell, M. V. Bell, R. J. Henderson, and D. R. Tocher. 1995. Requirement criteria for essential fatty acids. Journal of Applied Ichthyology 11:183–198.

Spooner, D. E., and C. C. Vaughn. 2006. Context-dependent effects of freshwater mussels on stream benthic communities. Freshwater Biology 51:1016–1024.

Strayer, D. 2008. Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance. University of California Press.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and J. S. Nichols. 2004. Changing Perspectives on Pearly Mussels, North America's Most Imperiled Animals. BioScience 54:429–439.

Strayer, D. L., and D. Dudgeon. 2010. Freshwater biodiversity conservation: Recent progress and future challenges. Freshwater Science 29:344–358.

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

Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27:409–423.

Vuorio, K., M. Tarvainen, and J. Sarvala. 2007. Unionid mussels as stable isotope baseline indicators for long-lived secondary consumers in pelagic food web comparisons. Fundamental and Applied Limnology / Archiv für Hydrobiologie 169:237–245.

Weber, A. M., J. E. Bauer, and G. Thomas Watters. 2017. Assessment of nutritional subsidies to freshwater mussels using a multiple natural abundance isotope approach. Freshwater Biology 62:615–629.

Westhoff, J. T., and A. E. Rosenberger. 2016. A global review of freshwater crayfish temperature tolerance, preference, and optimal growth. Reviews in Fish Biology and Fisheries 26:329–349.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation Status of Freshwater Mussels of the United States and Canada. Fisheries 18:6–22.

Yeager, M. M., D. S. Cherry, and R. J. Neves. 1994. Feeding and Burrowing Behaviors of Juvenile Rainbow Mussels, *Villosa iris* (Bivalvia:Unionidae). Journal of the North American Benthological Society 13:217–222.

CHAPTER 2
THE RELATIVE IMPORTANCE OF SUSPENDED VERSUS BENTHIC FOOD RESOURCES TO FRESHWATER MUSSELS IN CENTRAL TEXAS, USA

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2.1 Abstract

1. As unionid mussels continue to decline in North America much remains unknown regarding their primary food resources and feeding relationships. Our objective was to investigate spatial, temporal, intra- and interspecific variation in food resource use.

2. In this study, we quantified stable C ($\delta^{13}C$) and stable N ($\delta^{15}N$) isotopic signatures for five mussel species and their potential food resources across four drainages and three seasons in central Texas, USA.

3. Across all species and sites, Bayesian mixing models suggest C derived from coarse particulate organic matter (CPOM; bulk detrital leaf packs) contributed on average 51% to the mussel diet and C derived from suspended particulate organic matter (SPOM) contributed 41% on average to the mussel diet. Mussel stable isotope signatures exhibited minimal variation across species within a site, but significant differences across sites and seasons. Although significant differences in food source contributions were observed between sites and species, differences were relatively small and either CPOM or SPOM were consistently the primary food resource.

4. Mussels were potentially consuming microbial biofilms associated with CPOM pools, but preferential assimilation of detrital biofilms remains to be quantified. Carbon:nitrogen ratios of mussel soft tissue varied seasonally with summer ratios approximately 3 times higher on average than spring and autumn ratios, which is suggestive of poor food quality and thermal stress during the summer. Some species had significant positive relationships between

isotopic ratios and shell length, which indicates changes in food resource incorporation with increasing mussel size.

5. Together these data suggest the C in mussel tissues was of benthic and suspended origin. These findings provide further evidence that mussels effectively exploit benthic in addition to planktonic food resources which emphasizes the need to maintain riparian habitats and allochthonous inputs.

2.2 Introduction

Mussel diversity and abundance in North America have been declining for decades due to anthropogenic stressors such as impoundments, sedimentation, altered hydrology, impaired water quality, and water withdrawal (Williams et al., 1993). Increased awareness of the ecological function of mussels and their imperiled status has driven significant interest in elevating conservation efforts for these animals, including in-depth explorations of causes for decline and propagation/reintroduction programs (Strayer et al., 2019; Haag et al., 2019). Knowledge of mussel feeding relationships is necessary to further understand their role in ecosystem processes and to aid in expanding conservation efforts.

Mussels can dominate benthic biomass in riverine systems and are capable of filtering large volumes of water (Vaughn et al., 2008; Haag, 2012). The nutrient and energy transfer that mussels facilitate through their feeding activity links the benthic and pelagic interfaces of aquatic systems and can affect primary and secondary production across trophic levels (Vaughn et al., 2004; Howard & Cuffey, 2006; Spooner & Vaughn, 2006). Despite the ecological importance of unionid feeding activities, much is unknown regarding their food sources and feeding relationships. Mussels are primarily described as suspension feeders, with a diet comprised of algae, diatoms, rotifers, protozoans, detritus, bacteria, and dissolved organic carbon (Strayer,

2008; Vaughn et al., 2008; Haag 2012). Algae has been identified as a key constituent of mussel diets in river and lake habitats, providing important nutrients and composing a significant portion of ingested material in the gut (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Newton et al., 2013; Weber et al., 2017). In riverine habitats, bacteria may be of equal or greater importance than algae to the mussel diet (Nichols & Garling, 2000; Christian et al., 2004; Newton et al., 2013). Mussels also possess the digestive enzymes necessary to use detritus as a nutritional subsidy (Christian et al., 2004) and fatty acid studies have shown that detritus may comprise a significant component of mussel diets in certain habitats (Newton et al., 2013).

In addition to suspension-feeding, mussels also use deposit-feeding, accomplished by generating water currents within the mantle cavity to access benthic or buried organic material through the shell gape, allowing access to benthic food sources such as sediment-based organisms and detritus (Gatenby et al., 1996; Nichols et al., 2005). Juveniles regularly consume benthic organic matter through pedal feeding (sweeping their ciliated foot through the sediment; a form of deposit-feeding; Yeager et al., 1994) and organic particles associated with sediment are an important subsidy to the juvenile mussel diet (Gatenby et al., 1996, 1997). Similarly, some adult mussels have been shown to derive on average 80% of their diet from benthic sources (Raikow & Hamilton, 2001).

Unionid diets can show considerable spatial variation across habitats (Newton et al., 2013) and such spatial variation may be influenced by local conditions. For instance, mesohabitat type influences the accumulation of fine benthic organic particles – with backwaters and pools accumulating more fine organic material than riffles or runs (Grubaugh & Anderson, 1989). Streambed substrata (e.g. bedrock versus gravel) influence the ability of mussels to burrow and access organic benthic food resources. Localized land use can result in reduced

riparian vegetation, increased nutrient inputs, limited allochthonous inputs, increased autochthonous production, and increased inorganic turbidity, all of which can influence mussel feeding dynamics (Allan et al., 1997). In addition to spatial variability, unionid food sources exhibit temporal variability. A majority of benthic and suspended algae species are influenced by seasonal changes in temperature and light availability allowing for seasonal succession of algal population assemblages (Sheath & Burkholder, 1985). Further, allochthonous inputs of riparian detritus in the autumn in temperate climates provide organic inputs to the system through leaf litter and subsequent microbial colonization (Webster & Benfield, 1986). This microbial abundance increases with temperature (Boyd, 2019), providing additional food resources for unionids in spring and summer months.

Tracking the feeding and diets of mussels can be challenging. However, stable isotope approaches can be particularly insightful in disentangling the amorphous diets of these animals. Naturally-occurring stable isotopes in consumer soft tissue reflect the isotopic signatures of their food resources and provide an effective means to elucidate unionid trophic positions and diet (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Christian et al., 2004; Newton et al., 2013; Weber et al., 2017a). Dietary components are typically identified via carbon (C) stable isotope signatures [$^{13}C/^{12}C$, or delta ^{13}C ($\delta^{13}C$)] whereas trophic position is identified via nitrogen (N) isotopes ($^{15}N/^{14}N$, or $\delta^{15}N$; DeNiro & Epstein, 1978, 1981).

Variation in the carbon:nitrogen (C:N) ratios of freshwater consumers can provide further insight on consumer diet, particularly with assessing food quality (Sterner & Elser, 2002; Trochine et al., 2019) and origins (Sterner & Elser, 2002; Cross et al., 2005; Trochine et al., 2019). Food resources with low C:N ratios are of higher quality than resources with high C:N ratios (Trochine et al., 2019). Stoichiometry can also indicate origins of dietary items, as

allochthonous and autochthonous resources have considerably different C:N ratios, and similarly, benthic and pelagic resources can have different stoichiometry (Cross et al., 2005).

The degree to which bivalve diet changes with body size is not fully understood. There is evidence that bivalve $\delta^{13}C$ and $\delta^{15}N$ become more enriched in some species of freshwater mussels and brackish clams as organisms increase in size (Tai Tue et al., 2012; Yasuno et al., 2014). Such enrichment may be due to age-related changes in feeding strategies or metabolic effects of isotopic incorporation and physiological condition of the individual (Minagawa & Wada, 1984; Yeager et al., 1994; Gatenby et al., 1996). This suggests that individuals at different life stages or varying size classes within a life stage (i.e., small versus large adults) may be using different trophic niches and potential food resources, however, there is no consensus understanding of the body size to isotopic ratio relationship in bivalves.

In this study, we examined the extent to which adult unionid mussels consume suspended versus benthic food resources and whether usage patterns are stable or vary across seasons, sites, and species. We also investigated factors influencing differences in food resource use. Our objectives in this study were to: (1) determine whether primary diet components are stable or vary across seasons, sites, and species, (2) compare the relative importance of benthic and suspended carbon sources in order to gain insight into feeding mechanisms (deposit versus suspension feeding), and (3) evaluate relationships between isotopic signatures and adult body sizes.

2.3 Methods

2.3.1 Species and study sites

The initial design involved sampling three endemic, state-listed, taxa (*Cyclonaias petrina, C. houstonensis,* and *Lampsilis bracteata*) in two rivers each in Texas, USA (*C. petrina* in the Guadalupe and Colorado Rivers; *C. houstonensis* in the Navasota and Colorado Rivers; *L. bracteata* in the Guadalupe and Llano Rivers). However, taxonomic revisions occurred after the first season (Spring 2017) of field sampling (Burlakova et al., 2018; Johnson et al., 2018; Inoue et al., 2019) that resulted in a modification of the design. *Cyclonaias petrina* in the Guadalupe River was reclassified as *C. necki*, thus we added a site (Llano River) to ensure *C. petrina* was sampled in two rivers. *Cyclonaias houstonensis* was reclassified as *C. pustulosa* in the Colorado River and in the Navasota River, which maintained two sites for this taxa. *Lampsilis bracteata* in the Guadalupe River was reclassified as *L. bergmanni* resulting in a single site for both *Lampsilis spp.*, with *L. bracteata* being sampled in the Llano River. Thus, the final design included a total of five species, two of which were sampled in two rivers each while the remaining three species were sampled in only a single river each for a total of seven species-site combinations (Table 1).

2.3.2 Mussel sampling

Each species-site combination was sampled in spring (April 2017), summer (July 2017), and autumn (October 2017). On each sampling date, we collected ten individuals per species within a given river by snorkeling and searching benthic sediments by hand. Length (posterior to anterior margin of the shell; mm), width (dorsal to ventral margin of the shell; mm), height (most inflated portion of the shell; mm), and total wet mass (shell plus soft tissue; g) were measured for each individual. To obtain tissues for stable isotope analyses, mussels were opened using a pair of flat-tipped, reverse-action pliers and two sublethal tissue samples were taken from foot tissue

using a 1.5 mm × 4.5 mm biopsy punch (nasal biopsy tool #453733, Karl Storz, Tuttlingen, Germany; Fritts et al., 2015). Mussels were allowed to recuperate streamside in water (1–2 h) and subsequently returned alive to the streambed from where they were collected. Tissue samples were put on ice in the field, subsequently frozen, and transported to Auburn University, AL, USA for processing. Tissue samples were dried at 80°C to a constant mass, ground using a mortar and pestle, weighed (nearest 10^{-5} g), and placed in 4 mm × 6 mm tin capsules (Costech Analytical, Valencia, CA, USA).

2.3.3 Potential food source and water quality sampling

Hypothesized food sources [suspended particulate organic matter (SPOM), fine particulate organic matter (FPOM), and coarse particulate organic matter (CPOM; benthic detritus)] were sampled at the same sites and seasons as for the mussels. At each site, SPOM was sampled by collecting ten 1–2 L samples at mid-depth and pre-filtering through 55 μm mesh to remove larger particles (Vaughn et al., 2008). Pre-filtered water was then vacuum-filtered through a pre-combusted (450 °C for 4 h) 47 mm Whatman® glass fiber (GF/F) filter (nominal pore size = 0.7 μm) to isolate suspended solids between 0.7 and 55 μm, reflecting the size fraction typically consumed by mussels (Post, 2002; Strayer, 2008; Vaughn et al., 2008). Filters were frozen and transported to Auburn University where they were dried at 80 °C to a constant mass and fumigated in 3 N H_3PO_4 for 8 h to remove carbonates (Harris et al., 2001).

Five to ten mid-channel surface sediment samples were collected for FPOM (detritus, algae, bacteria, and fungi mixed with sand) at each site and season using a turkey baster (~25 mL/sample). Samples were pre-filtered (55 μm), filtered (nominal pore size = 0.7 μm), and

transported to Auburn University in the same manner as the water samples and dried to a constant mass at 80 °C. The sediment layer was then removed from the filter, fumigated in 3 N H_3PO_4 for 8 h to remove carbonates (Harris et al., 2001), ground to a fine powder using a mortar and pestle, weighed (nearest 10^{-5} g), and encapsulated in 4 mm × 6 mm tin capsules.

Approximately 85 g of CPOM (leaf packs and other detritus) were collected at each site and season, frozen, transported to Auburn University, fumigated, ground, and encapsulated in same manner as for the FPOM samples. All prepared isotopic samples of mussel tissues and food resources were shipped to Washington State University Stable Isotope Core Laboratory for $\delta^{13}C$ and $\delta^{15}N$ analysis.

Isotope ratios are reported in parts per thousand (‰) relative to standards [Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N for nitrogen], defined in delta notation as:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample} / R_{standard} - 1) \times 10^3$$

where $R = {}^{13}C / {}^{12}C$ or ${}^{15}N / {}^{14}N$, respectively (DeNiro & Epstein, 1978, 1981).

Water temperature, conductivity, and pH were measured at each sampling location with an Oakton PC Testr multiparameter meter (Oakton Instruments, Vernon Hills, IL, USA). Discharge was recorded from the nearest United States Geological Survey (USGS) gage at the time of sample collection.

2.3.4. Statistical analyses

We used a combination of linear models and supportive Bayesian mixing models to address our objectives. First, to determine whether isotopic signatures varied across seasons, sites, and species (objective 1) and to identify possible relationships between isotopic signatures

and consumer size (objective 3), we used a three-way analysis of covariance (ANCOVA) to test the effects of species, season, site, and their interactions with shell length as a covariate on mussel $\delta^{13}C$ and $\delta^{15}N$. Second, to identify whether food quality varied across seasons, sites, and species (objective 1), we used a three-way analysis of variance (ANOVA) to test the effects of species, season, site, and their interactions on mussel C:N ratios. When species, season, site, or their interactions were significant, a Tukey's Honestly Significantly Different (HSD) test was conducted for the post-hoc analysis. Statistical significance was set at $p < 0.05$. All analyses of variance tests were performed using SAS® version 9.4 (SAS, 2013).

To account for variation in lipid content between consumers and food resources, all $\delta^{13}C$ values were mathematically corrected using the linear relationships between $\delta^{13}C$ and C:N for mussels and $\delta^{13}C$ and % carbon for food resources (Post et al., 2007). All mussel $\delta^{13}C$ values were corrected for lipid influence using C:N ratios as follows:

$$\delta^{13}C_{corrected} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}N$$

All food resource $\delta^{13}C$ values were corrected for lipid influence using % carbon as follows:

$$\delta^{13}C_{corrected} = \delta^{13}C_{untreated} - 3.02 + 0.09 \times \% \text{ carbon}$$

Corrected $\delta^{13}C$ values were used for all statistical analyses.

To determine the relative contribution of each potential food source to mussel diets, we used a Bayesian tracer mixing model framework (MixSIAR; R Core Team, 2013; Stock & Semmens, 2016). MixSIAR accounts for hierarchical structure (food chain and individual trophic niche), uncertainty in the mixture (mussel tissue), and source (food sources) variance (Semmens et al., 2009; Stock et al., 2018). This analysis was used to determine whether primary diet components varied spatially (objective 1) and to provide insight into food resources (objective 2). Season was not included as a variable in MixSIAR models due to inadequate seasonal sample

sizes for some food resources. The MixSIAR model used two tracers ($\delta^{13}C$ and $\delta^{15}N$) and three food sources (CPOM, FPOM, and SPOM).

To evaluate whether our isotope mixing model was valid, we used a Monte Carlo simulation of the resource mixing region to evaluate the probability that consumer stable isotope contributions were satisfied by the resource polygon (Smith et al., 2013). The point-in-polygon method is an iterative approach that uses a user-defined discrimination factor to generate mixing polygons with the distribution of the putative food sources. The proportion of the resource polygon that has a solution is given as frequentist probabilities that the putative resource data and associated MixSIAR model can calculate source contributions to explain a consumer's isotopic signature (Smith et al., 2013). The discrimination factors that were determined to be mathematically justified via the point-in-polygon method and were used in the MixSIAR models were originally extrapolated from previous studies on unionids (Post, 2002) and then modified to satisfy the resource polygon geometry (Smith et al., 2013; Brett, 2014) as trophic discrimination can vary based on the physiology, taxonomy, or environment of the target species (Phillips et al., 2014). The nitrogen trophic enrichment factor was doubled to ensure that our consumers fell within the resource mixing region. (Smith et al., 2013; Brett, 2014). Thus the specific C discrimination factor was estimated to be 0.4‰ ± 1.3 *SD* and N was 6.8‰ ± 2 *SD*, with these discrimination factors being used in all models. After using the point-in-polygon method to evaluate the validity of our mixing model with consumer and food source data for each site, we removed consumers that had less than a 5% chance of having their isotopic signature explained by the source contributions (Figure 2, right graph for each site).

Four mixing models incorporating different combinations of variables (site and species) were initially tested and compared using deviance information criterion (DIC) scores (Table 2;

Phillips et al., 2014; Stock & Semmens, 2016; Stock et al., 2018). Model convergence comparisons were made by assessing Gelman-Rubin diagnostics and Geweke diagnostics (Phillips et al., 2014; Stock & Semmens, 2016; Stock et al., 2018). Two priors for food source contribution were compared for use in the model: an uninformative prior and a literature-based prior attributing 60% contributions from SPOM, 20% from FPOM, and 20% from CPOM (Hornbach et al., 1984; Raikow & Hamilton, 2001; Nichols et al., 2005; Weber et al., 2017).

2.4 Results

2.4.1 Water quality

In general, the Guadalupe and Brazos River sites had lower water temperatures than the Colorado or Llano sites (Table 3). pH was lowest for any given season in the Navasota River and typically highest in the Colorado River (Table 3). Conductivity and discharge were highest in the Colorado River and lowest in the Navasota River (Table 3).

2.4.2 Mussel isotopic values

Seasonal Variation - There was significant seasonal variability in stable isotope signatures (Table 4). Controlling for the effects of species, site, and shell length, mussels were more carbon depleted in the spring than in autumn (ANCOVA: $t_{151} = 4.26$, $p < 0.0001$) and more carbon depleted in autumn than in summer seasons (ANCOVA: $t_{151} = 7.3$, $p = <0.0001$). Mussels were more nitrogen-enriched in the spring (ANCOVA: $t_{151} = 6.22$, $p < 0.0001$) and summer (ANCOVA: $t_{151} = 5.93$, $p < 0.0001$) than in the autumn.

Spatial Variation - Across all seasons, there was a difference in mussel $\delta^{13}C$ signatures among sites, with mussels in the Llano River the most carbon enriched and mussels in the Colorado River the most carbon depleted (Table 4). Mussels were significantly different in their $\delta^{15}N$ signatures across sites, with individuals in the Colorado River the most nitrogen-enriched and individuals in the Llano River the least nitrogen-enriched (Table 4).

There were strong intraspecific differences in carbon enrichment among sites, but few interspecific differences within a given site (Table 4). Across all seasons, *C. petrina* was more carbon-depleted and nitrogen-enriched in the Colorado River as compared to *C. petrina* in the Llano River (ANCOVA: $t_{155} = 4.27$, $p = 0.0007$; $t_{155} = 23.24$, $p < 0.0001$ for $\delta^{13}C$ and $\delta^{15}N$, respectively). Similarly, *C. pustulosa* was more nitrogen-enriched in the Colorado River as compared to the Navasota River (ANCOVA: $t_{155} = 3.44$, $p = 0.0128$). Where multiple species were sampled at a given site (Guadalupe, Colorado, and Llano Rivers), all mussels had nearly identical isotopic signatures for both $\delta^{13}C$ and $\delta^{15}N$, except for $\delta^{13}C$ signatures in the Guadalupe River (Table 4; Fig. 3–6).

Shell Length – Shell length (the covariate) had significant positive relationships with $\delta^{13}C$ (ANCOVA: $F_{161} = 3.79$, $p = 0.0002$) and $\delta^{15}N$ ($F_{161} = 6715$, $p < 0.0001$). Across all seasons and sites, shell length was significantly related to $\delta^{13}C$ for all species except *L. bracteata*. Shell length was also significantly related to $\delta^{15}N$ for *C. necki* (ANCOVA: $t_{161} = 7.79$, $p < 0.0001$), *C. petrina* (ANCOVA: $t_{161} = 3.39$, $p = 0.0001$), *C. pustulosa* (ANCOVA: $t_{161} = 13.85$, $p < 0.0001$) and *L. bergmanni* (ANCOVA: $t_{161} = 6.97$, $p < 0.0001$).

2.4.3 Carbon:Nitrogen ratios

There was no interspecific difference in C:N ratios, but C:N ratios did vary across sites (Table 4). There was significant seasonal variation in C:N ratios. Across all species and sites, C:N ratios were highest in the summer (Table 4). For all species except *C. necki*, summer C:N ratios were 2.6–3.4 times higher than spring and autumn C:N ratios (Table 4; Fig. 2). There was no significant difference in *C. necki* C:N ratios across seasons, with *C. necki* having the lowest ratio in the summer (3.67) and the highest ratio in the spring (3.81).

2.4.4 Food source contributions

The model analyzing species and site as fixed effects was the best fitting model, determined by the lowest DIC scores (Table 2). The MixSIAR model obtained the bestconvergence when using an uninformative prior estimate of food source contributions determined by Geweke diagnostics, Gelman-Rubin diagnostics, and DIC score model parameter estimates (Table 2). Based on our point-in-polygon analysis (Smith et al., 2013), seven of 32 consumer values were removed from the model for the summer season in the Navasota River and two of 24 consumer values were removed from the model for the spring and the autumn seasons from the Colorado River (Figure 2). Two of 31 consumer values were removed from the spring and the summer seasons in the Llano River, one of 21 values was removed from the spring season for C. necki and two of 26 values from the summer for L. bergmanni in the Guadalupe River (Figure 2).

Across all sites and species, bulk CPOM pools and SPOM were the dominant carbon sources with FPOM making relatively minor contributions. In the Navasota River, mussels and

CPOM had minimal seasonal variation and CPOM was carbon depleted as compared to the *C. pustulosa* (Fig 4a–c). CPOM dietary contributions to *C. pustulosa* in the Navasota River were 65.2% ± 13.1 *SD* and SPOM contributions were 16.5% ± 14.4 *SD.* The FPOM contributions to *C. pustulosa* in the Navasota River were 18.4% ± 10.9 *SD* (Fig. 4d).

In the Colorado River, CPOM and SPOM had the closest isotopic signature to mussels, with FPOM consistently carbon enriched as compared to consumers (Fig 5a–c). *Cyclonaias petrina* had mean CPOM contributions of 37.9% ± 19.9 *SD* and *C. pustulosa* had mean CPOM contributions of 47.5% ± 15.9 *SD*. The SPOM contributions for both *C. petrina* and *C. pustulosa* were 51.6% ± 24.1 *SD* and 41.7 ± 19.9 *SD*, respectively. The FPOM contributions for both species were approximately ten percent% (Fig. 5d).

In the Llano River, SPOM had a carbon signature closer to mussels in spring than in autumn and summer, contributing to greater SPOM contribution (Fig 6a–c). Generally, CPOM was carbon depleted and FPOM was consistently carbon enriched compared to mussels (Fig 6a–c). At this site, CPOM contributions to *C. petrina* were 39.1% ± 19.9 *SD*, and SPOM was 58.5% ± 20.6 *SD*. The FPOM contribution was 2.4% ± 2.3 *SD*. For *L. bracteata*, dietary contribution of CPOM was 52.7% ± 18.7 *SD* and the dietary contribution of SPOM was 43.3% ± 20.8 *SD*. The contribution from FPOM was 4.0% ± 5.2 *SD* (Fig. 6d).

In the Guadalupe River, SPOM and FPOM were consistently carbon enriched as compared to mussels and CPOM had the closest carbon signature to consumers at this site, with the exception of *L. bergmanni* in the summer season (Fig 7a–c). Mean CPOM dietary contribution was 83.7% ± 4.5 *SD* for *C. necki* and 32.3% ± 11.7 *SD* for *L. bergmanni*. Contributions of SPOM were 9.8% ± 4.6 *SD* and 65.5% ± 12.1 *SD* for *C. necki* and *L. bergmanni,* respectively. Contributions of FPOM were less than 7% for both species (Fig. 7d).

2.4.5 Nitrogen-enrichment of consumers

For all seasons, mussels exhibited $\delta^{15}N$ enrichment relative to their respective food sources, and for most seasons, mussels were several trophic levels above their primary identified food source of CPOM or SPOM (Figs. 4–7). In the Guadalupe River, *C. necki* (11.05‰ ± 0.29 *SD* $\delta^{15}N$) was nitrogen-enriched above CPOM by 8.69‰ and *L. bergmanni* was nitrogen-enriched above SPOM by 8.56‰. In the Colorado River and *C. pustulosa* (12.81‰ ± 0.79 *SD* $\delta^{15}N$) was nitrogen-enriched above CPOM by 6.43‰ and *C. petrina* (13.19‰ ± 0.90 *SD* $\delta^{15}N$) was nitrogen-enriched above SPOM by 6.55‰. *Cyclonaias petrina* (6.54‰ ± 0.19 *SD* $\delta^{15}N$) from the Llano River was enriched above SPOM by 5.16‰ and *L. bracteata* (7.29‰ ± 0.40 *SD* $\delta^{15}N$) was enriched above CPOM by 3.17‰. *Cyclonaias pustulosa* (11.92‰ ± 0.49 *SD* $\delta^{15}N$) of the Navasota River was enriched by 6.82‰ above CPOM.

2.5 Discussion

Our data suggest that mussels in these Texas rivers rely on benthic in addition to suspended organic matter. The primary carbon source for *C. necki*, *C. pustulosa*, and *L. bracteata* across each river was consistently CPOM. *Cyclonaias petrina* and *L. bergmanni* had a primary carbon source of SPOM, with secondary carbon contributions coming from CPOM. While *C. petrina* and *C. pustulosa* had similar primary carbon sources across sites, there was site-specific variation in C:N ratios and isotopic signature with notable nitrogen-enrichment above potential food resources and relationships between isotopes and shell size. These patterns suggest that mussels are using a benthic feeding mode to access detrital material, in addition to suspension

feeding, and provide insight into food quality, sources of stress, and consumer size-related resource use.

2.5.1 Stable isotope variation and food source contributions

The isotopic signatures of mussels in this study were generally not species-specific and varied with site. There were significant differences in isotopic signatures of mussel species across sites, but these differences were reflected in similar isotopic fractions between mussels and CPOM or SPOM, resulting in CPOM or SPOM consistently being identified as the predominant food source across sites.

While mussel isotopic signatures varied across sites, species within a site had nearly identical isotopic signatures in autumn and spring but not in the summer. This summer difference was potentially driven by the observed variation in summer C:N ratios, which causes changes in corrected $\delta^{13}C$ values. Further, variation in $\delta^{15}N$ signatures of basal resources across systems due to local geographical and anthropogenic influences could account for the variability of $\delta^{15}N$ in mussel tissues (Peipoch et al., 2012). Previous isotope studies have found freshwater mussels inhabiting the same site to have similar isotopic signatures, with minimal variation across seasons (Weber et al., 2017). This further suggests that the primary influences on mussel stable isotope composition are site-specific or drivers such as nutrient cycling and the dominant source of C (e.g., allochthonous versus autochthonous) as opposed to intraspecies competition. The variation of feeding in our study can be attributed to changes in corrected carbon signatures in the summer season, which is an artifact of

high C:N ratios during this season. If summer carbon values were not corrected they would show less variation and carbon enrichment, and CPOM would likely be the predominant food source for most species across all sites.

While mussels have traditionally been categorized as filter feeders, recent studies using isotopes have indicated a significant reliance on benthic food resources and the physiological ability to access benthic material and digest plant material associated with detritus (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Christian et al., 2004; Nichols et al., 2005; Weber et al., 2017). Allochthonous inputs into riverine systems may contribute to one-third of mussel biomass, and allochthonous vegetation was a necessary food resource to explain isotopic signatures in five mussel species sampled in a seventh-order stream (Weber et al., 2017). Our results further suggest that benthic, allochthonous resources may be of equal or higher importance than planktonic sources to mussel diets in riverine systems. We found the mean contribution of CPOM to mussel diets averaged 51% across all sites and species whereas SPOM contributed 41%, on average, to mussel diets. This aligns with previous studies showing up to 80% of mussel diets may be derived from benthic materials (Raikow & Hamilton, 2001). The importance of CPOM as a substantial dietary source in these study systems illustrates important conservation and management implications for unionids. In addition to general water quality, it is important to maintain the integrity of riparian habitats and their allochthonous inputs to surface waters.

Bivalves can use deposit-feeding to access benthic food resources such as CPOM (McMahon & Bogan, 2001; Nichols et al., 2005) and may preferentially assimilate the living microbial communities associated with particulate organic matter,

communities comprised of up to 86% microbial biomass (Nichols & Garling, 2000; Christian et al., 2004). Mussels at all sites except the Llano River exhibited nitrogen-enrichment that may be indicative of diets where the primary food source is conditioned and subsequently enriched by microbes (Goedkoop et al., 2006) such as the microbial biofilms conditioning and decomposing activities associated with leaf detritus. However, because contributions of detrital biofilm were not separated from the detritus itself, we cannot confirm preferential assimilation of microbes associated with CPOM. The relative contribution of the detrital pool and associated microbial communities that mussels are using as their primary carbon subsidy likely varies spatially and seasonally and warrants further study.

2.5.2 Food limitation and thermal stress

Consistent patterns of nitrogen-enrichment of mussel tissues relative to primary food resources suggest that quality and/or quantity of the dominant food resource (CPOM and SPOM) was frequently limiting, particularly in July. Typically, $\delta^{15}N$ of a consumer is enriched ~3.4‰ above its primary food resource (DeNiro & Epstein, 1981). In our study, *L. bracteata* was enriched 3.2‰ above CPOM in the Llano River, which is similar to the expected 3.4‰ level of enrichment (DeNiro & Epstein, 1981; Post, 2002). However, for all other species, $\delta^{15}N$ in mussel tissue was enriched by 5.16–8.69‰ above their respective dominant food resource, either CPOM or SPOM. Nitrogen-enrichment above one trophic level (i.e., >3.4‰) can be caused by "self" trophic fractionation due to catabolism of tissues that leads to metabolic retention of $\delta^{15}N$ in the organism (Cherel et al., 2005). Such increased enrichment has been shown in oysters and fish and is indicative of nutrient and environmental stress (Bowes et al., 2014; Patterson &

Carmichael, 2018). Whether such self-fractionation occurs in freshwater mussels has not been documented.

We saw a sharp increase in July C:N ratios, relative to April and October, suggesting nutrient and environmental stress were particularly high in the summer. Energetic demand of mussels increases with temperature during the summer months, putting them at increased risk of food limitation (Haney et al., 2020). Thermally sensitive species such as *C. pustulosa* deplete energetic stores and increase N excretion with increasing temperatures (Spooner & Vaughn, 2008). As energy budgets decline towards the "individual threshold for growth" (point where assimilation balances metabolic requirements and growth is zero) C:N ratios increase because C is not a limiting resource, and N is being excreted at a higher rate than C (Sterner & Elser, 2002; Frost et al., 2004). Taken together, our results suggest that the co-dominant CPOM and SPOM resources were not of sufficient quality and/or quantity and most species were subjected to thermal stress and food limitation in July. Interestingly, in the one system (Llano River) where $\delta^{15}N$ was not enriched, mussels assimilated SPOM to a greater degree than in other systems. Increased use of SPOM may have alleviated food quality/quantity issues in the Llano River to some extent although Llano mussels still experienced a spike in C:N in July. The only species (*C. necki*) that did not exhibit a July spike in C:N ratios also had relatively low metabolic rates in a previous study (Haney et al., 2020), suggesting that decreased energy demand may partially offset food limitation issues in the summer, although this species still exhibited $\delta^{15}N$ enrichment throughout the year.

2.5.3 Size trends

All species showed a general trend of C and N enrichment with increasing shell length. Similar patterns have been shown in previous studies on unionids and may be due to multiple mechanisms (Fritts et al., 2013; Yasuno et al., 2014). For example, younger, rapidly growing individuals may retain more dietary protein whereas older, slower-growing individuals exhibit higher isotopic incorporation (Rio et al., 2009). Based on the size range of mussels sampled in this study, almost all individuals were at or beyond the onset of maturity suggesting that the body size/ isotopic ratio correlation is not due to ontogenetic changes (Seagroves et al., 2019; Dudding et al., 2020). Enrichment in stable isotope signatures with increasing size may indicate that larger mussels are preferentially ingesting and assimilating more δ^{13}C and δ^{15}N enriched components of bulk plankton or microbial pools (Carmichael et al., 2008; Yasuno et al., 2014). These findings provide evidence that there is a relationship between δ^{15}N and δ^{13}C and body size in adult unionid mussels — phenomena that have only been exhibited in some bivalve mollusks. Further research must be done to understand this relationship and whether isotopic incorporation or preferential particle selection changes with increasing adult body size.

2.5.4 Current caveats in stable isotope methodologies for unionid feeding studies

There are several caveats to our analysis and issues with standard stable isotope methodology that should be addressed for future studies on unionid feeding ecology. Currently, mathematical equations exist to correct δ^{13}C signatures of aquatic animal samples for lipid composition using associated C:N values – but these equations assume a linear relationship

between lipids and C:N and have only been tested in aquatic animal samples with a C:N ratio of ≤ 6.9 (Post et al., 2007). In this study, we corrected summer mussel $\delta^{13}C$ signatures despite the average C:N ratio being 10.41, therefore our summer $\delta^{13}C$ isotopic data should be interpreted with caution as the method used for correction was extrapolated beyond the previously measured range of the linear lipid and C:N relationship. Further investigation of mathematical correction must be conducted for individuals with a C:N ratio ≥ 6.9 so that corrected $\delta^{13}C$ data can be validated.

A second issue exists regarding the MixSIAR model assumptions that consumers must fit within putative resource polygon geometry. Without carbon correction, our consumers were considerably more depleted than all food sources sampled, and therefore fell outside of the resource polygon. Despite carbon correction and excluding consumers that had less than 5% probability of having a solution found with mixing models, the majority of our consumers were not centered within our resource polygon and were still relatively carbon depleted relative to the food resources. This is further evidence that lipid correction equations must be expanded for consumers with high C:N ratios and further investigation is required to explain the overall pattern of carbon depletion found in our consumers. Additionally, when three food resources align linearly as opposed to aligning in a triangle, it is mathematically impossible to distinguish the use of the resource in the middle from a mixed-use of the food sources — as in the case in the Navasota, Colorado, and Guadalupe Rivers. For our study, consumers are offset between the SPOM (middle) and CPOM (left) signatures, indicating that both SPOM and CPOM contribute to the mussel diet as shown by the MixSIAR models.

A similar issue occurred with nitrogen enrichment, as the nitrogen signatures of our consumers were enriched more than one trophic level above our sampled food resources. This

resulted in the trophic enrichment factor of our mixing model being two times greater than in previous studies to satisfy the resource polygon geometry, suggesting that polygon geometry assumptions and trophic discrimination factors for unionids need further consideration. As our consumers did not fall within our food resource polygon, there is the possibility that we missed potential food resources. While this could explain the discontinuity between the signatures of consumers and sources, the food sources we collected were analogous to those collected by other studies that found solutions (Nichols & Garling, 2000; Post, 2002; Christian et al., 2004; Weber et al., 2017). One important point to address is that mixtures like FPOM and SPOM are dynamic and potentially of variable nutritional content and there is the possibility that mussels are differentially assimilating different components of the bulk resource. However, it should also be noted that such nutritional variation would be expected to be reflected in the variation associated with resource signatures, and although there are a few instances of carbon variation in putative resources, most resource pool replicates in this study showed minimal variation in carbon signatures.

Finally, it is recommended that trophic discrimination factors used in MixSIAR models reflect the biology, taxonomy, and environment of the target species being analyzed (Phillips et al., 2014). To satisfy model assumptions, we were unable to calculate discrimination factors tailored specifically to aquatic, benthic ectotherms, as this would not satisfy resource polygon geometry. There must be further quantification of appropriate discrimination factors which satisfy mixing model assumptions but are also ecologically relevant to the study taxa. The interspecific, temporal, and spatial isotopic data collected in this study represents real scenarios where existing lipid correction and mixing model methodologies may not be suitable.

2.5.5 Potential conservation implications

As freshwater mussel populations are declining across North America, it is critical to understand the ecological and physiological processes behind their diet and feeding. This study has provided additional evidence to contest the long-lasting paradigm of mussels as exclusively filter feeders (Coker et al., 1921; Allen, 1921). While mussels use suspended feeding mechanisms and food resources, the modeling in this research shows a significant contribution of benthic subsidies, in the form of CPOM, to the mussel diet. The specific components of the detrital and associated microbial pool that are being assimilated by the mussels as nutritional sources have yet to be identified. An important question related to benthic versus suspended feeding mechanisms is whether mussels are filtering microbial biofilms that have been sloughed from CPOM, and subsequently resuspended and filtered from the water column, or are directly feeding on benthic materials. Sloughed biofilms may only be suspended for short distances above the river bottom, depending on bottom substrate size and flow. If so, direct benthic feeding is likely a fundamental strategy wherein mussels may be feeding on sloughed biofilms at the sediment-water interface. These would not have shown up in our SPOM samples. Regardless of feeding mode, diet appears to be less dependent on species identity, and more dependent on local context, with different species having similar isotopic signatures at a given location.

This study suggests that at least some mussel species exhibit size-related changes in $\delta^{15}N$ and $\delta^{13}C$ signatures, which could be indicative of changes in feeding patterns throughout the life of the organism. Additionally, we identified a potential biochemical indicator of stress which is $\delta^{15}N$ that is enriched several trophic levels above basal food resources. This could be indicative of metabolic stress, potentially corresponding with instances of increased temperatures and low dissolved oxygen. The relationships between environmental stressors and patterns in isotopic

signatures of mussels are currently not well understood and warrant further study. Our study highlights the need to maintain riparian habitats and their associated detrital inputs into riverine systems and focuses on benthic substrates as a potential provider of nutrition for freshwater mussels as opposed to simply habitat. Further understanding of the relationship between environmental stressors, isotopic signatures, and nutritional subsidies to mussels can allow for more focused management and propagation efforts for these imperiled organisms.

2.6 References

Allan D., Erickson D. & Fay J. (1997). The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* **37**, 149–161. https://doi.org/10.1046/j.1365-2427.1997.d01-546.x

Allen W.R. (1921). Studies of the biology of freshwater mussels: Experimental studies of the food relations of certain Unionidae. *The Biological Bulletin* **40**, 210–241. https://doi.org/10.2307/1536464

Bowes R.E., Lafferty M.H. & Thorp J.H. (2014). Less means more: nutrient stress leads to higher δ15N ratios in fish. *Freshwater Biology* **59**, 1926–1931. https://doi.org/10.1111/fwb.12396

Boyd C.E. (2019). *Water Quality: An Introduction*. Springer Nature.

Brett M.T. (2014). Resource polygon geometry predicts Bayesian stable isotope mixing model bias. *Marine Ecology Progress Series* **514**, 1–12. https://doi.org/10.3354/meps11017

Burlakova L., Karatayev A., Froufe E., Bogan A.E. & Lopes-Lima M. (2018). A new freshwater bivalve species of the genus *Cyclonaias* from Texas (Unionidae: Ambleminae: Quadrulini). *The Nautilus* **132**, 7

Carmichael R.H., Hattenrath T., Valiela I. & Michener R.H. (2008). Nitrogen stable isotopes in the shell of Mercenaria mercenaria trace wastewater inputs from watersheds to estuarine ecosystems. *Aquatic Biology* **4**, 99–111. https://doi.org/10.3354/ab00106

Cherel Y., Hobson K.A., Bailleul F. & Groscolas R. (2005). Nutrition, Physiology, and Stable Isotopes: New Information from Fasting and Molting Penguins. *Ecology* **86**, 2881–2888. https://doi.org/10.1890/05-0562

Christian A.D., Smith B.N., Berg D.J., Smoot J.C. & Findlay R.H. (2004). Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca:Unionidae) in 2 small Ohio streams. *Journal of the North American Benthological Society* **23**, 101–113. https://doi.org/10.1899/0887-3593(2004)023<0101:tpapfs>2.0.co;2

Coker R.E., Shira A.F., Clark H.W. & Howard A.D. (1921). *Natural History and Propagation of Fresh-water Mussels*. U.S. Government Printing Office.

Cross W.F., Benstead J.P., Frost P.C. & Thomas S.A. (2005). Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology* **50**, 1895–1912. https://doi.org/10.1111/j.1365-2427.2005.01458.x

DeNiro M.J. & Epstein S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

DeNiro M.J. & Epstein S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**, 343–351. https://doi.org/10.1016/0016-7037(78)90199-0

Dudding J.F., Hart M., Khan J.M., Robertson C.R., Lopez R. & Randklev C.R. (2020). Reproductive life history of 2 imperiled and 1 widely-distributed freshwater mussel species from the southwestern United States. *Freshwater Science* **39**, 156–168. https://doi.org/10.1086/707774

Fritts A.K., Peterson J.T., Wisniewski J.M. & Bringolf R.B. (2015). Nonlethal assessment of freshwater mussel physiological response to changes in environmental factors. *Canadian Journal of Fisheries and Aquatic Sciences* **72**, 1460–1468. https://doi.org/10.1139/cjfas-2014-0565

Fritts M.W., Fritts A.K., Carleton S.A. & Bringolf R.B. (2013). Shifts in stable-isotope signatures confirm parasitic relationship of freshwater mussel glochidia attached to host fish. *Journal of Molluscan Studies* **79**, 163–167. https://doi.org/10.1093/mollus/eyt008

Frost P.C., Xenopoulos M.A. & Larson J.H. (2004). The stoichiometry of dissolved organic carbon, nitrogen, and phosphorus release by a planktonic grazer, *Daphnia*. *Limnology and Oceanography* **49**, 1802–1808. https://doi.org/10.4319/lo.2004.49.5.1802

Gatenby C., Parker B. & Neves R.J. (1997). Growth and survival of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae) reared on algal diets and sediment. *American Malacological Bulletin* **14**, 57–66

Gatenby C.M., Neves R.J. & Parker B.C. (1996). Influence of Sediment and Algal Food on Cultured Juvenile Freshwater Mussels. *Journal of the North American Benthological Society* **15**, 597–609. https://doi.org/10.2307/1467810

Goedkoop W., Åkerblom N. & Demandt M.H. (2006). Trophic fractionation of carbon and nitrogen stable isotopes in *Chironomus riparius* reared on food of aquatic and terrestrial origin. *Freshwater Biology* **51**, 878–886. https://doi.org/10.1111/j.1365-2427.2006.01539.x

Grubaugh J.W. & Anderson R.V. (1989). Upper Mississippi River: seasonal and floodplain forest influences on organic matter transport. *Hydrobiologia* **174**, 235–244. https://doi.org/10.1007/BF00008163

Haag W.R. (2012). *North American Freshwater Mussels*. Cambridge University Press, New York, NY.

Haag W.R., Culp J.J., McGregor M.A., Bringolf R. & Stoeckel J.A. (2019). Growth and survival of juvenile freshwater mussels in streams: Implications for understanding enigmatic mussel declines. *Freshwater Science*, 000–000. https://doi.org/10.1086/705919

Haney A., Abdelrahman H. & 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. https://doi.org/10.1007/s10750-019-04138-4

Harris D., Horwáth W.R. & van Kessel C. (2001). Acid fumigation of soils to remove carbonates prior to total organic carbon or CARBON-13 isotopic analysis. *Soil Science Society of America Journal* **65**, 1853–1856. https://doi.org/10.2136/sssaj2001.1853

Hornbach D.J., Way C.M., Wissingl T.E. & Burky A.J. (1984). Effects of particle concentration and season on the filtration rates of the freshwater clam, *Sphaerium striatinum* Lamarck (Bivalvia: Pisidiidae). *Hydrobiologia* **108**, 83–96

Howard J.K. & Cuffey K.M. (2006). The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biology* **51**, 460–474. https://doi.org/10.1111/j.1365-2427.2005.01507.x

Inoue K., Harris J.L., Robertson C.R., Johnson N.A. & Randklev C.R. (2019). A comprehensive approach uncovers hidden diversity in freshwater mussels (Bivalvia: Unionidae) with the description of a novel species. *Cladistics* **36**, 88–113. https://doi.org/10.1111/cla.12386

Johnson N.A., Smith C.H., Pfeiffer J.M., Randklev C.R., Williams J.D. & Austin J.D. (2018). Integrative taxonomy resolves taxonomic uncertainty for freshwater mussels being considered for protection under the U.S. Endangered Species Act. *Scientific Reports* **8**, 1–1. https://doi.org/10.1038/s41598-018-33806-z

McMahon R. & Bogan A.E. (2001). Mollusca: Bivalvia. In: *Ecology and classification of North American freshwater invertebrates*. (Eds J.H. Thorp & A.P. Covich), Academic Press, New York.

Minagawa M. & Wada E. (1984). Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. *Geochimica et Cosmochimica Acta* **48**, 1135–1140. https://doi.org/10.1016/0016-7037(84)90204-7

Newton T.J., Vaughn C.C., Spooner D.E., Nichols S.J. & Arts M.T. (2013). Profiles of Biochemical Tracers in Unionid Mussels Across a Broad Geographical Range. *Journal of Shellfish Research* **32**, 497–507. https://doi.org/10.2983/035.032.0229

Nichols S.J. & Garling D. (2000). Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. *Canadian Journal of Zoology* **78**, 871–882. https://doi.org/10.1139/z99-256

Nichols S.J., Silverman H., Dietz T.H., Lynn J.W. & Garling D.L. (2005). Pathways of food uptake in native (Unionidae) and introduced (Corbiculidae and Dreissenidae) freshwater bivalves. *Journal of Great Lakes Research* **31**, 87–96. https://doi.org/10.1016/S0380-1330(05)70240-9

Patterson H.K. & Carmichael R.H. (2018). Dissolved oxygen concentration affects δ15N values in oyster tissues: implications for stable isotope ecology. *Ecosphere* **9**, e02154. https://doi.org/10.1002/ecs2.2154

Peipoch M., Martí E. & Gacia E. (2012). Variability in d15N natural abundance of basal resources in fluvial ecosystems: A meta-analysis. *Freshwater Science* **31**, 1003–1015. https://doi.org/10.1899/11-157.1

Phillips D.L., Inger R., Bearhop S., Jackson A.L., Moore J.W., Parnell A.C., *et al.* (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* **92**, 823–835. https://doi.org/10.1139/cjz-2014-0127

Post D.M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **83**, 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post D.M., Layman C.A., Arrington D.A., Takimoto G., Quattrochi J. & Montaña C.G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* **152**, 179–189. https://doi.org/10.1007/s00442-006-0630-x

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Raikow D.F. & Hamilton S.K. (2001). Bivalve diets in a midwestern U.S. stream: A stable isotope enrichment study. *Limnology and Oceanography* **46**, 514–522. https://doi.org/10.4319/lo.2001.46.3.0514

Rio C.M. del, Wolf N., Carleton S.A. & Gannes L.Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* **84**, 91–111. https://doi.org/10.1111/j.1469-185X.2008.00064.x

SAS (2013). SAS Institute Inc., Cary, North Carolina, USA.

Seagroves L.A., Barnhart M.C., Hardy T. & Schwalb A.N. (2019). Reproductive ecology of the threatened and endemic freshwater mussel *Lampsilis bracteata*. *Aquatic Conservation: Marine and Freshwater Ecosystems* **29**, 1216–1226. https://doi.org/10.1002/aqc.3154

Semmens B.X., Ward E.J., Moore J.W. & Darimont C.T. (2009). Quantifying inter-and intra-population niche variability using hierarchical bayesian stable isotope mixing models. *PLoS ONE* **4**, 1–9. https://doi.org/10.1371/journal.pone.0006187

Sheath R.G. & Burkholder J.M. (1985). Characteristics of softwater streams in Rhode Island II. Composition and seasonal dynamics of macroalgal communities. *Hydrobiologia* **128**, 109–118. https://doi.org/10.1007/BF00008730

Smith J.A., Mazumder D., Suthers I.M. & Taylor M.D. (2013). To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. *Methods in Ecology and Evolution* **4**, 612–618. https://doi.org/10.1111/2041-210X.12048

Spooner D.E. & Vaughn C.C. (2008). A trait-based approach to species' roles in stream ecosystems: climate change, community structure, and material cycling. *Oecologia* **158**, 307–317. https://doi.org/10.1007/s00442-008-1132-9

Spooner D.E. & Vaughn C.C. (2006). Context-dependent effects of freshwater mussels on stream benthic communities. *Freshwater Biology* **51**, 1016–1024. https://doi.org/10.1111/j.1365-2427.2006.01547.x

Sterner R.W. & Elser J.J. (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press.

Stock B.C., Jackson A.L., Ward E.J., Parnell A.C., Phillips D.L. & Semmens B.X. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* **2018**, 1–27. https://doi.org/10.7717/peerj.5096

Stock B.C. & Semmens B.X. (2016). MixSIAR GUI User Manual. *Version 3.1*, 1–42. https://doi.org/10.5281/zenodo.47719

Strayer D. (2008). *Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance*. University of California Press.

Strayer D.L., Geist J., Haag W.R., Jackson J.K. & Newbold J.D. (2019). Essay: Making the most of recent advances in freshwater mussel propagation and restoration. *Conservation Science and Practice* **1**, e53. https://doi.org/10.1111/csp2.53

Tai Tue N., Hamaoka H., Sogabe A., Quy T.D., Nhuan M.T. & Omori K. (2012). Food sources of macro-invertebrates in an important mangrove ecosystem of Vietnam determined by dual stable isotope signatures. *Journal of Sea Research* **72**, 14–21. https://doi.org/10.1016/j.seares.2012.05.006

Trochine C., Díaz Villanueva V., Balseiro E. & Modenutti B. (2019). Nutritional stress by means of high C:N ratios in the diet and starvation affects nitrogen isotope ratios and trophic fractionation of omnivorous copepods. *Oecologia* **190**, 547–557. https://doi.org/10.1007/s00442-019-04438-5

Vaughn C.C., Gido K.B. & Spooner D.E. (2004). Ecosystem Processes Performed by Unionid Mussels in Stream Mesocosms: Species Roles and Effects of Abundance. *Hydrobiologia* **527**, 35–47. https://doi.org/10.1023/B:HYDR.0000043180.30420.00

Vaughn C.C., Nichols S.J. & Spooner D.E. (2008). Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society* **27**, 409–423. https://doi.org/10.1899/07-058.1

Weber A.M., Bauer J.E. & Thomas Watters G. (2017). Assessment of nutritional subsidies to freshwater mussels using a multiple natural abundance isotope approach. *Freshwater Biology* **62**, 615–629. https://doi.org/10.1111/fwb.12890

Webster J.R. & Benfield E.F. (1986). Vascular plant breakdown in freshwater ecosystems. *Annual Review or Ecology and Systematics* **17**, 567–594. https://doi.org/10.1146/annurev.es.17.110186.003031

Williams J.D., Warren M.L., Cummings K.S., Harris J.L. & Neves R.J. (1993). Conservation Status of Freshwater Mussels of the United States and Canada. *Fisheries* **18**, 6–22. https://doi.org/10.1577/1548-8446(1993)018<0006:CSOFMO>2.0.CO;2

Yasuno N., Shindo K., Takagi Y., Kanaya G., Shikano S., Fujimoto Y., *et al.* (2014). Ontogenetic changes in the trophic position of a freshwater Unionidae mussel. *Fundamental and Applied Limnology* **184**, 341–349

Yeager M.M., Cherry D.S. & Neves R.J. (1994). Feeding and Burrowing Behaviors of Juvenile Rainbow Mussels, *Villosa iris* (Bivalvia: Unionidae) *Journal of the North American Benthological Society* **13**, 217–222

2.7 Tables

Table 1 Taxonomy [current taxonomic status and initial taxonomic status with the original study design; (Burlakova *et al.*, 2018; Johnson *et al.*, 2018; Inoue *et al.*, 2019)], collection sites, and decimal degrees (DD) coordinates. Size range (mm) and sample size (*n*) for each species × site combination of focal taxa is listed along with size at onset of maturity for each species. Size at onset of maturity estimates was extracted from (Dudding *et al.*, 2020)[1] and (Seagroves *et al.*, 2019)[2]. For size at maturity estimates denoted with an *, information was unavailable for these species, so size was adapted from *Cyclonaias pustulosa* for *C. petrina* and *Lampsilis bracteata* for *L. bergmanni*.

Initial species	**Current taxonomic status**	**Collection site**	**Latitude Longitude (DD)**	**Range of shell length (mm; *n*)**	**Size at onset of maturity**
Cyclonaias petrina	*C. petrina*	Colorado River	29.556197 -96.402160	46.3 – 89.7 (23)	33 mm*
	C. petrina	Llano River	30.39267 -99.19214	33.0 – 56.0 (9)	33 mm*
	C. necki	Guadalupe River	29.93953 -98.94846	36.9 – 59.8 (21)	36 mm[1]
C. houstonensis	*C. pustulosa*	Colorado River	29.556197 -96.402160	35.5 – 59.7 (24)	33 mm[1]
	C. pustulosa	Navasota River	31.15155 -96.19501	34.0 – 54.0 (32)	33 mm[1]
Lampsilis bracteata	*L. bracteata*	Llano River	30.39267 -99.19214	25.0 – 60.7 (31)	30 mm[2]
	L. bergmanni	Guadalupe River	29.93953 -98.94846	42.8 – 60.0 (26)	30 mm*

Table 2 MixSIAR model parameter estimates used for model selection criteria. Optimum Gelman-Rubin diagnostics have the greatest proportion of variables below the R cutoff <1.05 (~100%) and optimum Geweke diagnostics should have all parameters <5% ± 1.96. Gelman-Rubin and Geweke diagnostics indicate whether a model has converged. Lowest deviance information criterion (DIC) score indicates the best fitting model. The best DIC score, and thus best-fitting model used in this study, is denoted with an *.

Model ID	**Model**	**N**	**Gelman Rubin Diagnostic**		**Geweke Diagnostic**		**DIC score**
			n	**%**	**n**	**%**	
1	Null Model	156	156	100	2	1.28	486.31
2	Species	181	181	100	6	3.49	396.12
3	Site	176	176	100	11	6.44	188.89
4	**Species + Site**	176	176	100	8	4.74	119.52*

Table 3 Seasonal water temperature, pH, conductivity, and discharge at study collection sites in spring, summer, and fall (2017) during mussel and food source sampling.

Site	Strahler stream order	Season	Water temperature (°C)	pH	Conductivity (μS/cm)	Discharge (m^3/s)
Guadalupe River	5th	Spring 2017	22.6	8.3	471	3.54
		Summer 2017	26.5	8.4	497	1.56
		Fall 2017	16.0	8.7	510	1.59
Navasota River	5th	Spring 2017	21.7	7.5	340	1.10
		Summer 2017	27.7	7.9	283	0.65
		Fall 2017	12.7	8.3	248	0.31
Llano River	6th	Spring 2017	24.1	7.9	329	3.91
		Summer 2017	31.5	8.7	354	2.51
		Fall 2017	19.7	8.5	388	1.70
Colorado River	7th	Spring 2017	24.5	8.5	500	37.10
		Summer 2017	32.7	9.2	584	34.69
		Fall 2017	20.6	8.4	765	34.83

Table 4 Three-way analysis of covariance (ANCOVA) results for freshwater mussel $\delta^{13}C$, $\delta^{15}N$ with species, season, and sites as main effects and interactions. Three-way analysis of variance (ANOVA) results for freshwater mussel C:N ratios with species and site as effects and two-way interactions. Mean estimates and standard error (*SE*) are presented for all categorical effects analyzed for $\delta^{13}C$, $\delta^{15}N$, and C:N. Statistical differences in mean $\delta^{13}C$, $\delta^{15}N$ or C:N ratio are denoted with different letters (Tukey's HSD) at $p < 0.05$ if (**bold**).

Effect	Variables	$\delta^{13}C$		$\delta^{15}N$		C:N ratio	
		Mean (‰) ± *SE*	$F_{(df1,df2)}$ *p*	Mean (‰) ± *SE*	$F_{(df1,df2)}$ *p*	Mean ± *SE*	$F_{(df1,df2)}$ *p*
Species	***C. necki***	-28.22 ± 0.11^{c}	$14.8_{(4,151)}$ **< 0.0001**	11.05 ± 0.06^{b}	$165.61_{(4,151)}$ **< 0.0001**	3.74 ± 0.03	$2.06_{(4,151)}$ 0.089
	C. petrina	-25.98 ± 0.64^{b}		11.32 ± 0.55^{b}		5.83 ± 0.60	
	C. pustulosa	-25.11± 1.14^{bc}		12.30 ± 0.10^{a}		6.96 ± 1.08	
	L. bergmanni	-24.49 ± 1.14^{bc}		10.92 ± 0.11^{b}		7.53 ± 1.21	
	L. bracteata	-23.16 ± 1.11^{a}		7.29 ± 0.07^{c}		6.50 ± 1.13	
Season	**Fall**	-27.09 ± 0.23^{b}	$86.85_{(2,151)}$ **< 0.0001**	9.71 ± 0.36^{b}	$22.98_{(2,151)}$ **< 0.0001**	3.70 ± 0.03^{c}	$45.48_{(2,151)}$ **< 0.0001**
	Spring	-28.21 ± 0.16^{c}		11.23 ± 0.30^{a}		3.85 ± 0.03^{b}	
	Summer	-21.22 ± 1.07^{a}		11.19 ± 0.25^{a}		10.41 ± 1.06^{a}	
Site	**Guadalupe River (GR)**	-26.16 ± 0.69^{b}	$5.78_{(4,155)}$ **0.0002**	10.98 ± 0.07^{c}	$202.43_{(4,155)}$ **< 0.0001**	5.84 ± 0.72^{b}	$44.78_{(4,159)}$ **< 0.0001**
	Colorado River (CR)	-26.31 ± 0.62^{c}		12.99 ± 0.13^{a}		6.52 ± 0.55^{a}	
	Llano River (LR)	-23.71 ± 0.87^{a}		7.12 ± 0.08^{d}		5.86 ± 0.89^{b}	
	Navasota River (NR)	-24.08 ± 1.79^{ab}		11.92 ± 0.09^{b}		7.41 ± 1.80^{ab}	
Species × Site	***C. necki* × GR**	-28.22 ± 0.11^{cd}	$4.84_{(3,155)}$ **0.0008**	11.05 ± 0.06^{c}	$5.2_{(3,155)}$ **0.0019**	3.74 ± 0.03	$2.41_{(3,159)}$ 0.069
	***C. petrina* × CR**	-26.12 ± 0.90^{d}		13.19 ± 0.19^{ab}		6.67 ± 0.77	
	***C. petrina* × LR**	-25.61 ± 0.07^{abc}		6.54 ± 0.06^{e}		3.66 ± 0.06	
	***C. pustulosa* × CR**	-26.49 ± 0.88^{cd}		12.81 ± 0.16^{a}		7.41 ± 1.80	
	***C. pustulosa* × NR**	-24.08 ± 1.79^{abc}		11.92 ± 0.09^{b}		6.37 ± 0.80	
	***L. bergmanni* × GR**	-24.49 ± 1.^{14ab}		10.92 ± 0.11^{c}		7.53 ± 1.21	
	***L. bracteata* × CR**	-23.16 ± 1.11^{a}		7.29 ± 0.07^{d}		6.50 ± 1.13	
Species × Season	***C. necki* × Fall**	-27.83± 0.16^{bef}	$5.19_{(8,151)}$ **<0.0001**	11.14 ± 0.07^{bc}	$26.92_{(8,151)}$ **< 0.0001**	3.79 ± 0.03^{ace}	$5.1_{(8,151)}$ **< 0.0001**
	***C. necki* × Spring**	-28.76 ± 0.18^{ef}		11.15 ± 0.17^{bc}		3.81 ± 0.06^{ce}	
	***C. necki* × Summer**	-28.00 ± 0.08^{de}		10.96 ± 0.05^{c}		3.67 ± 0.04^{de}	
	***C. petrina* × Fall**	-26.63 ± 0.54^{cde}		7.86 ± 0.70^{e}		3.75 ± 0.07^{de}	
	***C. petrina* × Spring**	-29.32 ± 0.11^{f}		13.41 ± 0.14^{a}		3.90 ± 0.06^{bcd}	
	***C. petrina* × Summer**	-21.86 ± 0.97^{ab}		13.38 ± 0.29^{a}		10.24 ± 0.93^{ab}	
	***C. pustulosa* × Fall**	-28.13 ± 0.25^{def}		11.91 ± 0.19^{bc}		3.72 ± 0.06^{de}	
	***C. pustulosa* × Spring**	-28.56 ± 0.17^{ef}		12.40 ± 0.20^{ad}		3.87 ± 0.05^{de}	
	***C. pustulosa* × Summer**	-19.22 ± 2.61^{a}		12.46 ± 0.12^{ab}		12.64 ± 2.60^{a}	
	***L. bergmanni* × Fall**	-27.83 ± 0.15^{cde}		11.12 ± 0.11^{cd}		3.56 ± 0.06^{e}	
	***L. bergmanni* × Spring**	-28.41 ± 0.24^{def}		11.06 ± 0.12^{bc}		3.90 ± 0.06^{ace}	
	***L. bergmanni* × Summer**	-20.88 ± 1.80^{ab}		10.75 ± 0.19^{c}		11.34 ± 1.91^{ac}	
	***L. bracteata* × Fall**	-25.26 ± 0.13^{abc}		7.18 ± 0.11^{e}		3.69 ± 0.06^{de}	
	***L. bracteata* × Spring**	-26.22 ± 0.11^{bcd}		7.38 ± 0.16^{e}		3.77 ± 0.07^{de}	
	***L. bracteata* × Summer**	-17.60 ± 2.75^{a}		7.28 ± 0.08^{e}		12.29 ± 2.77^{a}	

2.8 Figures

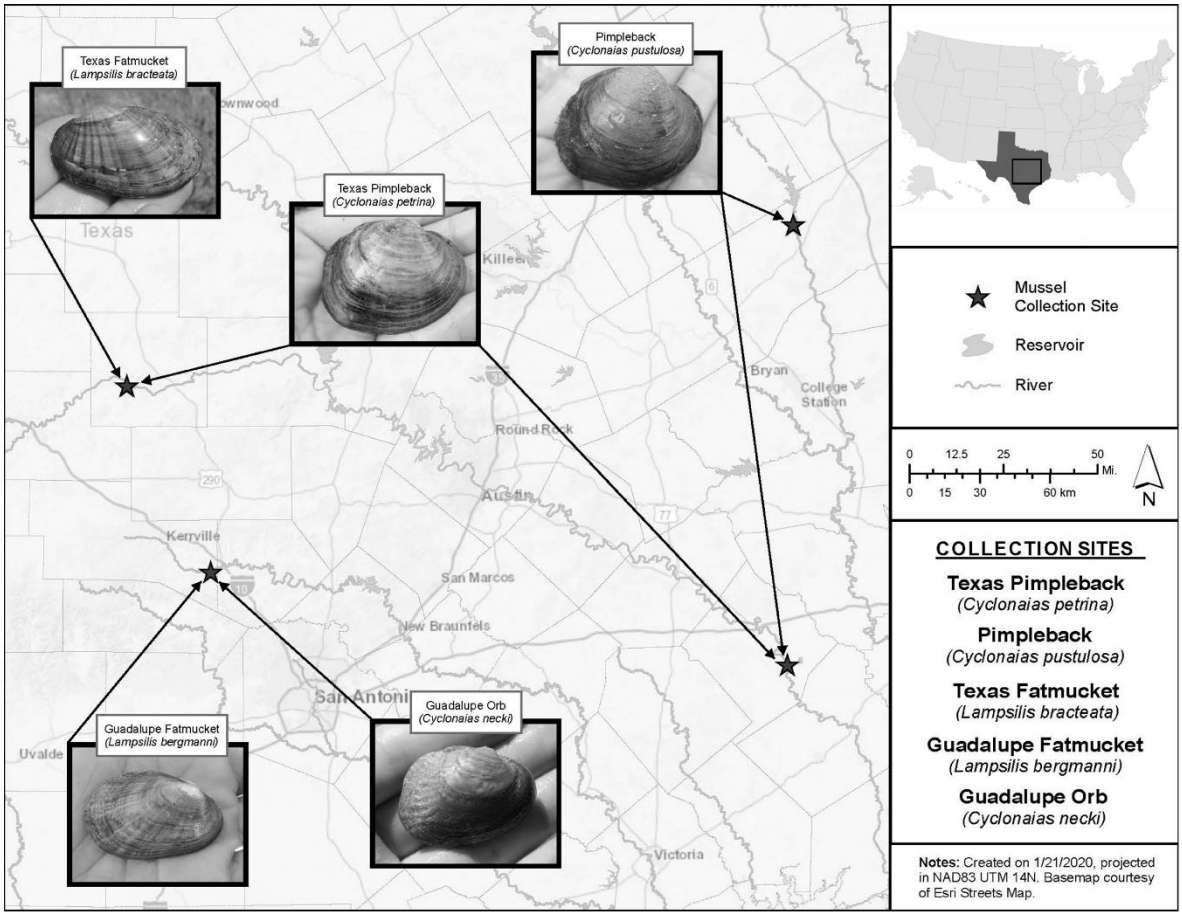

Figure 1 Map of Texas, USA with focal taxa, study sites, and river drainages.

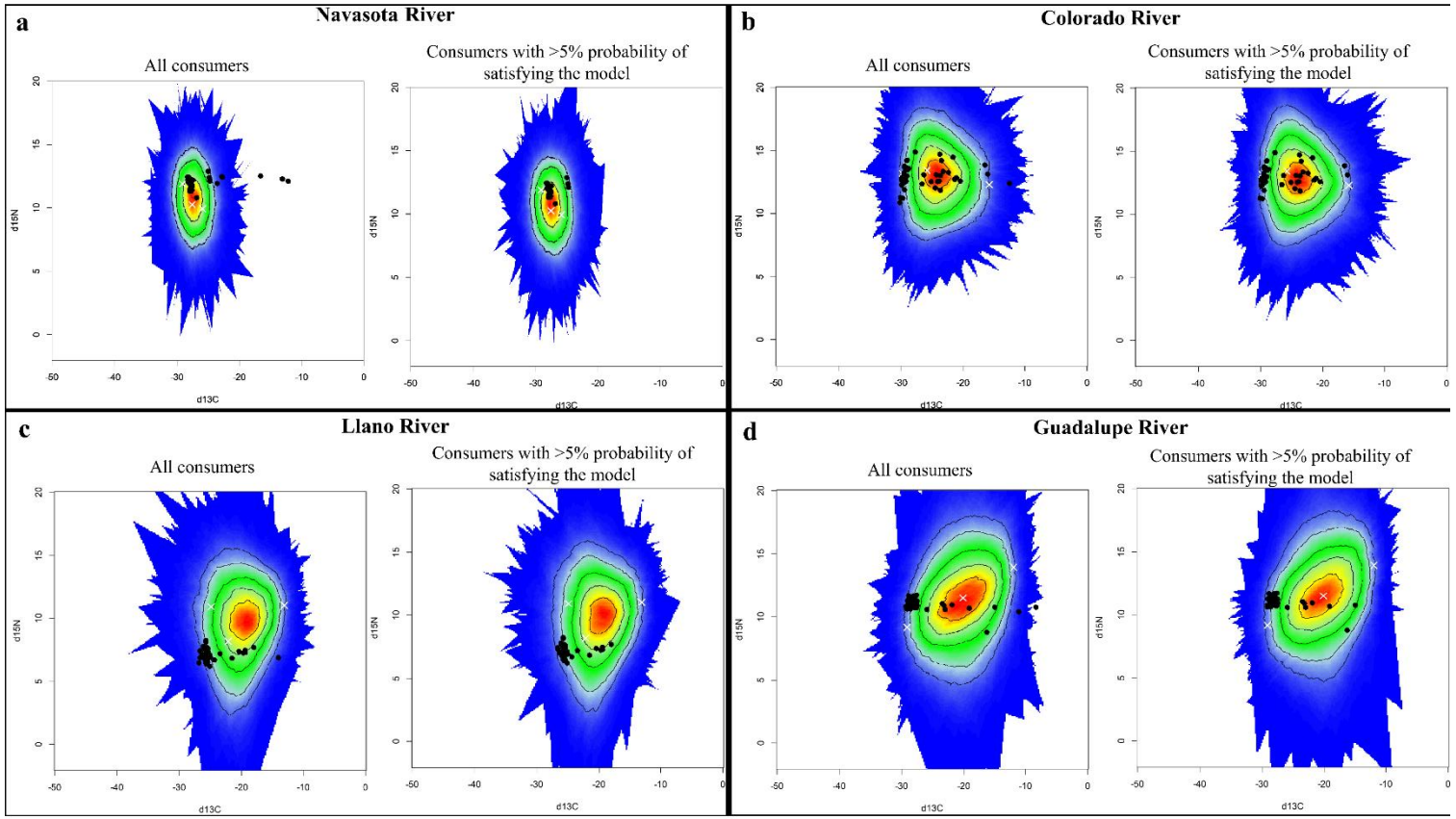

Figure 2 Simulated mixing regions for the Navasota River (a), Colorado River (b), Llano River (c), and Guadalupe River (d) with "all consumers" or only consumers within 95% of the mixing region (the outermost, dark blue, contour). The position of unionid consumers (black dots) and the average sources signatures (white crosses) are shown for each site. Contours represent probabilities within the mixing region at the 5% outermost contour and every 10% interval. For each site's "all consumers" polygon, mussels that lie outside the 95% mixing region were excluded from the "consumers with >5% probability of satisfying the model" mixing polygon. Consumers outside the 95% mixing region (outside the contours) were not used in this MixSIAR model as they need an alternative model to explain their isotopic signatures.

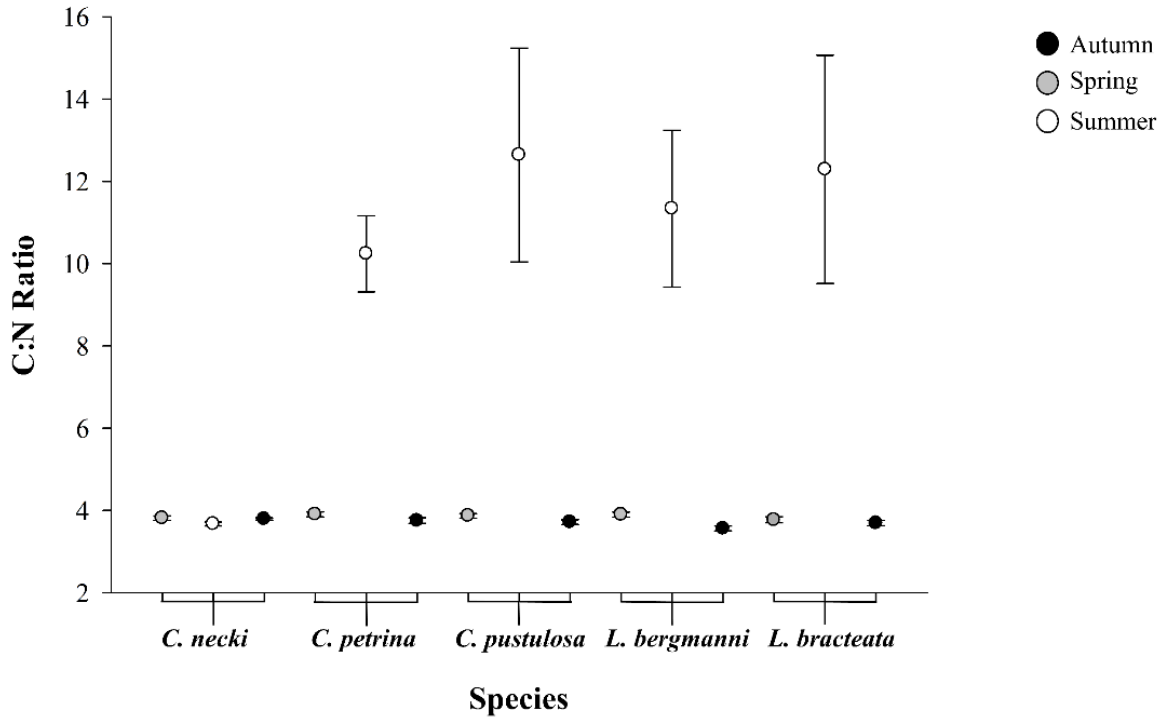

Figure 3 Carbon:nitrogen ratios for tissues of *Cyclonaias necki, C. petrina, C. pustulosa, Lampsilis bergmanni*, and *L. bracteata* across autumn (October), spring (April), and summer (July) seasons in 2017. Each point is the average for a species within a season and error bars represent the associated standard errors.

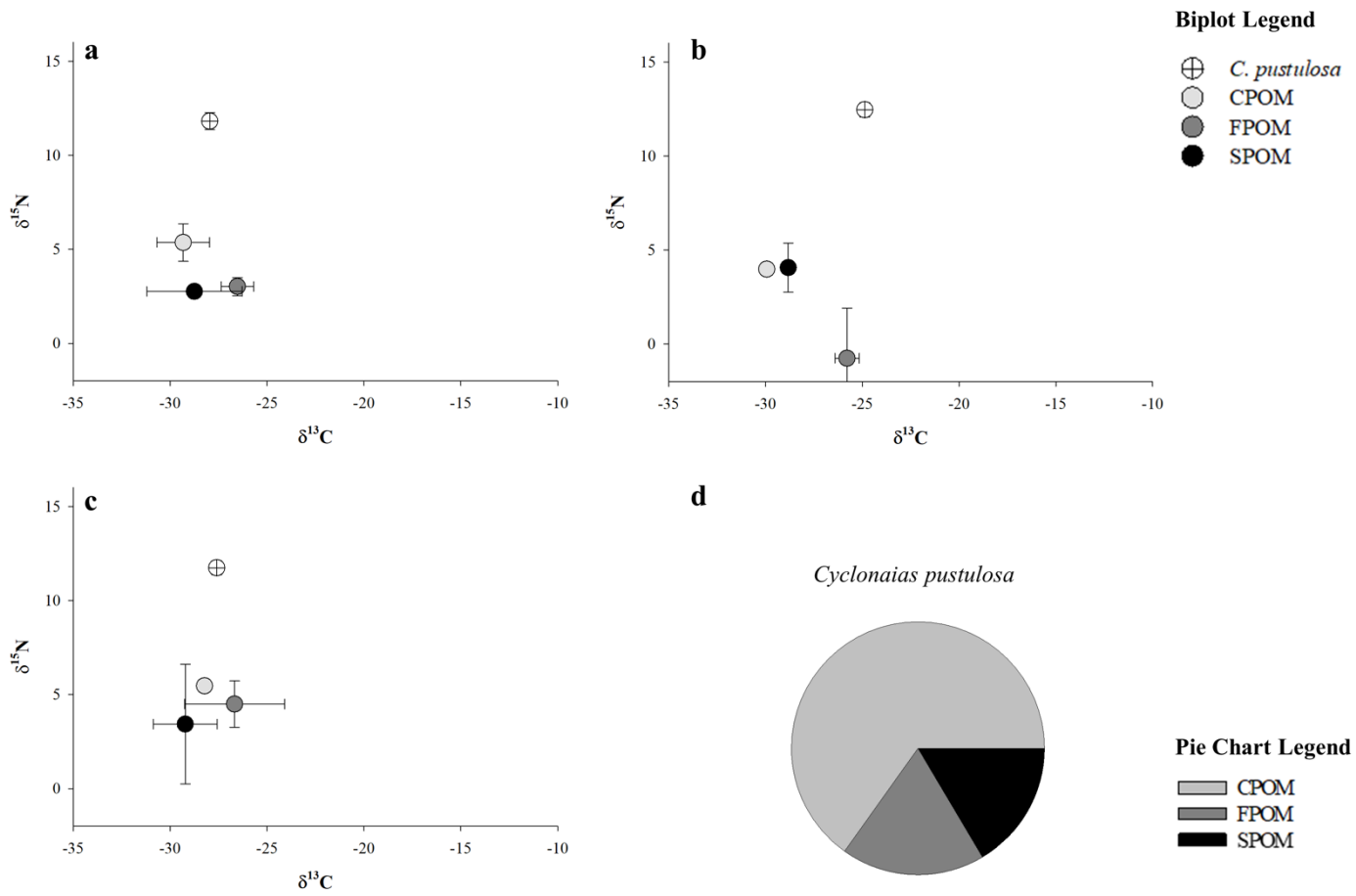

Figure 4 Stable CN isotope biplots for tissue of *Cyclonaias pustulosa* and food sources [coarse particulate organic matter (CPOM; benthic detritus), fine particulate organic matter from benthic sediments (FPOM), suspended particulate organic matter (SPOM)] at the Navasota River site across (a) spring (April), (b) summer (July), and (c) autumn (October) seasons in 2017. Each point is an average within a season and error bars represent the associated standard deviations. Annual estimated dietary sources for (d) *C. pustulosa* are represented with a pie chart. This chart reflects mean estimate values of potential carbon and nitrogen contribution to mussel tissue composition determined using Bayesian mixing models in MixSIAR.

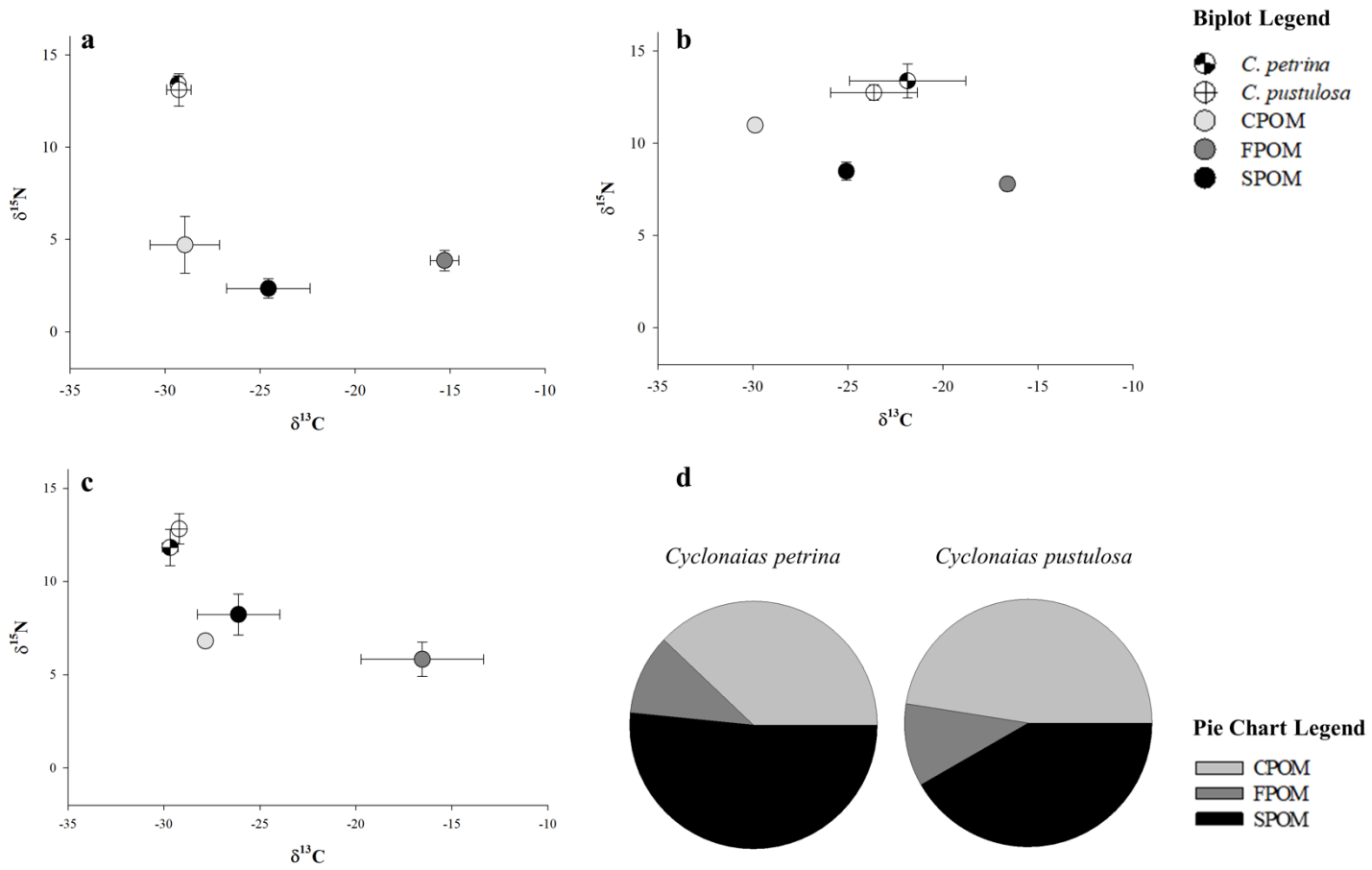

Figure 5 Stable CN isotope biplots for tissue of *Cyclonaias pustulosa* and *C. petrina*; and food sources [coarse particulate organic matter (CPOM; benthic detritus), fine particulate organic matter from benthic sediments (FPOM), suspended particulate organic matter (SPOM)] within the Colorado River site across (a) spring (April), (b) summer (July), and (c) autumn (October) seasons in 2017. Each point is an average within that season and error bars represent the associated standard deviations. Annual estimated dietary sources for (d) *C. pustulosa* and *C. petrina* are represented with pie charts. Charts reflect mean estimate values of potential carbon and nitrogen contribution to mussel tissue composition determined using Bayesian mixing models in MixSIAR.

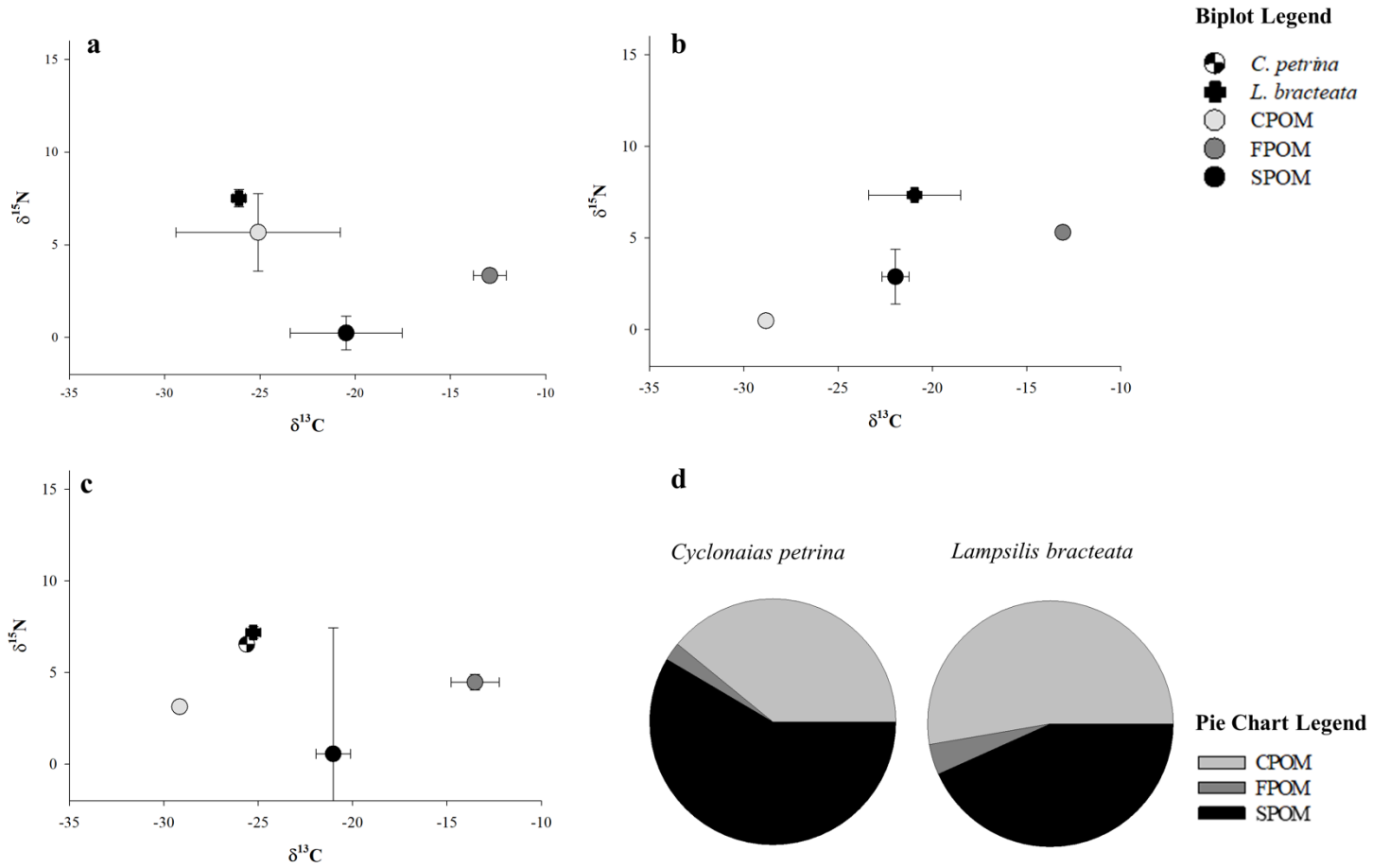

Figure 6 Stable CN isotope biplots for tissue of *Lampsilis bracteata* and *Cyclonaias petrina*; and food sources [coarse particulate organic matter (CPOM; benthic detritus), fine particulate organic matter from benthic sediments (FPOM), suspended particulate organic matter (SPOM)] at the Llano River site across (a) spring (April), (b) summer (July), and (c) autumn (October) seasons in 2017. Each point is an average within that season and error bars represent the associated standard deviations. Annual estimated dietary sources for (d) *L. bracteata* and *C. petrina* are represented with pie charts. Charts reflect mean estimate values of potential carbon and nitrogen contribution to mussel tissue composition determined using Bayesian mixing models in MixSIAR.

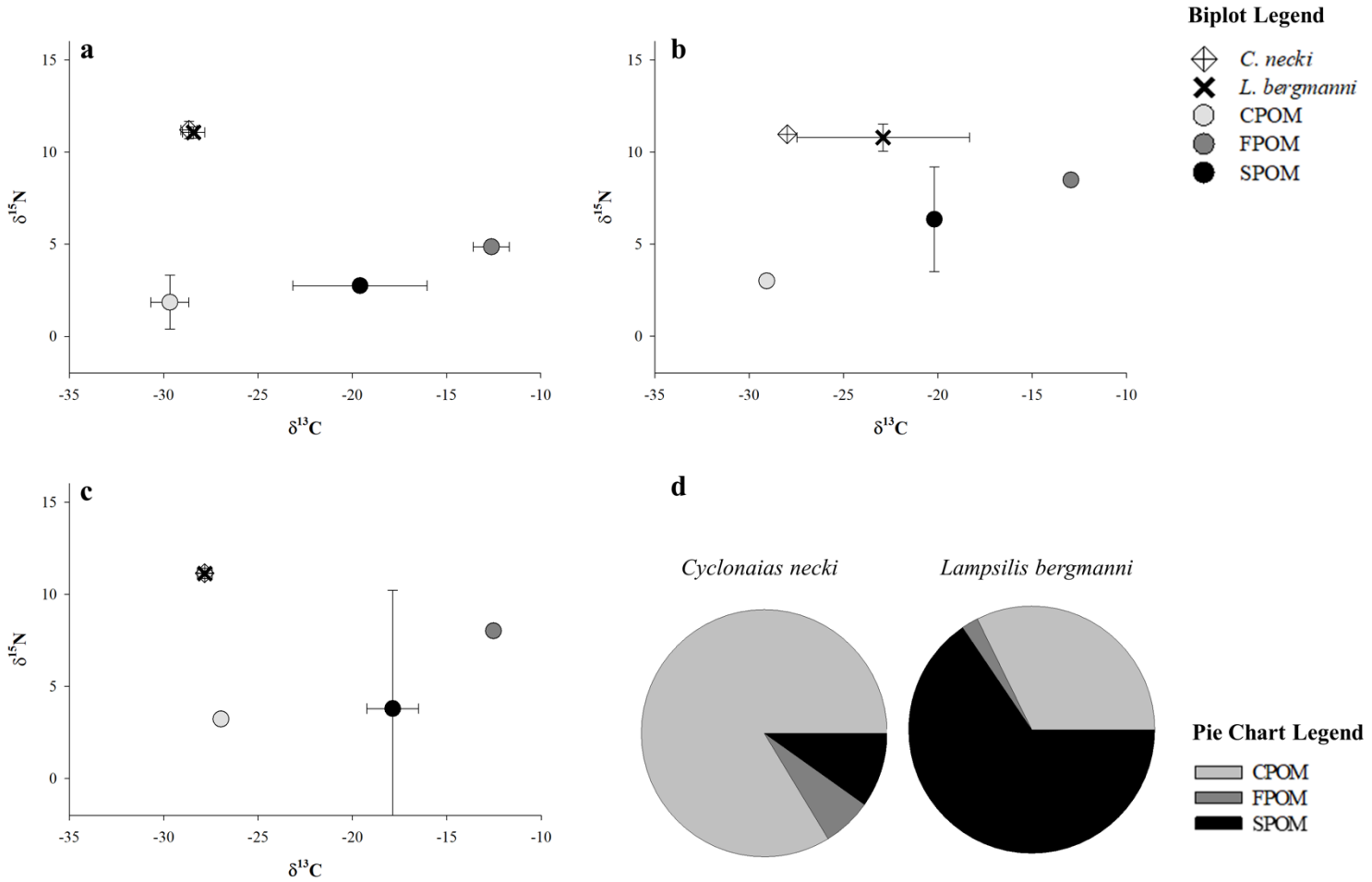

Figure 7 Stable CN isotope biplots for tissue of *Cyclonaias necki* and *Lampsilis bergmanni*; and food sources [coarse particulate organic matter (CPOM; benthic detritus), fine particulate organic matter from benthic sediments (FPOM), suspended particulate organic matter (SPOM)] at the Guadalupe River site across (a) spring (April), (b) summer (July), (c) and autumn (October) seasons in 2017. Each point is an average within a season and error bars represent the associated standard deviations. Annual estimated dietary sources for (d) *C. necki* and *L. bergmanni* are represented with pie charts. Charts reflect mean estimate values of potential carbon and nitrogen contribution to mussel tissue composition determined using Bayesian mixing models in MixSIAR.

CHAPTER 3
FEEDING ECOLOGY OF THREE FRESHWATER MUSSEL SPECIES (FAMILY: UNIONIDAE) IN A NORTH AMERICAN LENTIC SYSTEM

Manuscript in review in *Hydrobiologia*

3.1 Abstract

Freshwater mussels are typically considered to be primarily filter-feeders with the ability to pedal feed, however, there is limited detailed information regarding interspecific differences in food resources and feeding modes. The objective of this study was to investigate interspecific variation in food resource usage among a mussel assemblage in a lentic system associated with a reservoir drawdown. We quantified stable Carbon ($\delta^{13}C$) and stable Nitrogen ($\delta^{15}N$) isotopic signatures for three mussel species (*Elliptio pullata*, *Utterbackiana hartfieldorum*, and federally-listed *Fusconaia escambia*) and their potential food resources in Gantt Lake, a reservoir on the Conecuh River in southeastern Alabama, USA. For all species, Bayesian mixing models suggested C derived from limnetic, benthic fine particulate organic matter (FPOM) contributed on average 99% to the mussel diet. Carbon associated with littoral FPOM, limnetic and littoral suspended particulate organic matter (SPOM) and coarse particulate organic matter (CPOM; bulk detrital leaf packs) contributed <1%. Mussel isotopic signatures had minimal variation across species. *Elliptio pullata* that were collected live but emersed for eight weeks after the reservoir drawdown event were nitrogen-enriched compared to *E. pullata* that had been collected immediately during the drawdown event, which provides evidence of catabolism during emersion. All species were presumably consuming microbial and algae communities within FPOM pools. These data support the hypothesis that benthic sources can be a dominant food resource for unionids and that stranded unionids rely on internal energy stores to survive emersion.

3.2 Introduction

Freshwater mussels (Unionidae) can consume a wide range of food resources including live algae, bacteria, and particulate organic material. Individual constituents of mussel diets have been identified through several methodological approaches such as algae, rotifers, protozoans, detritus, bacteria, and dissolved organic carbon (Strayer, 2008; Vaughn et al., 2008; Haag, 2012). In both river and lake habitats, diatoms (Bacillariophyceae) and green algae (Chlorophyta) can be found in high concentrations in the mantle cavity and gut of mussels, suggesting preferential feeding of these dietary items (Nichols & Garling, 2000). Despite the positive selection for planktonic algae, bacteria are often of equal or greater importance to mussel diets in both riverine and lake systems (Nichols & Garling, 2000; Christian et al., 2004; Newton et al., 2013). Bacteria are found in seston but also dominate microbial communities associated with benthic detrital pathways (Krumins et al., 2013; Boyd, 2019).

Although typically described as suspension feeders, unionids are capable of exploiting benthic-derived food resources (Raikow & Hamilton, 2001; Nichols et al., 2005) and some studies have shown that both juvenile and adult unionids derive more than 50% of their diet from benthic sources (Yeager et al., 1994; Gatenby et al., 1996, 1997; Fogelman et al., 2022). The positive relationship observed between juvenile and adult *Elliptio complanata* growth and sediment organic content in lentic systems (Cyr, 2020) suggests that mussels can utilize benthic food sources when suspended sources are limited. Further, sympatric mussels are capable of partitioning food resources, likely via differences in gill morphology (Galbraith et al., 2009). Thus, evaluating the diets of multiple mussel species while considering a combination of potential food items could elucidate mechanisms driving rarity, allowing for sympatry, or other distributional phenomena.

It is difficult to evaluate the diets of freshwater mussels. Particles uptaken through filtration may be rejected as psuedofeces prior to ingestion, and particles ingested may pass through the digestive tract without being assimilated (Vaughn et al., 2008; Haag, 2012). Carbon and nitrogen stable isotopes and stoichiometric ratios can provide useful insight into aspects of primary food sources, trophic position, food quality, and stress of unionid consumers (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Christian et al., 2004; Newton et al., 2013; Weber et al., 2017; Fogelman et al., 2022). Typically dietary constituents are inferred via carbon (C) stable isotope signatures ($^{13}C/^{12}C$, or $\delta^{13}C$) and trophic position is inferred via nitrogen (N) isotopes ($^{15}N/^{14}N$, or $\delta\,^{15}N$; DeNiro & Epstein, 1978, 1981).

In addition to elucidating trophic position, nitrogen isotope ratios can be used to detect food limitations or stress in consumers. Nitrogen isotopic enrichment above one trophic level (i.e., >3.4‰) can be caused by "self" trophic fractionation due to catabolism of tissues that leads to metabolic retention of $\delta^{15}N$ in the organism (Cherel et al., 2005). This enrichment has been shown in unionids, oysters, and fish and is indicative of nutrient and environmental stress (Bowes et al., 2014; Patterson & Carmichael, 2018). There is also evidence that bivalve isotopic signatures can change with shell size, indicating varying trophic niches or food resources at different size ranges (Tai Tue et al., 2012, Yasuno et al., 2014), but there is limited consensus on the mechanisms behind these relationships. Additionally, consumer and food source C:N ratios can provide insight into food quality and origins (Sterner & Elser 2002, Cross et al., 2005, Trochine et al., 2019). Food resources with low C:N ratios are of higher quality than resources with high C:N ratios (Trochine et al., 2019). Food source stoichiometry can also indicate origins of dietary items as C:N ratios can differ between allochthonous and autochthonous or between benthic and suspended resource pools (Cross et al., 2005).

In this study, we compare the extent to which adults of three mussel species assimilate suspended versus benthic food resources in a lentic system, and whether usage patterns vary across species and sizes. Our specific objectives were to: (1) determine whether primary diet components vary across species, (2) compare the relative importance of benthic and suspended carbon sources to gain insight into feeding mechanisms (deposit versus suspension feeding), (3) evaluate relationships between isotopic signatures and body sizes, and (4) compare isotopic signatures between recently-immersed and emersed (8 weeks) individuals.

3.3 Methods

3.3.1 Study site and species

Gantt Lake is a reservoir impounded in 1922 on the Conecuh River in southeast Alabama, USA (Smillie, 1927; Williams et al., 2009). Gantt Lake has a surface area of 1,668 km^2 and the substrate associated with mussel habitat is a mixture of sand and clay and organic detritus (Williams et al., 2009; Miller et al., 2021). Three mussel species were sampled from Gantt Lake for isotope analysis during a planned drawdown for dam maintenance. *Fusconaia escambia* (Clench & Turner, 1956) is a federally threatened species (United States Fish and Wildlife Service, 2010) restricted to the Conecuh/Escambia and Yellow River drainages in Alabama and Florida. *Utterbackiana hartfieldorum* (Williams et al., 2009) is a less common species with a similar distribution in the southeastern United States, including Mississippi, Alabama, Florida, and Georgia. In Alabama, *U. hartfieldorum* is listed as critically imperiled but has an apparently secure national status (NatureServe, 2021). *Elliptio pullata* (Lea, 1856) is a common species found in the southeast United States, including Alabama, Florida, and Georgia.

3.3.2 Mussel Sampling

All three species were sampled from Gantt Lake in September 2019 during a planned drawdown event for maintenance of the reservoir's hydroelectric dam. Twelve individuals per species were collected from exposed substrates within 1–2 d of emersion at approximately 2 m pre-drawdown depth (Miller et al., 2021). Length (posterior to anterior margin of shell; mm), height (dorsal to ventral margin of shell; mm), and total wet mass (shell plus soft tissue; g) were measured for each individual. To obtain tissues for stable isotope analyses, mussels were opened using a pair of flat-tipped, reverse-action pliers and one to two sublethal tissue samples were taken from the foot tissue (nasal biopsy tool #453733, Karl Storz, Tuttlingen, Germany). *Elliptio pullata* and *U. hartfieldorum* were frozen, preserved, and deposited in the Invertebrate Collection at Troy University, Alabama, USA. *Fusconaia escambia* were allowed to recover on-site and then translocated as a condition of a biological opinion (BO# 2016-F-0576) between the Federal Energy Regulatory Commission, PowerSouth Energy Cooperative (federal licensee), and U.S. Fish and Wildlife Service (USFWS). Tissue samples were dried at 80°C to a constant mass, ground using a mortar and pestle, weighed (nearest 10^{-5} g), and placed in a 4 mm x 5 mm tin capsule (Costech Analytical, Valencia, CA, USA). In November 2019, 12 additional live *E. pullata* were recovered (Miller et al., 2021) that had been emersed but partially buried in exposed lakebed substrates for the previous 8 weeks since the initial Gantt Lake drawdown event. All 8-week emersed *E. pullata* were processed for stable isotopes in the same manner as initial, recently-immersed mussels. For the purposes of this study, we refer to the mussels initially collected within 48 h of stranding as "recently-immersed" as ≤ 48 h is likely not enough time to substantially change isotopic signatures from the immersed state. We refer to those collected 8 weeks after stranding as "emersed".

3.3.3 Potential food resource and water quality sampling

Hypothesized food sources [suspended particulate organic matter (SPOM), fine particulate organic matter (FPOM), and coarse particulate organic matter (CPOM; benthic detritus)] were sampled 1 week prior to the beginning of lake drawdown and sampling of mussels. Water samples for SPOM were collected from the limnetic and littoral zones one week before the drawdown. Samples taken from the littoral zone were collected <15 m from shore and samples taken from the limnetic zone were taken nearer the channel approximately 100 m from shore. Seven to eight, 1-L water column samples were collected for littoral samples (<1 m subsurface) and limnetic samples were collected with a 2.2 L Van Dorn horizontal water sampler (Wildco® Alpha) at approximately 9 m depth. All water samples were pre-filtered onsite through 55 µm mesh to remove larger particles (Vaughn et al., 2008). Prefiltered water was then transported back to the lab on ice and stored at -80°C. Samples were then thawed and vacuum-filtered through a pre-combusted (450°C for 4 h) 47 mm Whatman® glass fiber (GF/F) filter (nominal pore size = 0.7 µm) to isolate suspended solids between 0.7 and 55 µm, reflecting the size fraction typically consumed by mussels (Post, 2002; Strayer, 2008; Vaughn et al., 2008). Filters were dried at 80°C to a constant mass and fumigated in 3 N H_3PO_4 for 8 h to remove carbonates (Harris et al., 2001).

Five, shallow, surface-sediment samples were collected from the littoral zone (≤ 15 m from shore and <1 m subsurface) for littoral FPOM (detritus, algae, bacteria, and fungi mixed with sand) using a turkey baster (~25 mL/sample) and five deep surface-sediment samples were collected for limnetic FPOM at approximately 10 m using a 3.5 L Ekman grab sampler (Wildco®). Samples were pre-filtered (55 µm) and vacuum filtered (0.7 µm) in the same manner as the water samples. After vacuum filtration, FPOM samples were dried to a constant mass at

80°C. The dried sediment was then removed from the filter, fumigated in 3 N H_3PO_4 for 8 h to remove carbonates (Harris et al., 2001), ground to a fine powder using a mortar and pestle, weighed (nearest 10^{-5} g), and encapsulated in 4 mm × 6 mm tin capsules.

Approximately 85 g of submerged CPOM (mixed leaf packs and amorphous organic detritus) were collected from the littoral zone and transported in the same manner as SPOM and FPOM samples. Coarse particulate organic matter was then fumigated, ground, and encapsulated in the same manner as the FPOM samples. All prepared isotopic samples of mussel tissues and food resources were shipped to Washington State University Stable Isotope Core Laboratory for $\delta^{13}C$ and $\delta^{15}N$ analysis.

Isotope ratios are reported in parts per thousand (‰) relative to standards [Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N for nitrogen], defined in delta notation as:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample} / R_{standard} - 1) \times 10^3$$

where $R = {}^{13}C / {}^{12}C$ or ${}^{15}N / {}^{14}N$, respectively (DeNiro & Epstein, 1978, 1981).

3.3.4 Statistical analyses

To account for variation in lipid content between consumers and food resources, all $\delta^{13}C$ values were mathematically-corrected using the linear relationships between $\delta^{13}C$ and C:N for mussels or % carbon for food resources (Post et al., 2007). All mussel $\delta^{13}C$ values were corrected for lipid influence using C:N ratios as follows:

$$\delta^{13}C_{corrected} = \delta^{13}C_{untreated} - 3.32 + 0.99 \text{ x C:N}$$

All food resource $\delta^{13}C$ values were corrected for lipid influence using % carbon as follows:

$$\delta^{13}C_{corrected} = \delta^{13}C_{untreated} - 3.02 + 0.09 \text{ x \% carbon}$$

Corrected $\delta^{13}C$ values were used for all statistical analyses. Raw isotopic data are publicly available on Auburn University's scholarly repository, AUrora, at https://aurora.auburn.edu/handle/11200/50018 and directly from the corresponding author.

We used a combination of linear models and supportive Bayesian mixing models to address our objectives. First, to determine whether isotopic signatures varied across species (objective 1) or across recently-immersed and 8-week emersed individuals (objective 4) we used a one-way analysis of variance (ANOVA) to test the effects of species on mussel $\delta^{13}C$ and $\delta^{15}N$. Second, to identify possible relationships between isotopic signatures and consumer size (objective 3), we used linear regression analysis. Third, we used a one-way ANOVA to test the effect of species and recent versus 8-week emersion on mussel C:N ratios (objectives 1 and 4). The Shapiro–Wilk test was utilized for normality analysis of the variables, and Levene's test was used to evaluate the homogeneity of variance (HOV). Statistical analysis tests were performed on rank-transformed data if normality and/or HOV assumptions were violated ($p < 0.05$). If there were significant differences, post-hoc analyses were performed using the Tukey's Studentized Range test. When species effect was significant, a Dunn's test was conducted for the post-hoc analysis. Statistical significance was set at $p < 0.05$. All analyses of variance tests were performed using SigmaPlot Version 13.0 (SigmaPlot, 2014).

To determine the relative contribution of each potential food source to mussel diets (objective 3), we used a Bayesian tracer mixing model framework (MixSIAR; R Core Team, 2013; Stock & Semmens, 2016). MixSIAR accounts for hierarchical structure associated with food chains, uncertainty in the consumer tissue mixture, and food source variability (Semmens et al., 2009; Stock et al., 2018). This analysis was used to determine whether primary diet components varied across species (objective 1) and to provide insight into dietary carbon sources

(objective 2). The MixSIAR model used two tracers ($\delta^{13}C$ and $\delta^{15}N$) and five food sources (CPOM, littoral FPOM, limnetic FPOM, littoral SPOM, and limnetic SPOM). To evaluate whether our isotope mixing model was valid, we used a Monte Carlo simulation of the resource mixing region to evaluate the probability that our consumers' stable isotope contributions were satisfied by the resource polygon (Smith et al., 2013). The point-in-polygon method utilizes iterations to generate mixing polygons with the distribution of the dietary food sources using a user-chosen discrimination factor (Smith et al., 2013). The proportion of the polygon that has a solution is given as a frequentist probability that the proposed mixing model data can accurately calculate source contributions to explain a consumer's isotopic signature (Smith et al., 2013). Carbon discrimination used in the mixing model was 0.4‰ ± 1.3 *SD* and N was 3.4‰ ± 1 *SD* (Post, 2002). After using the point-in-polygon method to evaluate the validity of our mixing model, we excluded consumers that had <5% chance of having their isotopic signature explained by the source contributions in the MixSIAR analysis (Figure 1).

Two models, a null model and a model using species as a variable, were initially tested and compared using deviance information criterion (DIC) scores (Table 1; Phillips et al., 2014; Stock & Semmens, 2016; Stock et al., 2018). Model convergence comparisons were made by assessing Gelman-Rubin diagnostics and Geweke diagnostics (Phillips et al., 2014; Stock & Semmens, 2016; Stock et al., 2018). Two priors for food source contribution were compared for use in the model: an uninformative prior and a literature-based prior attributing 41% contributions from both limnetic and littoral SPOM, 8% from both limnetic and littoral FPOM, and 51% from CPOM (Table 1; Fogelman et al., 2022).

3.4 Results

3.4.1 Mussel isotopic values and carbon:nitrogen ratios

There were no significant differences across recently-immersed species in $\delta^{15}N$ signatures, but there were significant differences in $\delta^{13}C$ and C:N ratios between *F. escambia* and recently-immersed *E. pullata*. *Fusconaia escambia* was more carbon depleted than recently-immersed *E. pullata* and *F. escambia* exhibited a significantly lower C:N ratio relative to recently-immersed *E. pullata*. *Utterbackiana hartfieldorum* exhibited intermediate $\delta^{13}C$ and C:N ratios and was not significantly different from either of the other species (Table 2). Eight-week emersed *E. pullata* were nitrogen-enriched relative to recently-immersed *E. pullata* but did not differ in $\delta^{15}N$ from the other two recently-immersed species (Table 2).

Shell length had a significant negative relationship with $\delta^{13}C$ ($F_{1,10} = 70.65$, $p < 0.001$, $R^2 = 0.783$) and a significant positive relationship with $\delta^{15}N$ ($F_{1,10} = 24.76$, $p < 0.001$, $R^2 = 0.683$) for *U. hartfieldorum* (Figure 2). There was no relationship between shell length and isotopic signatures for any other species.

3.4.2 Food source contributions

The best-fitting model analyzed species as a fixed effect with a literature-based informative prior. This informative prior had a lower DIC score than the null model or a model using an uninformative prior (Table 1). Based on results of the point-in-polygon analysis, no consumer values were removed for any of the species (Figure 1). For all species, limnetic FPOM was the dominant carbon source with littoral FPOM, limnetic and littoral SPOM, and CPOM comprising relatively minor contributions. Fine particulate organic matter was carbon-enriched

and nitrogen-depleted compared to all mussel species and exhibited approximately one trophic level of fractionation from consumers (Figure 3). Limnetic FPOM dietary contributions to recently-immersed *E. pullata* were 98.7% ± 1.8 *SD*. For 8-w emersed *E. pullata* limnetic FPOM contributions were 99.4% ± 0.7 *SD.* Limnetic FPOM contributions for *U. hartfieldorum* and *F. escambia* were 99.0 ± 1.3 *SD* and 99.1 ± 1.3 *SD*, respectively. For all species, whether recently-immersed or 8-week emersed, littoral FPOM, limnetic and littoral SPOM, and CPOM contributions were <1% (Table 3).

3.5 Discussion

We quantified food resources in three mussel species via stable isotopes in Gantt Lake, Alabama and found that mussels primarily relied on benthic, as opposed to suspended, organic food resources. The primary carbon source for *E. pullata*, *U. hartfieldorum*, and *F. escambia* was consistently dominated by fine particulate organic matter associated with limnetic benthic (i.e., profundal) sediments. Littoral fine particulate organic matter, course particulate organic matter, and suspended particulate organic material contributed less than 1% to dietary carbon. This suggests that all three species utilized a benthic feeding mode to access detrital and organic material associated with benthic sediments.

Despite mussels being predominately classified as filter feeders, many recent studies using stable isotopes have shown that mussels can exploit benthic materials as food sources (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Christian et al., 2004; Nichols et al., 2005; Weber et al., 2017; Fogelman et al., 2022). Detrital material can serve as the primary food source in smaller lotic systems (Fogelman et al., 2022) while larger (especially lentic) systems may provide adequate suspended food resources in addition to detrital sources. Mussels are capable of

digesting detrital material (Christian et al., 2004) and allochthonous material has been shown to contribute 1/3–1/2 of mussel biomass and dietary carbon in riverine systems (Weber et al., 2017; Fogelman et al., 2022). Freshwater sediments can trap and store organic and inorganic particles that can form the base of aquatic food webs (Krumins et al., 2013) and there is a positive relationship between mussel growth rate and sediment organic content (Cyr, 2020). The isotopic signatures of mussel species in this study overlapped, with organic particles associated with limnetic benthic FPOM as the dominant food source. This pattern is consistent with prior stable isotope studies on unionids where sympatric individuals have similar isotopic signatures irrespective of taxonomy (Weber et al., 2017; Fogelman et al., 2022). This broad dietary overlap could be attributed to the effects of site-specific drivers (e.g., nutrient cycling, dominant carbon sources) on feeding (Peipoch et al., 2012).

Freshwater mussels can deposit-feed to access benthic food resources such as FPOM and CPOM and it has been suggested that they preferentially assimilate the microbial communities associated with benthic organic matter (Nichols & Garling, 2000; Christian et al., 2004; Nichols et al., 2005). In aquatic systems, “green” pathways have carbon entering the system as algae living on the sediment surface, and “brown” pathways have the majority of carbon entering the system through decomposition of organic materials (e.g. bacteria; Krumins et al., 2013). In shallow freshwater lakes, there is a direct coupling between sediment food webs and primary producers such as benthic algae (Krumins et al., 2013). Mussels in Gantt Lake may be relying on both “green” and “brown” pathways when using FPOM as a primary food source. The relative contribution of algae or bacteria from FPOM was not quantified in this study and warrants further investigation. Additionally, food resource sampling in this study did not capture temporal variability in “green” and “brown” pathways that may be contributing to the mussel diet.

It was surprising that the dominant food source of these mussels was benthic organic matter sampled from the limnetic zone, while most mussels and mussel species generally inhabited littoral zones. While mussels were not collected where the predominant food source was sampled, the signature of this deep-water benthic sediment most closely represents the signature of the mussel tissue. Although not directly quantified, limnetic sediments were grey to black in color and uniformly fine in texture (i.e., hypoxic/anoxic "muck") whereas littoral sediments were brown in color and positioned within a sand matrix. Littoral and limnetic samples came from representative sites and not across the entire lake, so the true spatial extent of these sediment "types" is not fully known. It is certainly possible that the limnetic sediments are found closer to shore than what we sampled and overlap with mussel distributions. Accumulated benthic organic matter in the limnetic zone is likely comprised of previous algal bloom biomass and other nutrient-rich organic matter. These sediments can get routinely suspended (Evans, 1994; Bloesch, 1995), and such episodic events can have strong effects on subsequent ephemeral algal proliferation (Dzialowski et al., 2008). Although the precise mechanisms remain unclear, episodic resuspension events may provide deep sediment organic matter availability (or the nutrients thereof) to littoral mussels and be particularly important in their growth and development.

Eight-week emersed *E. pullata* had a significantly higher nitrogen isotopic signature than *E. pullata* individuals that were collected immediately after drawdown. The enrichment of $\delta^{15}N$ of emersed versus recently-immersed *E. pullata* could be caused by nutritional and environmental stress. "Self" trophic fractionation can occur in consumers due to catabolism of tissues and subsequent metabolic retention of $\delta^{15}N$ (Cherel & Hobson, 2007; Bowes et al., 2014). Such a physiological process has been used to explain high trophic discrimination factors in

bivalves (Patterson & Carmichael, 2018; Fogelman et al., 2022). It is likely that during 8-weeks of emersion, *E. pullata* was experiencing significant nutritional and environmental stress and utilizing its tissue stores for nutrition in the absence of an ability to deposit or suspension feed. However, the hypothesis of increased catabolism by emersed mussels was not supported by C:N ratios. Increased C:N ratios are indicative of increased N excretion and depletion of energy stores (Sterner & Elser, 2002), but C:N ratios of emersed *E. pullata* were not significantly different than those of recently-immersed *E. pullata* in our study, even after 8 weeks out of water and presumably no access to external food sources. This suggests that N-enrichment is a more sensitive indicator of catabolism in stranded mussels than C:N ratios, and/or that they efficiently senesce when stranded and require only a small amount of internal energy stores for basic maintenance.

We detected a negative relationship between shell size and C-depletion and a positive relationship between shell size and N-enrichment for *U. hartfieldorum*, but not the other species sampled in this study. Previous studies have reported relationships between shell size and isotopic signatures, but have generally shown both C- and N-enrichment with increasing shell length (Fritts et al., 2013; Yasuno et al., 2014; Fogelman et al., 2022). The reason for this discrepancy among studies is unknown but it suggests that in some systems such as Gantt Lake, *U. hartfieldorum* may preferentially assimilate more C-depleted and N-enriched fractions of organic pools in benthic sediments as they grow. *Utterbackiana hartfieldorum* sampled in this study fell into two size/age classes (40–50 mm and 80–100 mm) although age was not determined in this study. The pattern in length versus isotopic enrichment/depletion may be due to diet shifts associated with observed ontogenetic niche shifts in habitat use between young (shallow habitats) and older (deep habitats) individuals (Miller, pers. obs.). The relationship

between $\delta^{15}N$ and $\delta^{13}C$ and body size has only been investigated for a few freshwater mussel species (Fritts et al., 2013; Yasuno et al., 2014; Fogelman et al., 2022). Further research is necessary to discern the physiological mechanisms and relationships behind isotopic incorporation, preferential food particle selection, and adult mussel growth.

A central question when developing conservation plans for mussels is whether all species in a community have similar environmental needs or whether they need to be managed differently. In this study, the answer was unequivocal with regard to food resources. All species showed evidence of using FPOM as a major food resource — indicating that management of benthic food resource quality would benefit multiple species. It also suggests that either this food source is not limiting, or the environment is sufficiently unstable, to support multiple species as we do not see food source partitioning across sympatric species in this system. Additionally, we found evidence that *E. pullata* were undergoing catabolism during 8 weeks of emersion. Further investigations into the relationships between energy stores and survival of stranded mussels may be extremely useful for our understanding of how mussel survival is affected by dewatering events. As freshwater mussel populations continue to decline in North America, it is critical to understand their feeding ecology and the relative importance and quality of the benthos as a provider of food resources. Continued investigation of nutritional pathways to mussels across a range of systems and environmental conditions will allow for more effective management and conservation efforts for these imperiled species.

3.6 References

Bloesch, J. 1995. Mechanisms, measurement and importance of sediment resuspension in lakes. Marine and Freshwater Research 46:295–301.

Bowes, R. E., M. H. Lafferty, and J. H. Thorp. 2014. Less means more: Nutrient stress leads to higher δ15N ratios in fish. Freshwater Biology 59:1926–1931.

Boyd, C. E. 2019. Water Quality: An Introduction. Springer Nature.

Cherel, Y., and K. Hobson. 2007. Geographical variation in carbon stable isotope signatures of marine predators: A tool to investigate their foraging areas in the Southern Ocean. Marine Ecology Progress Series 329:281–287.

Cherel, Y., K. A. Hobson, F. Bailleul, and R. Groscolas. 2005. Nutrition, Physiology, and Stable Isotopes: New Information from Fasting and Molting Penguins. Ecology 86:2881–2888.

Christian, A. D., B. N. Smith, D. J. Berg, J. C. Smoot, and R. H. Findlay. 2004. Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca:Unionidae) in 2 small Ohio streams. Journal of the North American Benthological Society 23:101–113.

Cross, W. F., J. P. Benstead, P. C. Frost, and S. A. Thomas. 2005. Ecological stoichiometry in freshwater benthic systems: Recent progress and perspectives. Freshwater Biology 50:1895–1912.

Cyr, H. 2020. Site exposure, substrate, depth, and the thermocline affect the growth of native unionid mussels in a stratified lake. Freshwater Science 39:773–790.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:343–351.

Dzialowski, A. R., S.-H. Wang, N.-C. Lim, J. H. Beury, and D. G. Huggins. 2008. Effects of sediment resuspension on nutrient concentrations and algal biomass in reservoirs of the Central Plains. Lake and Reservoir Management 24:313–320.

Evans, R. D. 1994. Empirical evidence of the importance of sediment resuspension in lakes. Hydrobiologia 284:5–12.

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

Fritts, M. W., A. K. Fritts, S. A. Carleton, and R. B. Bringolf. 2013. Shifts in stable-isotope signatures confirm parasitic relationship of freshwater mussel glochidia attached to host fish. Journal of Molluscan Studies 79:163–167.

Galbraith, H. S., S. E. Frazier, B. Allison, and C. C. Vaughn. 2009. Comparison of gill surface morphology across a guild of suspension-feeding unionid bivalves. Journal of Molluscan Studies 75:103–107.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1996. Influence of Sediment and Algal Food on Cultured Juvenile Freshwater Mussels. Journal of the North American Benthological Society 15:597–609.

Gatenby, C., B. Parker, and R. J. Neves. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae) reared on algal diets and sediment. American Malacological Bulletin 14:57–66.

Haag, W. R. 2012. North American Freshwater Mussels. Cambridge University Press, New York, NY.

Harris, D., W. R. Horwáth, and C. van Kessel. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or CARBON-13 isotopic analysis. Soil Science Society of America Journal 65:1853–1856.

Krumins, J. A., D. van Oevelen, T. M. Bezemer, G. B. De Deyn, W. H. G. Hol, E. van Donk, W. de Boer, P. C. de Ruiter, J. J. Middelburg, F. Monroy, K. Soetaert, E. Thebault, J. van de Koppel, J. A. van Veen, M. Viketoft, and W. H. van der Putten. 2013. Soil and Freshwater and Marine Sediment Food Webs: Their Structure and Function. Bioscience 63:35–42.

Miller, J., N. T. A. Quach, M. Patel, and A. Guillaumet. 2021. Survivability of Mussels, Targeting Federally Listed Species, Over a 13-Week Period During the Drawdown of Gantt Reservoir. A Report to PowerSouth Energy Cooperative and U.S. Fish and Wildlife Service.

Newton, T. J., C. C. Vaughn, D. E. Spooner, S. J. Nichols, and M. T. Arts. 2013. Profiles of Biochemical Tracers in Unionid Mussels Across a Broad Geographical Range. Journal of Shellfish Research 32:497–507.

Nichols, S. J., and D. Garling. 2000. Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. Canadian Journal of Zoology 78:871–882.

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

Patterson, H. K., and R. H. Carmichael. 2018. Dissolved oxygen concentration affects δ15N values in oyster tissues: Implications for stable isotope ecology. Ecosphere 9:e02154.

Peipoch, M., E. Martí, and E. Gacia. 2012. Variability in δ15N natural abundance of basal resources in fluvial ecosystems: A meta-analysis. Freshwater Science 31:1003–1015.

Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92:823–835.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703–718.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montaña. 2007. Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Raikow, D. F., and S. K. Hamilton. 2001. Bivalve diets in a midwestern U.S. stream: A stable isotope enrichment study. Limnology and Oceanography 46:514–522.

Semmens, B. X., E. J. Ward, J. W. Moore, and C. T. Darimont. 2009. Quantifying inter-and intra-population niche variability using hierarchical bayesian stable isotope mixing models. PLoS ONE 4:1–9.

SigmaPlot. 2014. SyStat Software, Inc. San Jose, California, USA.

Smillie, W. G. 1927. Studies of an Epidemic of Malaria at the Gantt Impounded Area, Covington County, Alabama. American Journal of Hygiene 7:40–72.

Smith, J. A., D. Mazumder, I. M. Suthers, and M. D. Taylor. 2013. To fit or not to fit: Evaluating stable isotope mixing models using simulated mixing polygons. Methods in Ecology and Evolution 4:612–618.

Sterner, R. W., and J. J. Elser. 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press.

Stock, B. C., A. L. Jackson, E. J. Ward, A. C. Parnell, D. L. Phillips, and B. X. Semmens. 2018. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. PeerJ 2018:1–27.

Stock, B. C., and B. X. Semmens. 2016. MixSIAR GUI User Manual. Version 3.1 1–42.

Strayer, D. 2008. Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance. University of California Press.

Tai Tue, N., H. Hamaoka, A. Sogabe, T. D. Quy, M. T. Nhuan, and K. Omori. 2012. Food sources of macro-invertebrates in an important mangrove ecosystem of Vietnam determined by dual stable isotope signatures. Journal of Sea Research 72:14–21.

Trochine, C., V. Díaz Villanueva, E. Balseiro, and B. Modenutti. 2019. Nutritional stress by means of high C:N ratios in the diet and starvation affects nitrogen isotope ratios and trophic fractionation of omnivorous copepods. Oecologia 190:547–557.

United States Fish and Wildlife Service. 2010. Federal Register. 75:69222–69294.

Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27:409–423.

Weber, A. M., J. E. Bauer, and G. Thomas Watters. 2017. Assessment of nutritional subsidies to freshwater mussels using a multiple natural abundance isotope approach. Freshwater Biology 62:615–629.

Williams, J., A. Bogan, and J. Garner. 2009. A new species of freshwater mussel, Anodonta hartfieldorum (Bivalvia: Unionidae), from the Gulf Coastal Plain drainages of Alabama, Florida, Louisiana, and Mississippi, USA. Nautilus -Greenville then Sanibel- 123:25–33.

Yasuno, N., K. Shindo, Y. Takagi, G. Kanaya, S. Shikano, Y. Fujimoto, T. Shimada, and E. Kikuchi. 2014. Ontogenetic changes in the trophic position of a freshwater Unionidae mussel. Fundamental and Applied Limnology 184:341–349.

Yeager, M. M., D. S. Cherry, and R. J. Neves. 1994. Feeding and Burrowing Behaviors of Juvenile Rainbow Mussels, *Villosa iris* (Bivalvia:Unionidae). Journal of the North American Benthological Society 13:217–222.

3.7 Tables

Table 1 MixSIAR model parameter estimates used for model selection criteria. Optimum Gelman-Rubin diagnostics have the greatest proportion of variables below the R cutoff <1.05 (~100%) and optimum Geweke diagnostics should have all parameters <5% ± 1.96. Gelman-Rubin and Geweke diagnostics indicate whether a model has converged. Lowest deviance information criterion (DIC) score indicates the best fitting model. The best DIC score, and thus best-fitting model used in this study, is denoted with an *.

Model ID	**Model**	**N**	**Gelman Rubin Diagnostic**		**Geweke Diagnostic**		**DIC score**
			n	**%**	**n**	**%**	
1	Null Model	60	58	97	3	5.00	5.55
2	Species, Uninformative Prior	96	56	58	3	3.13	39.00
3	Species, Informative Prior	96	96	100	3.33	3.47	-6.74*

Table 2 One-way analysis of variance (ANOVA) results for freshwater mussel $\delta^{13}C$, $\delta^{15}N$, and C:N with species as an effect. Mean estimates and standard error (*SE*) are presented for species effect analyzed for $\delta^{13}C$, $\delta^{15}N$, and C:N. Statistical differences among species in mean $\delta^{13}C$, $\delta^{15}N$ or C:N ratio are denoted with different superscript letters (Dunn's Test) at $p < 0.05$ if (**bold**).

	Mean (‰) ± *SE*					
Parameter	***U. hartfieldorum* (recently immersed)**	***F. escambia* (recently immersed)**	***E. pullata* (recently immersed)**	***E. pullata* (8-week emersed)**	**Test Statistic**	***p-value***
$\delta^{13}C$	-30.68 ± 0.22^{ab}	-31.07 ± 0.12^{a}	-30.53 ± 0.09^{b}	-30.54 ± 0.10^{ab}	$H_3 = 8.63$	**0.035**
$\delta^{15}N$	7.43 ± 0.18^{ab}	7.80 ± 0.16^{ab}	7.33 ± 0.10^{b}	7.80 ± 0.08^{a}	$H_3 = 11.24$	**0.010**
C:N	3.94 ± 0.04^{ab}	3.87 ± 0.04^{b}	4.33 ± 0.11^{a}	4.04 ± 0.06^{ab}	$H_3 = 15.64$	**0.001**

Table 3 Mean estimated dietary source contributions for recently-immersed *Utterbackiana hartfieldorum*, *Fusconaia escambia*, *Elliptio pullata*, and 8-week emersed *E. pullata* with standard deviation (*SD*). Dietary sources are coarse particulate organic matter (CPOM), littoral and limnetic fine particulate organic matter (FPOM), and littoral and limnetic suspended particulate organic matter (SPOM). This table reflects mean estimate values of potential carbon and nitrogen contributions to mussel tissue composition determined using Bayesian mixing models in MixSIAR.

Food Source	***U. hartfieldorum* (recently immersed)**	***F. escambia* (recently immersed)**	***E. pullata* (recently immersed)**	***E. pullata* (8-week emersed)**
CPOM	0.7 ± 1.2	0.7 ± 1.2	0.8 ± 1.4	0.4 ± 0.6
Littoral FPOM	0.1 ± 0.3	0 ± 0.2	0.1 ± 0.7	0 ± 0.2
Limnetic FPOM	99.0 ± 1.3	99.1 ± 1.3	98.7 ± 1.8	99.4 ± 0.7
Littoral SPOM	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.4	0.1 ± 0.1
Limnetic SPOM	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.6	0.1 ± 0.3

3.8 Figures

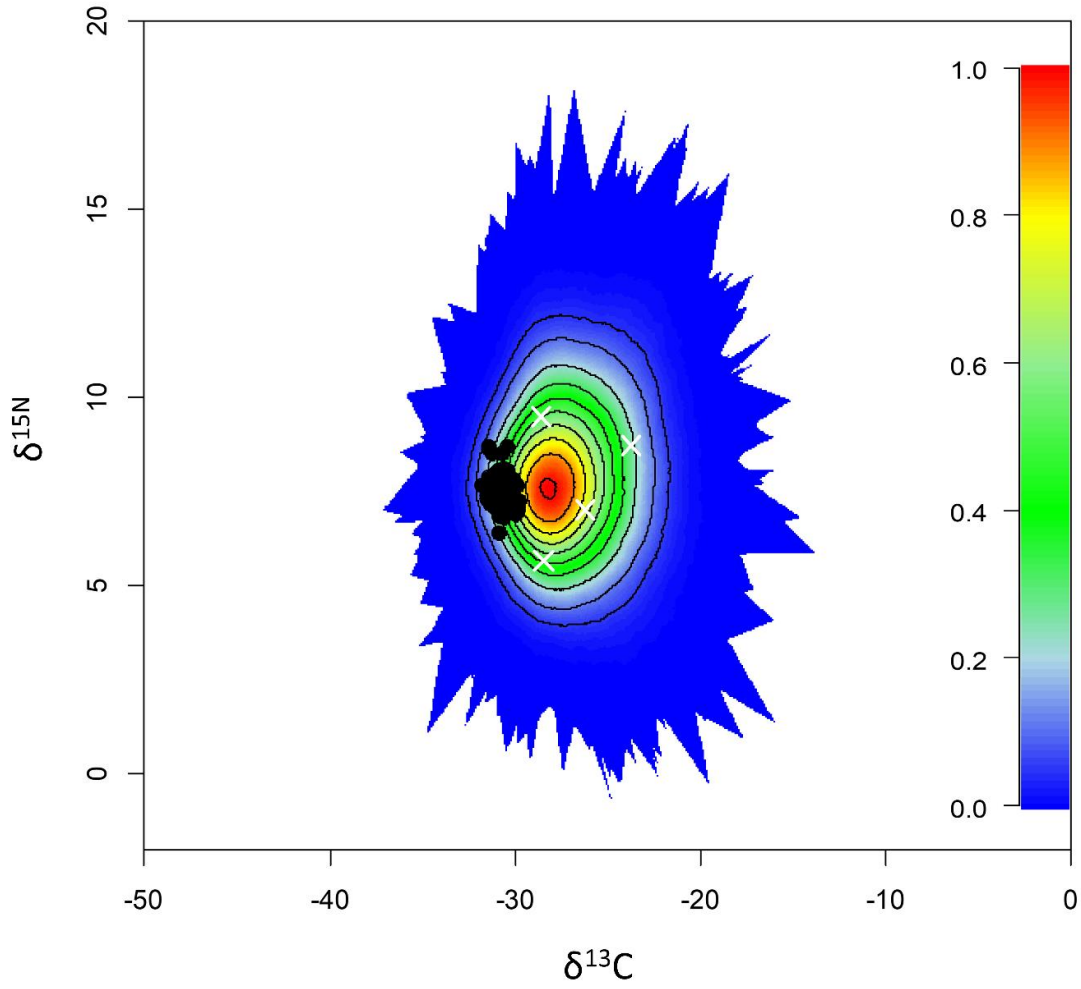

Figure 1 Simulated mixing regions for *Elliptio pullata*, *Utterbackiana hartfieldorum*, and *Fusconaia escambia* (black dots) and the average source signatures (white crosses). Consumers within 95% of the mixing region (outermost, dark blue, contour) are included, and consumers with <5% probability of satisfying the mixing model were excluded. Consumers outside the 95% mixing region are not used in the MixSIAR model as they need an alternative model to explain their isotopic signatures, although all in the current study fit the model with >20% probability. Contours represent probabilities within the mixing region at the 5% outermost contour and every 10% interval.

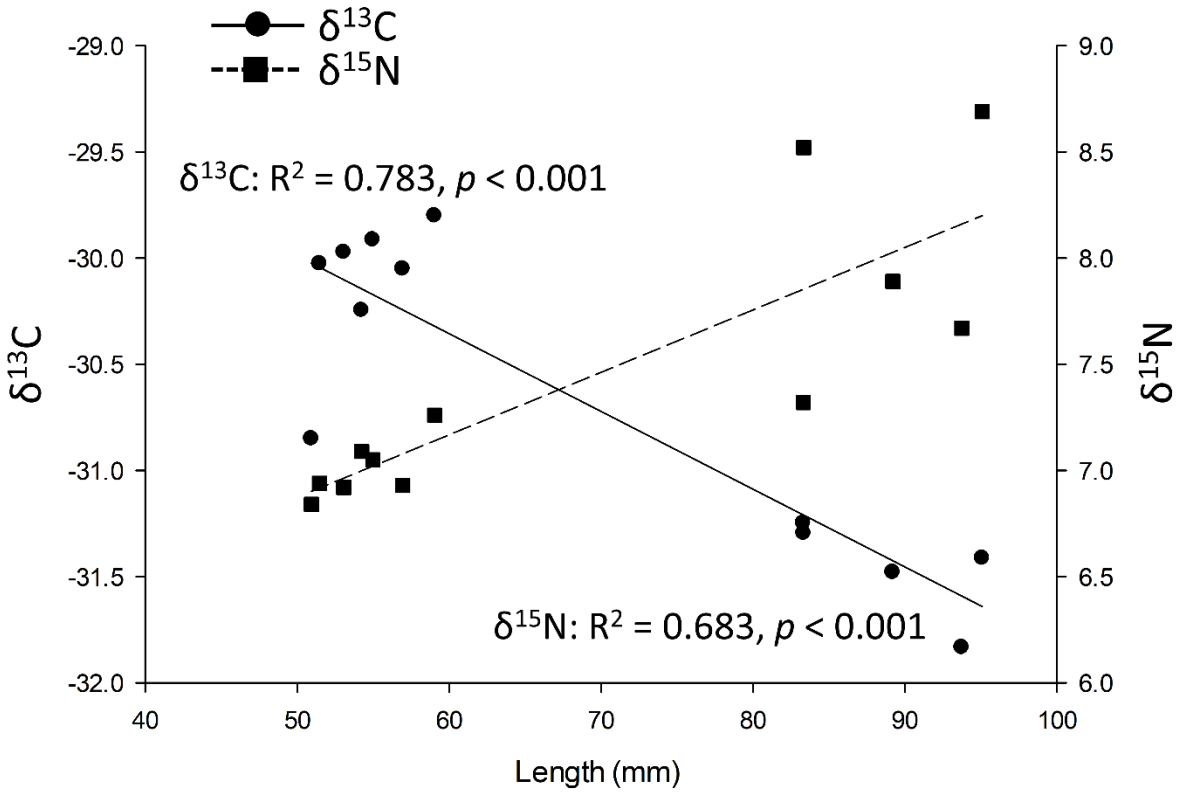

Figure 2 Scatter plot of the relationship between shell length (mm) and stable carbon and stable nitrogen isotopic signatures in *Utterbackiana hartfieldorum*.

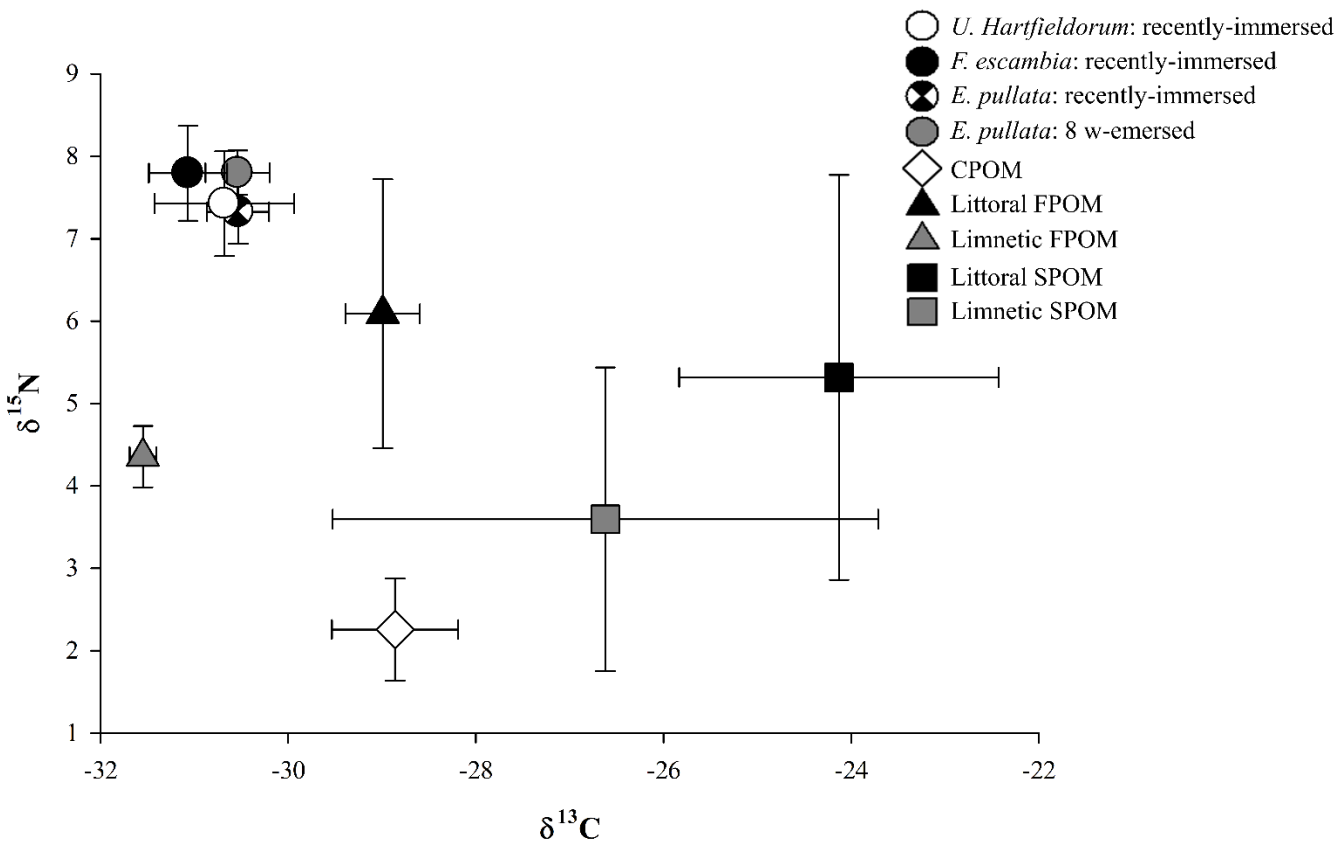

Figure 3 Stable CN isotope biplots for immersed and emersed *Elliptio pullata*, *Utterbackiana hartfieldorum*, and *Fusconaia escambia* and food sources [coarse particulate organic matter from benthic detritus (CPOM), fine particulate organic matter from benthic sediments (FPOM), suspended particulate organic matter (SPOM)]. Each point is an average and error bars represent the associated standard deviation.

CHAPTER 4

EVALUATING SPATIAL AND TEMPORAL FATTY ACID VARIANCE TO INFORM FOOD RESOURCES OF UNIONID MUSSELS

4.1 Abstract

Unionid mussels are increasingly imperiled in North America but much remains unknown about their feeding ecology. Quantitative analysis of fatty acids (FAs) can be used to provide insights into food quality, quantity, and origins. Our main objective was to measure seasonal patterns in FA composition of unionid mussels from five species (*Cyclonaias necki*, *C. petrina*, *C. pustulosa*, *Lampsilis bergmanni*, and *L. bracteata*) across four rivers in central Texas, USA whose primary dietary carbon source has previously been identified as coarse particulate organic matter associated with benthic detritus. Fatty acid profiles were used to elucidate primary dietary sources within basal detrital compartments in more detail, as well as provide insight into food quality and quantity. Mussels across seasons and drainages had 55% of their FA composition in common, but we also observed significant variations in FA profiles and ecologically relevant groupings of FAs across drainages. Essential FA profiles were dominated by eicosapentaenoic acid (EPA; 20:5n-3). Unlike previous FA studies on aquatic vertebrates and unionids, arachidonic acid (ARA; 20:4n-6) and docosahexanoic acid (DHA; 22:6n-3) were either absent or found in low concentrations which allowed for only limited inferences regarding food quality. The percent of total FAs that were algal derived ranged from 7–10% while the percent of total FAs that were bacterially derived was less than 1% indicating that source-specific algal-derived FAs contributed more to the mussel diet. Ratios of n-3 and n-6 polyunsaturated FAs suggest that mussels are utilizing both suspension and deposit-feeding modes of feeding to access bacterial and algal constituents of the diet. Fatty acid profiles of mussels differed more

across rivers than among species and seasons, indicating the highest food quantity for mussels in the Colorado River, and the lowest in the Navasota River, determined by total sums of n-3 and n-6 FAs, saturated FAs, monounsaturated FAs, polyunsaturated FAs, and total FAs. Further understanding of unionid dietary requirements and limitations can allow for more informed conservation and management efforts.

4.2 Introduction

Diet quality can have profound effects on consumer physiology, behavior, and ecological interactions (Burian et al., 2020). Unionids are generally considered to be suspension feeders with a diet comprised primarily of algae, bacteria, particulate, and dissolved organic materials. However, there is evidence that they also utilize benthic resources associated with detritus and sediments (Nichols & Garling, 2000; Christian et al., 2004; Vaughn et al., 2008; Newton et al., 2013; Fogelman et al., 2022). Within a system, there is dietary overlap and little evidence of different dietary specializations among sympatric species, but there is little detailed knowledge of mussel diet quality or overall nutrition (Fogelman et al., 2022). Understanding the dietary details of freshwater mussels potentially can elucidate mechanisms associated with their growth, local abundance, and distribution.

Stable carbon and nitrogen isotope signatures are biomarkers that can be used to evaluate trophic food webs in aquatic systems. Carbon and nitrogen stable isotopes in consumer soft tissues closely reflect the isotopic signatures of their diet (DeNiro & Epstein, 1978, 1981). Stable isotope analysis is an effective tool for mapping aquatic food webs but this technique has limitations when analyzing basal food compartments and differentiating between dietary sources with similar isotopic signatures (DeNiro & Epstein, 1981; Newton et al., 2013). Many basal food

compartments in an aquatic system consist of multiple components. For example, suspended particulate organic matter (SPOM) compartments are composed of inorganic sediment particles, plankton, bacteria, and detritus in a wide range of size fractions (Boyd, 2019). Use of stable isotope analysis has been successful in identifying contributions of food resource compartments to unionid mussels, but isolating specific fractions of basal resource compartments, individual dietary components, or inferences on food resource quality have remained elusive (Raikow & Hamilton, 2001; Weber et al., 2017; Fogelman et al., 2022,).

Quantitative fatty acid (FA) analysis is a technique that can identify specific consumer food components within basal resource compartments based on source-specific FAs and can estimate food quality based on concentrations of polyunsaturated FAs (PUFAs; Dalsgaard et al., 2003; Kelly & Scheibling, 2012; Newton et al., 2013; Fritts et al., 2018). Most animals cannot synthesize PUFAs *de novo*, and PUFAs are necessary for many critical physiological processes (Ahlgren et al., 2009). Fatty acids that cannot be produced biologically within an organism in sufficient quantities to meet physiological demands and thus must be obtained through diet are known as essential FAs (EFAs; Iverson et al., 2004; Kelly & Scheibling, 2012). The three most important PUFAs in vertebrates are eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6). The importance of relative concentrations of PUFAs in invertebrate diets is less well understood, but it is assumed that these same three PUFAs are also important in invertebrate diets (Arts et al., 2009; Jardine et al., 2020). Eicosapentaenoic acid, DHA, and ARA must be obtained from a consumer's diet or from the precursors α-linolenic acid (ALA; 18:3n-6; EPA and DHA precursor) and linoleic acid (LIN; 18:2n-6; ARA precursor) which are provided by algal and cyanobacterial components of the diet (Arts et al., 2009). Eicosapentaenoic acid, DHA, and ARA provide structural and functional

integrity of cell membranes, and are precursors of eicosanoids, which are highly biologically-active paracrine hormones (Sargent et al., 1999). In the freshwater mussel *Dreissena,* ARA has been shown to stimulate reproduction while EPA and DHA stimulate growth (Arts et al., 2009). Eicosapentaenoic acid and its formed eicosanoids also competitively inhibit and interfere with ARA eicosanoid production and function; thus the relative quantities of DHA, EPA, and ARA are important in a consumer diet. For fish, the optimal ratios of DHA:EPA are 2:1 and optimal ratios of EPA:ARA range from 1:1 to 10:1 (Sargent et al., 1999). Polyunsaturated FA ratios for aquatic invertebrates have generally been extrapolated from fish PUFA concentrations (Arts et al., 2009), however high concentrations of ARA have been detected in some unionids, suggesting that EPA:ARA ratios might be lower in unionid mussels than generally assumed based on patterns in fish (Ahlgren et al., 2009; Newton et al., 2013).

There are several FA metrics in addition to EFAs that can be used to distinguish between food origins (including bacterial and algal resources and whether consumers are using a deposit- or suspension-feeding). Because freshwater mussels are epibenthic organisms linking the pelagic and benthic interfaces of aquatic systems, it is important to consider their ability to access both benthic and suspended food sources — which may include a combination of algae, bacteria, fungi, and organic detritus (Raikow & Hamilton, 2001; Dalsgaard et al., 2003). A signature almost exclusive to chlorophyll-producing algae is the placement of a double carbon bond in the n-3 or n-6 position during the synthesis of PUFAs (Newton et al., 2013). Total PUFAs (n-3 and n-6 FAs) are higher in algal than in terrestrial or cyanobacterial sources (Ahlgren et al., 2009) which could be useful when evaluating allochthonous or autochthonous food source origins. Generally, in aquatic food webs, the n-3:n-6 ratio of consumers is >1 if food sources are autochthonous while the ratio is <1 in terrestrial food webs or if the food sources are

allochthonous in aquatic food webs (Arts et al., 2009). Short-chain monounsaturated FAs (MUFAs) and saturated FAs (SAFAs) are characteristic biomarkers for bacteria in terrestrial soil and benthic aquatic habitats (Cavigelli et al., 1995; Newton et al., 2013). Total algal-derived FAs (EFAs) and total bacterial-derived FAs (short odd chained FAs) can provide insight into the contribution of algal versus bacterial food sources to the consumer diet. Both the absolute concentrations and the dietary proportions between n-3 (EPA and DHA) and n-6 PUFAs (ARA, ALA, and LIN) are important indicators of food quality (Arts et al., 2009) available to consumers in littoral or lotic habitats. Consumers feeding on low-quality foods, such as decomposing particles, tend to have lower n-3:n-6 ratios compared to consumers that feed on higher-quality foods, such as fresh algae (Arts et al., 2009; Sargent, 1995; Milke et al., 2004).

This study aims to use quantitative FA analysis to identify the primary dietary constituents (bacteria or algae) of five species of freshwater mussels from central Texas, USA whose primary basal food resource has been identified through stable isotope analysis as coarse particulate organic matter (detritus; Fogelman et al., 2022). Mussels have the necessary enzymes to digest nutrient-poor detritus (Christian et al., 2004), but likely gain substantial dietary benefit from the assimilation of algae and bacteria associated with the benthos (Raikow & Hamilton, 2001). Fatty acid profiles of consumer tissues can potentially identify microbial components associated with organic material and associated benthic dietary components for mussels. Our objectives in this study were to (1) quantify the FA profiles of five species of freshwater mussels; (2) evaluate ecological drivers of FA variation including season, drainage, and species; and (3) evaluate specific dietary components within the basal food compartments previously determined with stable isotope studies.

4.3 Methods

4.3.1 Mussel sampling

We studied unionids in four Texas drainages (Navasota River, Colorado River, Llano River, and Guadalupe River). Four endemic, state-listed taxa *(Cyclonaias necki*, *C. petrina*, *Lampsilis bracteata*, and *L. bergmanni*); and one common, widespread taxon (*C. pustulosa*) were targeted. Three species were sampled from one river each, with *C. necki* and *L. bergmanni* sampled from the Guadalupe River and *L. bracteata* sampled from the Llano River. *Cyclonaias petrina* was sampled from the Colorado and Llano Rivers, and *C. pustulosa* was sampled from the Colorado and Navasota Rivers. Thus, the final design included a total of five species, two of which were sampled in two rivers each while the remaining three species were sampled in only a single river each, for a total of seven species-drainage combinations (Figure 1; Table 1).

Each species-drainage combination was sampled in spring (April), summer (July), and fall (October) of 2017. On each sampling date, we collected ten individuals per species at a given drainage by snorkeling and searching benthic sediments by hand. To obtain tissues for FA analysis, mussels were opened using a pair of flat-tipped, reverse-action pliers, and two sublethal tissue samples were taken from foot tissue using a 1.5 × 4.5 mm biopsy punch (nasal biopsy tool #453733, Karl Storz, Tuttlingen, Germany; Fritts et al., 2015). Mussels were allowed to recuperate streamside (1–2 h) and subsequently returned alive to the streambed from where they were collected. Tissue samples were put on ice in the field, subsequently frozen, and transported to Auburn University, Alabama, USA for processing. Tissue samples were stored at -20°C until processing (Rudy et al., 2016).

4.3.2 Laboratory analysis for fatty acids

Lipid were extracted, transesterified, and analyzed by gas chromatography mass spectrometry following previously described methods (Higgins et al., 2014). Briefly, mussel foot tissue was weighed individually (nearest 10^{-5} g), lyophilized, and ground into a powder using a mortar and pestle. Powdered tissue (2.5–10 mg) was placed into a 5 mL homogenization vial containing 50 μL of an evaporated 1 μg/μL nonadecanoic acid as an internal standard. Samples were homogenized with 4 mL of 1 M methanolic HCl and 4 mL 1.0 mm glass beads (Biospec Products, Bartlesville, Oklahoma, USA) to extract lipids. Samples were disrupted using a Mini-Beadbeater-16 (Biospec Products) for 30-second beating intervals at 3,450 oscillations/min for 8 cycles and cooled in between cycles. Beads, solids, and methylation solution were then transferred to a 15 mL PYREX™ tube with a Teflon-lined lid; and 1 mL of hexane was added and then vortexed to mix. The reaction within the PYREX™ tube with all previously mentioned reagents was incubated for 1 hour at 100°C to transesterify lipids. After incubation, 4 mL of 6% K_2PCO_3 solution was added to the tube to stop the reaction. The PYREX™ tubes were gently agitated by hand and allowed to sit until phase separation was achieved (~5 minutes). The hexane phase containing FAs was removed using a Pasteur pipette and transferred to a 2 mL crimp-top gas chromatography (GC) vial. All samples were stored at -80°C until injection on GC.

An Agilent HP 6890 GC coupled to an HP 5970 mass spectrometer and a DB-23 analytical column (J&W Scientific, Folsom, California, USA) was used for FA identification and concentration quantification (objective 1). Helium was used as a carrier gas with a column flow of 1.0 mL/min and 1 μL injection volume was used with splitless injection. The inlet temperature was 250°C and the column oven protocol was: 120°C ramp at 1.5°C per minute to 198°C then

hold at 198°C for 8 minutes. Qualitative FA identifications were completed using retention time of sample peaks compared to the retention time and ion comparison of known FA methyl esters (FAMEs) using a Supelco FAME 37 Standard (Sigma-Aldrich, Inc. St. Louis, Missouri, USA). To quantify the concentrations of each FAME found in individual mussel samples (objective 1), we used the sample peak area relative to an external canola oil standard (Sigma Aldrich, Inc. St. Louis, Missouri, USA). Concentration quantification calculations accounted for response and recovery of lipids during the transesterification and analysis processes, through comparison to an internal and external standard. The nomenclature *A:*Bn-*C* is used when reporting all FAs, where *A* is the number of carbon atoms, *B* is the number of double bonds, and *C* is the position of the first double bond relative to the terminal (n) methyl carbon atom. All FA data for mussels were reported in μg FA methyl ester/mg dry tissue mass.

4.3.3 Statistical analyses

We used a combination of multivariate and univariate analyses to address our objectives. First, to identify variability in FA profiles across species, drainage, or season (objective 2) we used multivariate statistics to evaluate dissimilarities in FAs (using the Euclidean distance-based matrix and 9,999 permutations). To determine the effects of species, drainage, season, and their interactions on mean FA composition and the variance of FA composition in consumers, we performed an analysis of similarity (ANOSIM) on raw FA concentrations. We conducted non-metric multidimensional scaling (NMDS) on the raw FA value similarity matrices to characterize differences in FA profiles across species, drainages, and seasons. All multivariate analyses were conducted using the vegan package in R (Oksanen et al., 2018). In ANOSIM, a global R was

computed such that 2 or more groups containing the same values have R = 0 (i.e., similarity within groups equals the similarity among groups), whereas groups of values that have nothing in common have R = 1.

We used a three-way analysis of variance (ANOVA) to test the effects of species, season, and drainage on univariate groupings of FAs (objective 2). We classified six groups of FAs including SAFAs, MUFAs, PUFAs, n-3 FAs, n-6 FAs, EFAs, and bacterial FAs (Table 2). Additionally, we evaluated the ratios of n-3:n-6 FAs, ARA:EPA, and EPA:DHA to make inferences on food quality (Table 2). The Shapiro–Wilk test was utilized for normality analysis of the variables, and Levene's test was used to evaluate the homogeneity of variance (HOV). Statistical analysis tests were performed on rank-transformed data if normality and/or HOV assumptions were violated ($p < 0.05$). If there were significant differences, post-hoc analyses were performed using the Tukey's Studentized Range test. Statistical significance was set at $p < 0.05$. All analyses of variance tests were performed using SAS® version 9.4 (SAS, 2013). Food quantity across species, drainages, and seasons was evaluated with the sums of the individual groupings of n-3 and n-6 FAs, SAFAs, MUFAs, PUFAs, and total FAs (Table 2; Newton et al., 2013). Variables with the greatest sum of the groupings were determined to have the most food quantity available to consumers and variables with the least sum of the groupings were determined to have the least food quantity.

4.4 Results

A total of 67 mussels were analyzed for FA composition. Twenty-two of 37 targeted FAs were detected in mussel tissue analyzed (Table 3). Saturated FAs detected were: 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, and 22:0. Monounsaturated FAs detected were: 15:1, 17:1, 18:1n-9c, 18:1n-9t; 20:1n-9 and 22:1n-9. Polyunsaturated FAs detected were: 18:3n-6, 18:3n-3 (ALA),

18:2n-6 (LIN), 20:5n-3 (EPA), 20:4n-6 (ARA), 22:6n-3 (DHA), and 22:2. The most abundant SAFAs, MUFAs, and PUFAs, regardless of species, season, or drainage were palmitic acid (C16:0), gondoic acid (C20:1n-9), and eicosapentaenoic acid (EPA; 20:5n-3), respectively. The least prevalent SAFAs, MUFAs, and PUFAs across all species, seasons, and drainages were behenic acid (22:0), erucic acid (22:1n-9), and calendic acid (18:3n-6). The most dominant n-3 FA was EPA and the most dominant n-6 FA was ALA.

There was a significant difference in mean FA profile composition across drainages (Figure 2; ANOSIM; $R^2 = 0.15$, $p = 0.001$). There was also a significant difference in mean FA profile composition across species and season (Figure 2; ANOSIM: $R^2 = 0.07$, $p = 0.026$ for species; $R^2 = 0.04$, $p = 0.004$ for season).

Total FAs, MUFAs, PUFAs, and EFA/algal FAs were consistent between species and seasons within a given drainage but differed among drainages. The Colorado River had the highest number of total FAs, SAFAs, MUFAs, PUFAs, and EFAs/algal FAs (Table 4). The Navasota River had the lowest number of total FAs, SAFAs, and MUFAs. While the Guadalupe and Navasota Rivers had the lowest numbers of total PUFAs and EFAs/algal FAs (Table 4). Conversely, SAFAs differed across species and seasons. The numbers of total SAFAs were the highest in *C. necki* but the lowest in *C. pustulosa* and *L. bergmanni* (Table 4). Total SAFAs were highest in spring and summer. Most FAs could not be assigned to algal or bacterial sources. The percent of total FAs that were primarily algal derived ranged from 7.2–9.6% across all species, seasons, and drainages. Fatty acids that were primarily derived from bacteria were consistent across species, seasons, and drainages at <1% of the total FAs. The remaining ~90% of total FAs could have come from both algae and bacteria.

Total n-3 FAs were consistent across species, seasons, and drainages. Total n-6 FAs were consistent across species but differed across seasons and drainages (Table 4). Fall had the lowest total FAs while summer had the highest total n-6 FAs. The Colorado River had more n-6 FAs compared to the Guadalupe, Llano, and Navasota Rivers. The n-3:n-6 ratio was different across species, seasons, and drainages, with the highest ratios found in *C. necki*, spring season, and the Guadalupe River, respectively. While the lowest n-3:n-6 ratios were found in *C. pustulosa*, fall season, and the Colorado River. Eicosapentaenoic acid:ARA ratios were consistent across species and seasons but differed across drainages with the Guadalupe River had the highest EPA:ARA ratios while the Colorado and Llano Rivers had the lowest. The DHA:EPA ratio was different across species, seasons, and drainages. The highest DHA:EPA ratios in species, seasons, and drainages were found in *C. pustulosa*, fall season, and the Navasota River, respectively, while the lowest ratios were found in *C. necki*, spring and summer seasons, and the Llano River, respectively.

Food quantity was evaluated as the greatest sum within the individual groupings of n-3 and n-6 FAs, SAFAs, MUFAs, PUFAs, and total FAs. The Colorado River mussels exhibited the greatest sum of total FAs, SAFAs, MUFAs, PUFA, and n-6 FAs, and thus the greatest food quantity available. The Navasota River was determined to have the lowest food quantity available, with the least amount of total FAs, SAFAs, and MUFAs in mussel tissue. Regardless of the drainage, food quantity was estimated to be greatest in summer and lowest in the fall.

4.5 Discussion

Few studies have evaluated the spatial, temporal, or specific variability of FAs in freshwater bivalves. We observed variation in FA profiles and ecologically relevant groupings of

FAs across drainages, although multivariate analyses evaluating differences within species, seasons, and drainages had low explanatory power due to overlap in the FA composition of mussels. While EPA dominated EFA profiles, ARA and DHA were either absent or found in low concentrations. Patterns in FA groupings generally indicated that the fall season, Colorado River, and *C. pustulosa* had the lowest quality of food available, but Navasota River and fall seasons had the lowest quantity of food available. The summer season, Guadalupe River, and *C. necki* had the greatest quality of food available while the Colorado River and summer season had the greatest quantity of food available. Algal-derived, source-specific FAs were 10 times more abundant than bacterial-derived FAs. Ratios of n-3 and n-6 PUFAs suggest that mussels are utilizing both suspension- and deposit-feeding modes of feeding to access bacterial and algal constituents of the diet.

Food quantity and quality: We found evidence for differences in food quantity across drainages and seasons. Across all drainages, total sums of n-3 and n-6 FAs, SAFAs, MUFAs, PUFAs, and total FAs indicated that the quantity of food available to mussels was greatest in the Colorado River and lowest in the Navasota River, with the Guadalupe and Llano Rivers being intermediate. Patterns in FAs also indicated seasonal differences with highest food quantity in the summer and lowest in the fall.

Amongst the targeted FAs, we found evidence for limited availability of ALA, an important precursor to DHA and EPA. Alpha linolenic acid was not found in *C.necki* (all seasons) or *L. bergmanni* (summer) in the Guadalupe River, *C. petrina* (fall) in the Colorado River, *C. petrina* (spring) in the Llano River, or *C. pustulosa* (spring) in the Navasota River. However, DHA and EPA were found in all species, drainages, and seasons despite the periodic absence of the precursor ALA. This could indicate that ALA is limiting, and consumers are

quickly converting all ALA stores into DHA and EPA necessary for physiological processes. It is unlikely that mussels were exclusively obtaining DHA and EPA from dietary sources as these are EFAs that are generally believed to be unavailable in basal food sources in sufficient amounts to support necessary physiological processes (Arts et al., 2009).

Food quality for many consumers can be inferred from ratios of DHA:EPA:ARA. However, because studies done on optimal DHA:EPA:ARA ratios have been primarily conducted on aquatic vertebrates, inferences may not hold true for unionids. In fish, optimal ratios of DHA:EPA are indicated as 2:1 (Arts et al., 2009). In our study, DHA:EPA ratios differed across species, seasons, and drainages with mussel DHA:EPA ratios ranging from 1:12.5 to 1:33. This would indicate poor food quality based on the fish literature, with the Colorado River, *C. pustulosa*, and the fall season exhibiting the lowest quality among drainage, species, and seasonal groupings.

Optimal EPA:ARA ratios for aquatic consumers in general range from 1:1–10:1 with EPA generally equal to or greater in concentration than ARA, with more balanced EPA:ARA ratios (lower) indicating higher food quality (Arts et al., 2009). Mussel EPA:ARA ratios in this study ranged from 8–30. This would indicate a range in food quality with the Navasota River, *C. pustulosa*, and the fall season have the highest food quality while the Llano River, *C. necki*, and the summer season have the lowest food quality. The reason for the relative increase in EPA:ARA in our mussels could be a result of limited ARA in available food sources.

In general, the EFA ratios found in mussels in this study do not fit the optimal and/or general ranges reported for fish. This may indicate poor food quality in our study drainages, but it may also indicate that freshwater vertebrate patterns are not representative of invertebrates. Previous studies have suggested that freshwater filter- and deposit-feeding zoobenthos show no,

or only traces, of DHA compared to benthic invertebrate predators (Goedkoop et al., 2000). It is possible that EPA can serve a similar role as DHA for mussels and that EPA is capable of supporting somatic growth and reproduction (Ahlgren et al., 2009; Guo et al., 2017). Nutritional studies similar to those conducted for commercially important aquaculture species would be of great use in determining optimal FA ratios for freshwater mussel taxa as well as desirable FA characteristics of food resources.

Food sources: Detritivores and herbivores typically exhibit only trace amounts of DHA (Arts et al. 2009). Mussels in our study exhibited low DHA, supporting the importance of algae and bacteria in their diet. Previous studies have suggested that bacteria may be a primary dietary source for mussels (Nichols & Garling, 2000; Christian et al., 2004). In this study, the mean algal:bacterial derived FAs for those that could be definitively assigned to either algae or bacteria was 10, with bacteria providing <1% of the assignable FAs. This is lower than the 4–16% bacterial FA contributions identified in other North American systems (Newton et al., 2013; Metcalfe-Smith et al., 2005). The remaining 90% of FAs could be derived from other food sources including bacteria, algae, fungi, or synthesized within the mussels.

Feeding mode (i.e. filter- vs deposit-feeding) can be inferred from n-3:n-6 ratios as well as relative amounts of DHA. Freshwater detritivore and/or herbivore species that exhibit a combination of filter and deposit-feeding have exhibited n-3:n-6 ratios of ~1. Those that are almost exclusively deposit-feeders have ratios of 0.15–0.22 while those that are predominately filter feeders have exhibited ratios of 5.8 (Arts et al., 2009; Goedkoop et al., 2000; Wacker & von Elert, 2004). In our study, mussels had n-3:n-6 ratios of 1.51–3.79 indicating that they are likely obtaining food via a combination of feeding modes with filter-feeding predominating. Mussels in the Colorado and Navasota Rivers had a lower n-3:n-6 ratio of 1.51–1.52,

respectively, suggesting that they relied less on filter-feeding than mussels in the Llano and Guadalupe rivers with higher n-3:n-6 ratios of 2.05 and 3.14, respectively. This aligns with the findings in Fogelman et al., (2022), where stable isotope analysis indicated mussels were obtaining food from a combination of suspended and benthic resources.

Essential FAs: Previous studies examining FAs of diverse, dense, fecund mussel assemblages have shown high concentrations of ARA (Newton et al., 2013) and suggested that freshwater mussel ARA does not vary seasonally and may be actively regulated at high levels (Hagar & Dietz, 1986; Kainz et al., 2010). Arachidonic acid can be synthesized by mussels via elongation and desaturation of LIN that is obtained from green algae and cyanobacteria. However, it has been postulated that this process is inefficient and may not satisfy organism needs (Gonzalez-Baro & Pollero, 1988; Cook & McMaster, 2002; Ahlgren et al., 1992). In our study, LIN was one of the most abundant FAs found across all drainages and species. Conversely, ARA was not very abundant and exhibit high seasonal variability. It was not detected in the summer or fall for *L. bergmanni* in the Guadalupe River, fall for *C. pustulosa* in the Colorado and Navasota River, or fall for *L. bracteata* in the Llano River. Arachidonic acid was ~3 times higher in the spring than summer or fall for *C. necki* in the Guadalupe River and *C. petrina* in the Colorado River.

The low abundance of ARA overall, and greater prevalence in the spring and summer seasons could be related to stress and/or reproductive cycles. One of the known physiological functions of ARA is cortisol formation, which allows fish to mitigate stress (Koven et al., 2003). Low levels of ARA could indicate that mussels in this study are not overly stressed during the fall season. An additional function of ARA that has been identified in marine bivalves and *Dreissena* is reproduction (Wacker & von Elert, 2004). Low ARA could indicate that individuals

are not undergoing gametogenesis during the fall sampling season as ARA has been found to be highly regulated during reproductive processes in aquatic invertebrates and passed to offspring (Carboni et al., 2013; Ginjupalli et al., 2015). Finally, because desaturation of elongation of EPA can inhibit the formation of eicosanoids from ARA (Arts et al., 2009), synthesis of EPA from ALA may be inhibiting production of ARA from LIN — which would indicate that mussels are selectively synthesizing EPA for somatic growth over ARA for reproduction.

Feeding ecology implications: Fatty acids in unionid mussels varied more among drainages than between species or seasons. Primary FAs specific to a single source were algal-derived as opposed to bacterial-derived. n-3:n-6 PUFA ratios indicated that mussels accessed these dietary constituents through a combination of suspension- and deposit-feeding mechanisms, with an emphasis on suspension feeding. What may be of importance to mussels is adequate quantities of food rather than a specific type of food, as they can use both algal and bacterial food resources and access them through multiple feeding modes. Mussels in these lotic Texas drainages have previously been identified as utilizing coarse particulate organic matter associated with leaf litter detritus as their primary carbon source, and this study suggests that the source-specific dietary markers and dietary fractions they are consuming are derived from algae more so than bacteria. As the mussels from this study were utilizing benthic resources (findings of Fogelman et al., (2022) and findings within this chapter), we suggest that they are utilizing diatoms (abundant in EPA and DHA; Zulu et al., 2018) within microbial communities associated with benthic detrital material. We suggest that mussels are adaptable in their dietary requirements across drainages, if they are receiving adequate amounts of EFAs required for reproduction and somatic growth. Because mussels within a drainage have similar FA profiles, management and conservation efforts can consider community-wide approaches of managing

food sources for unionids, such as ensuring sufficient quantity and quality of algal communities that support unionid growth and reproduction. Like heavily studied commercially important aquatic species, mussel conservationists must continue to investigate optimal FA profiles of unionid consumers *in situ* and *ex situ* to fully understand mussel nutrition requirements, especially in relation to optimal ratios of EFAs, PUFA synthesis, and how unionids specifically use individual PUFAs for discrete physiological functions.

4.6 References

Ahlgren, G., I.-B. Gustafsson, and M. Boberg. 1992. Fatty Acid Content and Chemical Composition of Freshwater Microalgae1. Journal of Phycology 28:37–50.

Ahlgren, G., T. Vrede, and W. Goedkoop. 2009. Fatty Acid Ratios in Freshwater Fish, Zooplankton and Zoobenthos – Are There Specific Optima? Pages 147–178 *in* M. Kainz, M. T. Brett, and M. T. Arts (editors). Lipids in Aquatic Ecosystems. Springer, New York, NY.

Arts, M. T., M. T. Brett, and M. j. Kainz. 2009. Lipids in Aquatic Ecosysytems. Springer, New York, NY.

Boyd, C. E. 2019. Water Quality: An Introduction. Springer Nature.

Burian, A., J. M. Nielsen, M. Winder. 2020. Food quantity-quality interactions and their impact on consumer behavior and trophic transfer. *The Bulletin of the Ecological Society of America* 101(2):e01661. https://doi.org/10.1002/bes2.1661

Carboni, S., A. D. Hughes, T. Atack, D. R. Tocher, and H. Migaud. 2013. Fatty acid profiles during gametogenesis in sea urchin (*Paracentrotus lividus*): Effects of dietary inputs on gonad, egg and embryo profiles. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 164:376–382.

Cavigelli, M. A., G. P. Robertson, and M. J. Klug. 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170:99–113.

Christian, A. D., B. N. Smith, D. J. Berg, J. C. Smoot, and R. H. Findlay. 2004. Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca:Unionidae) in 2 small Ohio streams. Journal of the North American Benthological Society 23:101–113.

Cook, H. W., and C. R. McMaster. 2002. Chapter 7 Fatty acid desaturation and chain elongation in eukaryotes. Pages 181–204 New Comprehensive Biochemistry. Elsevier.

Dalsgaard, J., M. St John, G. Kattner, D. Müller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46:225–340.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341–351.

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

Fritts, A. K., B. C. Knights, T. D. Lafrancois, J. M. Vallazza, L. A. Bartsch, M. R. Bartsch, W. B. Richardson, S. W. Bailey, R. M. Kreiling, and B. N. Karns. 2018. Evaluating potential effects of bigheaded carps on fatty acid profiles of multiple trophic levels in large rivers of the Midwest, USA. Food Webs 16:e00095.

Fritts, A. K., J. T. Peterson, J. M. Wisniewski, and R. B. Bringolf. 2015. Nonlethal assessment of freshwater mussel physiological response to changes in environmental factors. Canadian Journal of Fisheries and Aquatic Sciences 72:1460–1468.

Ginjupalli, G. K., P. D. Gerard, and W. S. Baldwin. 2015. Arachidonic acid enhances reproduction in Daphnia magna and mitigates changes in sex ratios induced by pyriproxyfen. Environmental Toxicology and Chemistry 34:527–535.

Goedkoop, W., L. Sonesten, G. Ahlgren, and M. Boberg. 2000. Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden: Seasonal variation and trophic indications. Canadian Journal of Fisheries and Aquatic Sciences 57:2267–2279.

Gonzalez-Baro, M., and R. Pollero. 1988. Lipid characterization and distribution among tissues of the freshwater crustacean Macrobrachium borellii during an annual cycle.

Guo, F., S. E. Bunn, M. T. Brett, and M. J. Kainz. 2017. Polyunsaturated fatty acids in stream food webs – high dissimilarity among producers and consumers. Freshwater Biology 62:1325–1334.

Hagar, A. F., and T. H. Dietz. 1986. Seasonal Changes in the Lipid Composition of Gill Tissue from the Freshwater Mussel Carunculina texasensis. Physiological Zoology 59:419–428.

Higgins, B. T., A. Thornton-Dunwoody, J. M. Labavitch, J. S. VanderGheynst. 2014. Microplate assay for quantification of neutral lipids in extracts from microalgae. Analytical Biochemistry 465:81-89.

Iverson, S., C. Field, W. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs 74:211–235.

Jardine, T. D., A. W. E. Galloway, M. J. Kainz. 2020. Unlocking the power of fatty acids as dietary tracers and metabolic signals in fishes and aquatic invertebrates. Philosophical Transactions of the Royal Society B 375:20190639. https://dx.doi.org/10.1098/rstb.2019.0639

Kainz, M. J., O. E. Johannsson, and M. T. Arts. 2010. Diet effects on lipid composition, somatic growth potential, and survival of the benthic amphipod Diporeia spp. Journal of Great Lakes Research 36:351–356.

Kelly, J. R., and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446:1–22.

Koven, W., R. van Anholt, S. Lutzky, I. Ben Atia, O. Nixon, B. Ron, and A. Tandler. 2003. The effect of dietary arachidonic acid on growth, survival, and cortisol levels in different-age gilthead seabream larvae (Sparus auratus) exposed to handling or daily salinity change. Aquaculture 228:307–320.

Metcalfe-Smith, J. L., D. J. McGoldrick, C. R. Jacobs, J. Biberhofer, M. T. Arts, G. L. Mackie, V. S. Jackson, D. S. Schloesser, T. J. Newton, E. M. Monroe & M. D. Drebenstedt. 2005. Creation of managed refuge sites for native freshwater mussels to mitigate impacts of the exotic zebra mussel in the delta area of Lake St. Clair. Final report. Environment Canada, Water Science and Technology Directorate (WSTD contribution no. 07-023). Burlington, Ontario: Government of Canada.

Milke, L. M., V. M. Bricelj, C. C. Parrish. 2004. Growth of postlarval sea scallops, *Placopecten magellanicus*, on microalgal diets, with emphasis on the nutritional role of lipids and fatty acids. Aquaculture 234: 293-317.

Newton, T. J., C. C. Vaughn, D. E. Spooner, J. Nichols, and M. T. Arts. 2013. Profiles of biochemical tracers in unionid mussels across a broad geographical range. Journal of Shellfish Research 32:497–507.

Nichols, S. J., and D. Garling. 2000. Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. Canadian Journal of Zoology 78:871–882.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, H. Wagner. 2018. Vegan: community ecology package. http://CRAN.Rproject.org/package=vegan

Raikow, D. F., and S. K. Hamilton. 2001. Bivalve diets in a midwestern U.S. stream: A stable isotope enrichment study. Limnology and Oceanography 46:514–522.

Rudy, M. D., M. J. Kainz, M. Graeve, S. M. Colombo, and M. T. Arts. 2016. Handling and Storage Procedures Have Variable Effects on Fatty Acid Content in Fishes with Different Lipid Quantities. PLOS ONE 11:e0160497.

Sargent, J., G. Bell, L. McEvoy, D. Tocher, and A. Estevez. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177:191–199.

SAS. 2013. SAS Institute Inc., Cary, North Carolina, USA.

Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27:409–423.

Wacker, A., and E. von Elert. 2004. Food quality controls egg quality of the zebra mussel Dreissena polymorpha: The role of fatty acids. Limnology and Oceanography 49:1794–1801.

Weber, A. M., J. E. Bauer, and G. T. Watters. 2017. Assessment of nutritional subsidies to freshwater mussels using a multiple natural abundance isotope approach. Freshwater Biology 62:615–629.

Zulu, N. N., K. Zienkiewicz, K. Vollheyde, and I. Feussner. 2018. Current trends to comprehend lipid metabolism in diatoms. Progress in Lipid Research 70:1–16.

4.7 Tables

Table 1 Species, collection drainages, and decimal degrees (DD) coordinates of studied unionids in four Texas drainages, USA.

Species	**Collection drainage**	**Latitude Longitude (DD)**
Cyclonaias petrina	Colorado River	29.556197 -96.402160
Cyclonaias petrina	Llano River	30.39267 -99.19214
Cyclonaias necki	Guadalupe River	29.93953 -98.94846
Cyclonaias pustulosa	Colorado River	29.556197 -96.402160
Cyclonaias pustulosa	Navasota River	31.15155 -96.19501
Lampsilis bracteata	Llano River	30.39267 -99.19214
Lampsilis bergmanni	Guadalupe River	29.93953 -98.94846

Table 2 Groupings of fatty acids (FAs) analyzed with univariate statistics and FAs used for ecological interpretations to evaluate food quality, quantity, and origins.

Terminology and ecological interpretations	**FAs within grouping and metrics used to evaluate food quality, quantity, and origins**
EFA; Essential FA and Algal FA	18:2n-6 (LIN), 18:3n-3 (ALA), 20:4n-6 (ARA), 20:5n-3 (EPA), and 22:6n-6 (DHA)
SAFA; saturated FA	12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, and 22:0
MUFA; monounsaturated FA	15:1, 17:1, 18:1n9c, 18:1n9t, 20:1n9, and 22:1n9
PUFA; polyunsaturated FA	18:2n-6, 18:3n-3, 18:3n-6, 20:4n-6, 20:5n-3, 22:6n-6, and 22:2
Bacterial Fatty Acids	15:0, and 17:0
Total Fatty Acids	Sum of all fatty acids in consumer tissue
Food quality	n-3:n-6 FAs, ARA:EPA, and EPA:DHA Higher ratios indicate greater food quality between compared variables (e,g., species, season)
Food quantity	Sum of n-3 FAs, n-6 FAs, SAFAs, MUFAs, PUFAs, and total FAs Higher sums indicate greater food quantity between compared variables (e,g., species, season)
Food origins	Terrestrial food webs or allochthonous inputs: n-3:n-6 <1 Aquatic food webs or autochthonous inputs: n-3:n-6 >1 Deposit feeders: n-3:n-6 of 0.15–0.22 Filter feeders: n-3:n-6 of ≥ 5.8 Deposit and filter feeders: n-3:n-6 = 0.23–5.8

Table 3 Fatty acid content (measured in µg FA/mg dry weight) among mussel species, seasons, and drainages. Mean fatty acid content, standard error (*SE*), total number of mussels sampled (N) and number of mussels individual fatty acids were detected in (*n*) are presented for all categories. If a fatty acid was not detected for a drainage, species, season combination it is denoted with a dash (—)

	Guadalupe River					**Colorado River**			
	Cyclonaias necki		***Lampsilis bergmanni***			***Cyclonaias petrina***		***Cyclonaias pustulosa***	
	Spring (N=4) Mean ± *SE* (*n*)	**Summer (N=8) Mean ± *SE* (*n*)**	**Spring (N=3) Mean ± *SE* (*n*)**	**Summer (N=3) Mean ± *SE* (*n*)**	**Fall (N=5) Mean ± *SE* (*n*)**	**Spring (N=4) Mean ± *SE* (*n*)**	**Fall (N=3) Mean ± *SE* (n)**	**Spring (N=6) Mean ± *SE* (*n*)**	**Fall (N=2) Mean ± *SE* (*n*)**
C12:0	—	—	—	—	—	—	—	0.02 (1)	—
C14:0	0.03 (1)	0.08 ± 0.05 (2)	0.02 (1)	0.07 (1)	0.10 ± 0.02 (5)	0.05 ± 0.02 (2)	—	0.02 (1)	0.07 ± 0.01 (2)
C15:0	0.04 ± 0.01 (3)	0.05 ± 0.01 (2)	0.02 (1)	0.08 (1)	0.17 ± 0.02 (5)	0.14 ± 0.04 (4)	0.03 ± 0.01 (3)	0.09 ± 0.04 (6)	0.15 ± 0.00 (2)
C15:1	0.34 ± 0.04 (3)	0.38 ± 0.07 (8)	0.03 (1)	0.23 ± 0.18 (2)	0.32 ± 0.04 (5)	1.25 ± 0.28 (4)	0.36 ± 0.09 (3)	0.62 ± 0.27 (6)	1.06 ± 0.13 (2)
C16:0	8.21 ± 0.69 (4)	11.48 ± 2.58 (8)	6.26 ± 0.53 (3)	7.50 ±1.57 (3)	7.53 ± 0.52 (5)	15.91 ± 2.84 (4)	5.70 ± 0.17 (3)	9.82 ± 1.69 (6)	6.62 ± 0.48 (2)
C17:0	0.14 ± 0.04 (4)	0.16 ± 0.05 (8)	0.04 ± 0.01 (3)	0.11 ± 0.09 (2)	0.17 ± 0.02 (5)	0.53 ± 0.11 (4)	0.13 ± 0.05 (3)	0.27 ± 0.11 (6)	0.23 ± 0.00 (2)
C17:1	0.35 ± 0.05 (3)	0.46 ± 0.10 (8)	0.18 ± 0.10 (3)	0.22 ± 0.16 (3)	0.64 ± 0.10 (5)	0.42 ± 0.10 (4)	0.07 ± 0.03 (2)	0.23 ± 0.09 (6)	0.19 ± 0.04 (2)
C18:0	7.51 ± 0.67 (4)	8.67 ± 0.69 (7)	5.54 ± 0.26 (3)	5.36 ± 1.15 (3)	5.89 ± 0.52 (5)	17.59 ± 2.96 (4)	4.68 ± 0.35 (3)	9.78 ± 1.66 (6)	4.98 ± 0.06 (2)
C18:1n-9c	5.24 ± 0.78 (3)	4.73 ± 0.83 (6)	1.23 ± 0.35 (3)	2.14 ± 1.87 (3)	4.40 ± 0.40 (5)	14.07 ± 2.60 (4)	4.30 ± 1.21 (3)	7.78 ± 2.49 (6)	5.41 (1)
C18:1n-9t	—	—	—	—	—	—	—	—	—
C18:2n-6	0.55 ± 0.19 (4)	1.73 ± 1.37 (8)	0.12 ± 0.06 (2)	0.55 (1)	1.02 ± 0.11 (5)	2.22 ± 0.50 (4)	0.56 ± 0.21 (3)	1.23 ± 0.47 (6)	1.47 ± 0.04 (2)
C18:3n-3	—	—	—	—	0.03 ± 0.01 (3)	0.06 ± 0.02 (3)	0.04 ± 0.01 (3)	0.07 ± 0.03 (2)	0.25 ± 0.03 (2)
C18:3n-6	—	—	—	—	—	—	—	—	—
C20:0	—	—	—	—	—	—	0.03 ± 0.01 (3)	—	—
C20:1n-9	5.50 ± 1.64 (4)	9.89 ± 1.47 (8)	2.21 ± 0.47 (3)	4.94 ± 0.47 (3)	6.40 ± 0.54 (5)	28.95 ± 5.38 (4)	7.20 ± 1.41 (3)	14.01 ± 3.82 (6)	8.63 ± 0.35 (2)
C20:4n-6	0.07 ± 0.03 (3)	0.02 (1)	0.04 (1)	—	—	1.40 ± 0.32 (4)	0.51 ± 0.18 (3)	0.58 ± 0.28 (6)	—
C20:5n-3	0.92 ± 0.27 (4)	1.14 ± 0.22 (8)	0.41 ± 0.12 (3)	0.78 ± 0.76 (2)	1.42 ± 0.19 (5)	5.00 ± 1.09 (4)	1.11 ± 0.37 (3)	2.25 ± 0.77 (6)	1.34 ± 0.42 (2)
C21:0	—	—	—	—	0.04 ± 0.02 (4)	0.05 ± 0.03 (4)	0.03 ± 0.01 (2)	0.25 ± 0.05 (2)	0.18 (1)
C22:0	—	—	—	—	—	—	0.01 (1)	—	—
C22:1n-9	—	—	—	—	—	—	0.03 (1)	—	—
C22:2	0.10 ± 0.03 (3)	0.18 ± 0.03 (6)	0.01 ± 0.00 (2)	0.19 (1)	0.20 ± 0.04 (4)	1.05 ± 0.25 (4)	0.17 ± 0.08 (3)	0.39 ± 0.19 (6)	0.45 ± 0.03 (2)
C22:6n-3	0.03 ± 0.01 (3)	0.05 ± 0.01 (6)	0.02 ± 0.00 (2)	0.05 (1)	0.11 ± 0.11 (5)	0.32 ± 0.09 (4)	0.05 (1)	0.17 ± 0.09 (6)	0.09 ± 0.04 (2)

Table 3 Continued.

	Llano River			Navasota River	
	Cyclonaias petrina	*Lampsilis bracteata*		*Cyclonaias pustulosa*	
	Fall (N=9) Mean ± *SE* (*n*)	**Spring (N=6) Mean ± *SE* (*n*)**	**Fall (N=9) Mean ± *SE* (*n*)**	**Spring (N=8) Mean ± *SE* (*n*)**	**Fall (N=8) Mean ± *SE* (*n*)**
C12:0	—	—	—	—	—
C14:0	—	—	0.09 ± 0.04 (8)	0.02 ± 0.01 (2)	0.05 ± 0.01 (8)
C15:0	0.02 ± 0.01 (2)	0.04 ± 0.02 (2)	0.19 ± 0.08 (6)	0.07 ± 0.01 (6)	0.12 ± 0.02 (8)
C15:1	0.06 ± 0.01 (3)	0.06 (1)	0.25 ± 0.06 (8)	0.36 ± 0.08 (8)	0.68 ± 0.14 (8)
C16:0	6.83 ± 0.83 (9)	7.54 ± 0.52 (6)	9.41 ± 1.96 (9)	6.89 ± 0.48 (8)	5.52 ± 1.23 (8)
C17:0	0.14 ± 0.03 (9)	0.11 ± 0.03 (6)	0.29 ± 0.05 (8)	0.14 ± 0.02 (8)	0.18 ± 0.03 (8)
C17:1	0.29 ± 0.07 (9)	0.50 ± 0.20 (5)	0.81 ± 0.20 (5)	0.37 ± 0.05 (8)	0.53 ± 0.06 (8)
C18:0	6.55 ± 0.77 (9)	6.59 ± 0.60 (6)	6.36 ± 1.42 (9)	5.86 ± 0.30 (7)	5.09 ± 1.12 (8)
C18:1n-9c	4.63 ± 0.56 (9)	3.66 ± 0.84 (6)	6.05 ± 0.66 (7)	4.14 ± 0.42 (8)	3.04 ± 0.44 (8)
C18:1n-9t	0.17 ± 0.04 (8)	—	0.18 ± 0.03 (6)	—	0.07 ± 0.03 (2)
C18:2n-6	0.63 ± 0.13 (9)	0.43 ± 0.16 (6)	1.23 ± 0.13 (8)	0.53 ± 0.14 (8)	0.95 ± 0.18 (8)
C18:3n-3	—	—	0.03 ± 0.00 (3)	—	0.04 ± 0.02 (4)
C18:3n-6	—	—	0.03 ± 0.01 (5)	—	0.01 (1)
C20:0	0.03 ± 0.02 (2)	—	0.02 (1)	—	0.05 ± 0.01 (2)
C20:1n-9	8.15 ± 0.89 (9)	6.02 ± 1.49 (6)	8.19 ± 0.55 (8)	7.30 ± 0.65 (8)	6.22 ± 0.91 (8)
C20:4n-6	0.06 ± 0.03 (2)	0.10 (1)	—	0.16 ± 0.07 (3)	—
C20:5n-3	1.24 ± 0.18 (9)	1.09 ± 0.31 (6)	1.64 ± 0.18 (7)	0.93 ± 0.24 (8)	1.06 ± 0.14 (8)
C21:0	0.12 (1)	—	0.06 ± 0.01 (5)	0.03 ± 0.02 (2)	0.14 ± 0.03 (8)
C22:0	—	—	—	—	—
C22:1n-9	—	—	—	0.17 (1)	—
C22:2	0.12 ± 0.03 (4)	0.06 ± 0.03 (4)	0.16 ± 0.04 (7)	0.15 ± 0.06 (6)	0.29 ± 0.05 (7)
C22:6n-3	0.03 ± 0.01 (4)	0.05 ± 0.02 (4)	0.08 ± 0.02 (8)	0.08 ± 0.03 (5)	0.13 ± 0.02 (8)

Table 4 Mean concentrations (reported in µg FA/mg dry weight tissue) and standard error (*SE*) for all each freshwater mussel fatty acid (FA) grouping per different species, seasons, and drainages. The studied FA groupings were total FA, total saturated FA (SAFA), total monounsaturated FA (MUFA), total polyunsaturated FA (PUFA), total essential FA (EFA)/algal FA and bacterial FA. Statistical differences [test statistics and degrees of freedom (df)] in mean FA concentration are denoted with different superscript letters (Tukey's HSD) at $p < 0.05$ (**bold**).

Effect	Variables	Total FA		Total SAFA		Total MUFA		Total PUFA		Total EFA/ Algal FA		Total Bacterial FA	
		Mean ± *SE*	**χ^2 (df) *p***	**Mean ± *SE***	**χ^2 (df) *p***	**Mean ± *SE***	**χ^2 (df) *p***	**Mean ± *SE***	**χ^2 (df) *p***	**Mean ± *SE***	**χ^2 (df) *p***	**Mean ± *SE***	**χ^2 (df) *p***
Species	***C. necki***	33.54 ± 3.68	$7.28_{(4)}$ *0.1217*	18.14 ± 1.37[a]	$10.02_{(4)}$ ***0.0400***	12.83 ± 1.90	$8.10_{(4)}$ *0.0879*	2.57 ± 1.02	$4.17_{(4)}$ *0.3837*	2.45 ± 1.00	$4.49_{(4)}$ *0.3441*	0.17 ± 0.04	$2.66_{(4)}$ *0.617*
	C. petrina	43.09 ± 7.91		18.17 ± 2.90[ab]		20.86 ± 4.12		4.06 ± 1.05		3.73 ± 0.92		0.28 ± 0.07	
	C. pustulosa	30.88 ± 3.67		13.80 ± 1.38[b]		14.26 ± 1.95		2.82 ± 0.52		2.57 ± 0.47		0.28 ± 0.04	
	L. bergmanni	23.05 ± 2.96		13.09 ± 0.86[b]		8.37 ± 1.82		1.75 ± 0.40		1.65 ± 0.37		0.21 ± 0.05	
	L. bracteata	29.54 ± 3.47		15.46 ± 2.07[ab]		12.72 ± 1.43		2.36 ± 0.33		2.25 ± 0.31		0.30 ± 0.08	
Season	**Fall**	28.09 ± 1.72	$1.04_{(2)}$ *0.5944*	13.41 ± 1.10[b]	$8.78_{(2)}$ ***0.0124***	12.57 ± 0.70	$0.11_{(2)}$ *0.9446*	2.52 ± 0.17	$3.82_{(2)}$ *0.1480*	2.35 ± 0.16	$3.72_{(2)}$ *0.1558*	0.29 ± 0.04	$5.84_{(2)}$ *0.0538*
	Spring	37.49 ± 4.99		17.41 ± 1.62[a]		16.88 ± 2.74		3.19 ± 0.69		2.94 ± 0.62		0.26 ± 0.05	
	Summer	32.39 ± 4.61		17.55 ± 1.72[a]		12.42 ± 2.37		2.67 ± 1.24		2.54 ± 1.21		0.17 ± 0.05	
Drainage	**Guadalupe River (GR)**	28.52 ± 2.59[ab]	$8.31_{(3)}$ ***0.0400***	15.73 ± 0.97[a]	$11.03_{(3)}$ ***0.0116***	10.70 ± 1.37[ab]	$9.22_{(3)}$ ***0.0266***	2.20 ± 0.58[b]	$10.99_{(3)}$ ***0.0118***	2.09 ± 0.47[b]	$11.10_{(3)}$ ***0.0112***	0.19 ± 0.03	$5.41_{(3)}$ *0.144*
	Colorado River (CR)	51.41 ± 8.86[a]		20.89 ± 3.08[a]		25.04 ± 4.68[a]		5.49 ± 1.16[a]		4.96 ± 1.04[a]		0.40 ± 0.08	
	Llano River (LR)	29.24 ± 2.32[ab]		14.75 ± 1.41[ab]		12.92 ± 1.03[ab]		2.20 ± 0.24[ab]		2.11 ± 0.22[ab]		0.24 ± 0.05	
	Navasota River (NR)	25.04 ± 2.06[b]		11.67 ± 1.22[b]		11.33 ± 0.95[b]		2.04 ± 0.30[b]		1.86 ± 0.27[b]		0.24 ± 0.04	

Table 5 Mean concentrations (reported in µg FA/mg dry weight tissue) and standard error (*SE*) for all each freshwater mussel fatty acid (FA) grouping per different species, seasons, and drainages. The studied FA groupings were n-3 FA, n-6 FA, n-3:n-6, eicosapentaenoic acid:arachidonic acid (EPA:ARA), and docosahexanoic acid:eicosapentaenoic acid (DHA:EPA). Statistical differences [test statistics and degrees of freedom (df)] in mean FA concentration are denoted with different superscript letters (Tukey's HSD) at $p < 0.05$ (**bold**).

Effect	Variables	n-3 Mean ± *SE*	n-3 χ^2 (df) *p*	n-6 Mean ± *SE*	n-6 χ^2 (df) *p*	n-3:n-6 Mean ± *SE*	n-3:n-6 χ^2 (df) *p*	EPA:ARA Mean ± *SE*	EPA:ARA χ^2 (df) *p*	DHA:EPA Mean ± *SE*	DHA:EPA χ^2 (df) *p*
Species	*C. necki*	1.10 ± 0.17	$3.72_{(4)}$ 0.4447	1.35 ± 0.91	$5.38_{(4)}$ 0.2503	3.44 ± 0.78^{a}	$11.11_{(4)}$ **0.0254**	29.65 ± 8.77	$8.16_{(4)}$ 0.086	0.03 ± 0.00^{b}	$16.34_{(4)}$ **0.0026**
	C. petrina	2.27 ± 0.55		1.47 ± 0.38		1.88 ± 0.19^{ab}		8.24 ± 3.89		0.04 ± 0.01^{ab}	
	C. pustulosa	1.48 ± 0.26		1.09 ± 0.22		1.55 ± 0.15^{b}		15.84 ± 9.50		0.08 ± 0.01^{a}	
	L. bergmanni	1.06 ± 0.23		0.74 ± 0.16		2.69 ± 0.83^{ab}		12.74		0.06 ± 0.01^{ab}	
	L. bracteata	1.36 ± 0.20		0.91 ± 0.15		1.90 ± 0.27^{ab}		10.62		0.04 ± 0.01^{ab}	
Season	Fall	1.37 ± 0.10	$1.23_{(2)}$ 0.5395	0.99 ± 0.08^{a}	$7.18_{(2)}$ **0.0276**	1.58 ± 0.11^{b}	$12.57_{(2)}$ **0.0019**	11.88 ± 6.86	$3.33_{(2)}$ 0.1888	0.08 ± 0.01^{a}	$10.45_{(2)}$ **0.0054**
	Spring	1.80 ± 0.36		1.18 ± 0.27^{ab}		2.25 ± 0.28^{ab}		13.69 ± 4.90		0.05 ± 0.01^{b}	
	Summer	1.11 ± 0.22		1.60 ± 1.22^{b}		3.79 ± 0.96^{a}		52.84		0.03 ± 0.00^{b}	
Drainage	Guadalupe River (GR)	1.08 ± 0.14	$8.19_{(3)}$ 0.0656	1.11 ± 0.54^{b}	$13.53_{(3)}$ **0.0036**	3.14 ± 0.57^{a}	$15.84_{(3)}$ **0.0012**	26.27 ± 7.59^{a}	$12.92_{(3)}$ **0.0048**	0.05 ± 0.01^{bc}	$16.32_{(3)}$ **0.001**
	Colorado River (CR)	2.86 ± 0.61		2.10 ± 0.43^{a}		1.51 ± 0.21^{b}		10.35 ± 6.75^{b}		0.06 ± 0.01^{ab}	
	Llano River (LR)	1.32 ± 0.14		0.80 ± 0.10^{b}		2.05 ± 0.20^{a}		20.97 ± 8.39^{ab}		0.04 ± 0.01^{c}	
	Navasota River (NR)	1.09 ± 0.15		0.77 ± 0.13^{b}		1.52 ± 0.12^{ab}		9.96 ± 2.62^{ab}		0.10 ± 0.01^{a}	

4.8 Figures

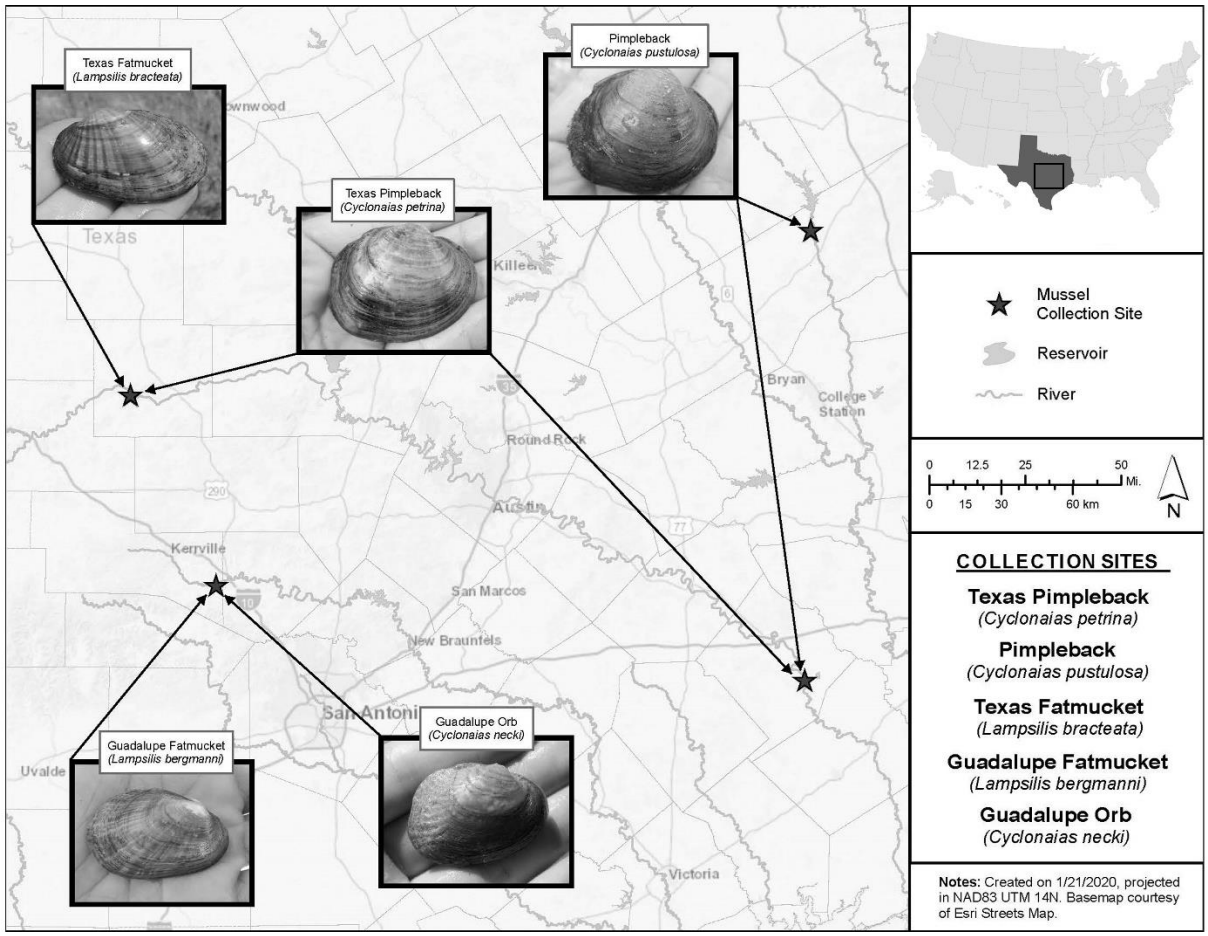

Figure 1 Map of Texas, USA with collection sites and river drainages

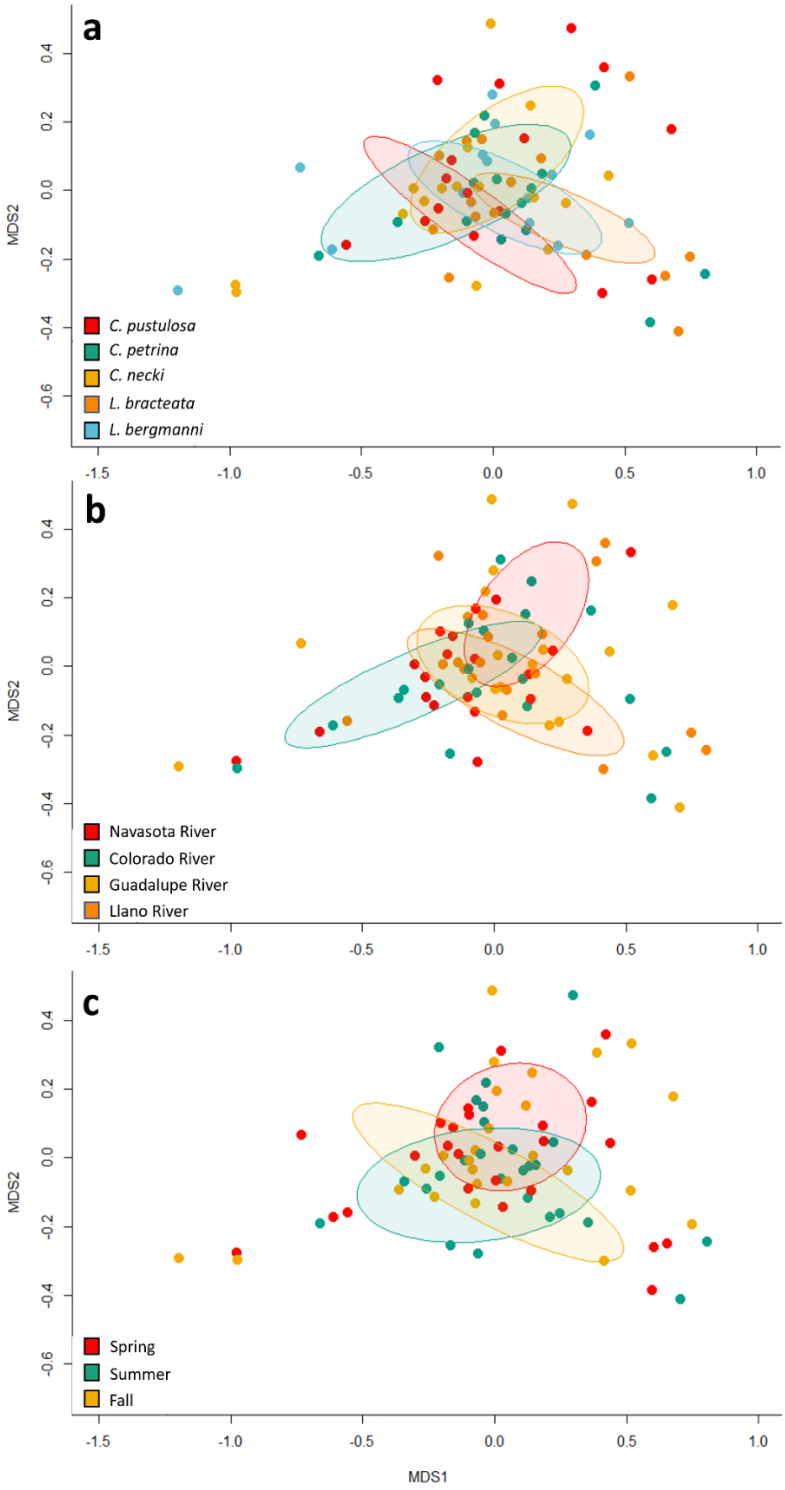

Figure 2 Nonmetric multidimensional scaling plot of fatty acids across the species *Cyclonaias pustulosa*, *C. petrina*, *C. necki*, *Lampsilis bracteata*, and *Lampsilis bergmanni* (a), Navasota River, Colorado River, Guadalupe River, and Llano River drainages (b), and spring, summer, and fall seasons (c). Ellipses represent multivariate dispersion of the weighted covariance matrix.

CHAPTER 5
A REVIEW OF NORTH AMERICAN FRESHWATER MUSSEL (BIVALVIA: UNIONIDA) LETHAL THERMAL TOLERANCE

5.1 Abstract

Freshwater mussels of the order Unionida are currently one of the most imperiled groups of organisms in the North American faunal region. Accurate risk assessments and development of effective management strategies for remaining populations require knowledge of thermal limits in the face of increasing surface water temperature due to climate change and various anthropogenic factors. We conducted a systematic literature review of unionid mussels (order Unionida, families Margaritiferidae and Unionidae) in the North American faunal region to (1) summarize lethal thermal tolerance data by life stage and taxonomy, (2) discuss ecological and climate change implications of existing lethal tolerance data (3) identify needs for future research. We identified lethal tolerance estimates for only 28 of 302 species in the families Unionidae and Margaritiferidae. The mean acute median lethal temperatures were 32.8°C for glochidia (19 species), 35.0°C for juveniles (13 species), and 36.3°C for adults (4 species). Generally, glochidia were less tolerant than juveniles or adults of the same species – but there were several exceptions. Generally, Amblemini had the highest acute and chronic thermal tolerance of all tribes followed by Anodontini, Pleurobemini, Lampsilini, and Quadrilini. Acclimation temperature affected lethal tolerance endpoints in less than half (52 of 145) of comparisons within species. Lethal tolerance data for additional species, combined with a comprehensive database of stream temperatures would be of great use in modeling the frequency and duration of lethal limit exceedance in North American systems and which taxa and populations are currently living at or near their upper lethal limits.

5.2 Introduction

Mean and extreme temperatures in North America are projected to continue increasing along with regional patterns of increased extreme precipitation and increased drought frequency and duration (IPCC, 2021). In August of 2020, temperature anomalies in the contiguous United States were 1.4°C above the 1901-2000 mean (NOAA National Centers for Environmental Information, 2021). With a 4°C increase in global temperatures relative to 1850–1900 baselines, a large portion of North America will experience an additional 30 days with daily maximum temperatures over 35°C (IPCC, 2021).

Freshwater habitats are already some of the most degraded on the Earth; aquatic organisms can be exposed to multiple stressors, including loss of suitable habitat and water quality parameters, loss of connectivity, and changes to thermal and flow regimes (Strayer & Dudgeon, 2010; Ganser *et al.*, 2013). Aquatic ectotherms are particularly vulnerable to changes in environmental temperatures (Fry, 1947; Westhoff & Rosenberger, 2016) because temperature is a critical factor affecting their behavior, physiology, reproduction, and distribution.

Freshwater mussels of the order Unionida are currently one of the most imperiled groups of organisms in the North American faunal region, and their imperilment is likely compounded by global climate change. Approximately 65% of freshwater mussels in North America are endangered, threatened, or vulnerable (Haag & Williams, 2014). Mussels are ecosystem engineers that contribute to many ecological processes in aquatic systems, including habitat alteration for other organisms, nutrient cycling, and streambed stabilization (Vaughn et al., 2004; Howard & Cuffey, 2006; Gangloff & Feminella, 2007; Vaughn, 2018). Mussels are particularly vulnerable to thermal stressors because they are sedentary, which limits their ability to seek refugia from stressful temperatures (Gough et al., 2012), and they have a complex parasitic life

cycle requiring host fishes that may have different thermal requirements (Pandolfo et al., 2012). However, there is a limited understanding of their thermal ecology on which to base conservation, restoration, propagation, and management strategies.

Unionid thermal ecology is a relatively new discipline compared to the thermal body of research for other aquatic ectotherms (e.g., fishes; Brett, 1952; Heath, 1967; Lowe & Heath, 1969; Sylvester, 1975; McFarlane et al., 1976; Becker & Genoway, 1979). Investigation of unionid thermal tolerance has developed only recently, with a single study published in the 1990s (Dimock & Wright, 1993) and most others published after 2010 (Pandolfo et al., 2010a; Pandolfo et al., 2010b; Galbraith et al., 2012, 2020; Archambault et al., 2013, 2014a,b; Ganser et al., 2013; Martin, 2016; Payton et al., 2016; Černá et al., 2018; Khan et al., 2019, 2020). In comparison, thermal tolerance studies on reptiles and sea hares started in the 1940s (Cowles & Bogert, 1944; Fry, 1947), and a great body of work had been completed for fish by the 1990s, allowing methodologies and thermal ecology framework to take shape (Lutterschmidt & Hutchison, 1997). While both their upper and lower thermal limits have been investigated for many ectotherms, most thermal tolerance work on unionids has been focused primarily on upper thermal limits.

Lethal thermal tolerance for aquatic ectotherms falls into two broad categories: static or dynamic studies. Static studies are those in which endpoints are evaluated after exposure to a constant temperature, and dynamic studies are those in which temperature is changed at a constant rate until endpoints are achieved (i.e., loss of equilibrium; Lutterschmidt & Hutchison, 1997). The lethal endpoint typically reported for static studies is lethal temperature (LT). The median lethal temperature (LT50) endpoint is the most frequently used for unionids – where the LT50 value represents the temperature expected to cause mortality in 50% of an exposed

population in a specified time (Reynolds & Casterlain, 1979). LT50 values are the most reported in unionid thermal tolerance literature, similar to other toxicological measures [e.g., median lethal concentrations (LC50) for chemicals]. These values are not inherently protective (i.e., expectation of being lethal to 50% of exposed animals), but they offer the greatest precision along a dose-response curve and are a standard measure of toxicity recognized by regulatory agencies such as the Environmental Protection Agency. More protective values sometimes are reported or requested by regulatory agencies (e.g., an LT05, the temperature at which only 5% of the population would experience mortality). However, values at the lower end of dose-response curves tend to have much wider confidence intervals and sometimes cannot be determined due to lack of partial mortality data (ASTM, 2013).

The lethal endpoint typically reported for dynamic studies is the critical thermal maximum (CTM), defined as "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death" (Cowles & Bogert, 1944). The general sequence of CTM endpoints for ectotherms are onset of muscle spasms, loss of righting response or equilibrium, and death (Lutterschmidt & Hutchison, 1997). Unionid CTM has been defined as the temperature at which the animal experiences extreme gaping behavior characterized by relaxed adductor muscles, parted mantle tissue and siphons, foot extension and is unresponsive to probing (Galbraith et al., 2012). Dynamic studies are advantageous because experiments can be completed quickly (e.g., within hours) and they require a small number of individuals for statistically relevant data (Bennett & Judd, 1992; Lutterschmidt & Hutchison, 1997; Galbraith et al., 2012).

When considering effects of global climate change and increasing surface water temperature on unionid mussels, it is imperative to synthesize current information on their

thermal limits. Once this is done, knowledge gaps, future research directions, and management strategies can be identified. The objectives of this literature review were to (1) summarize lethal thermal tolerance data for unionids by life stage and taxonomy, (2) discuss ecological and global change implications for existing lethal tolerance data (3) identify needs for future research and methodological standardization.

5.3 Methods

We conducted an initial literature search in February 2021 using specific search terms in Auburn University's Discovery database, which is a comprehensive literature portal. We searched the literature for all lethal thermal tolerance studies in which temperature was the only toxicant. The terms used were "unionid* OR margaritif* AND temperature OR thermal tolerance OR critical thermal maximum OR critical thermal minimum AND survival OR mortality OR reproduction." Search results were first examined by reading paper titles and if a paper had a title with relevant criteria, the abstract was then read. Publications were excluded if they were about freshwater mussels in other taxonomic orders besides Unionida (e.g., *Dreissena*) or if they included secondary stressors in addition to temperature (e.g. heavy metals, ammonia). From each paper in the initial search, we noted relevant citations and each of those papers was additionally included in our database for data extraction in an identical manner to publications that resulted from the initial database search. There is a body of thermal research for unionids that was not within the scope of this review, including sublethal effects of temperature (Baker & Hornbach, 2001; Jones et al., 2005; Pandolfo et al., 2010a; Archambault et al., 2013, 2014b; Ganser et al., 2013; Fritts et al., 2015; Aceves et al., 2018; Seagroves et al., 2019; Haney et al., 2020), thermal guilds (Spooner & Vaughn, 2008; Galbraith et al., 2010; Gough et al., 2012; Atkinson et al.,

2014; Gates et al., 2015), and paired toxicology tests which expose mussels to temperature and other stressors simultaneously (see Farris & Van Hassell, 2006 and studies therein).

Papers with relevant titles and abstracts were further examined to extract information of interest. All papers were categorized by taxa and life-stage evaluated. Data extracted from studies included, if applicable: LT50 and LT05 data, along with test duration, CTM and rate of temperature change, initial acclimation temperature, and its rate of change and duration. When reported, survival, average exposure temperature, and maximum exposure temperature were also recorded in addition to any treatment notes (e.g. immersed vs. emersed).

In unionid studies, acute exposures are generally those that occur for durations of less than 24 hours (glochidia) to 96 hours for juveniles and adults, while chronic exposures are those that occur for at least 21 days (ASTM, 2013). Because of the study durations in this review and the gap between the typical acute and chronic test time frames (i.e., 96 h and 21 days), we classified studies of < 7 days as acute, studies lasting 7–20 days as medium-term, and studies lasting ≥ 21days as chronic. Studies also were categorized by either "static" or "dynamic" exposure method. Static studies were those in which an animal was acclimated at a specific temperature, and then exposed at a different but stable temperature (Westhoff & Rosenberger, 2016). Dynamic studies are those in which the temperature that the animal is exposed to changes over time, which can include critical thermal maximum or ramping studies and studies where animals were exposed to ambient or diel temperature fluctuations (Westhoff & Rosenberger, 2016). Data were then organized by life stage, chronic or acute exposure, and taxa evaluated. When a species had no significant difference between treatment types (immersed vs. emersed, aeration vs. no aeration), acclimation temperature, or test length duration within a study, we calculated a mean of all lethal endpoints (i.e., LT50, LT05, or CTM) and 95% confidence

intervals. It was determined that there were no significant differences in treatment types, acclimation temperature, or test duration were determined if 95% confidence intervals from the original study did not overlap. If it was determined that there were no significant differences between treatment types, acclimation temperature, or test duration we combined endpoint values and calculated a new mean and associated confidence intervals. Any mean and associated confidence intervals calculated by the authors of this review are denoted in each table.

All species in the initial publications were assessed to confirm if the taxonomic identification is current. Taxonomic confirmation was conducted using the MUSSEL Project MUSSELp database (Graf & Cummings, 2021). We reported only the valid taxonomy in the results of this paper, but we have included both valid and nominal taxonomy used in publications in Appendix A.

5.4 Results and Discussion

5.4.1 Lethal Tolerances

We found LT data for 30 of the 302 species recognized in the North American faunal region — 28 of the 297 species in the family Unionidae (10%) and no species in the family Margaritiferidae. Twenty-eight of the 302 North American species currently recognized in Unionidae (Graf & Cummings, 2021) were represented in LT studies for one or more life stages. These species spanned the Amblemini, Lampsilini, Pleurobemini, and Quadrilini tribes in the subfamily Ambleminae and the Anodontini tribe in the subfamily Unioninae (Figure 1; Graf & Cummings, 2021). Lethal temperature studies are thus lacking for 90% of Unionidae species. Each species in Unionidae had LT data for one or more life stages (i.e., glochidia, juvenile, and adult), including three species for which LTs were tested on each life stage [*Amblema plicata*

(Archambault et al., 2014a; Khan et al., 2020), *Alasmidonta varicosa* (Pandolfo et al., 2010a; Galbraith et al., 2012), and *Lampsilis fasciola* (Archambault et al., 2013)].

Because thermal data is lacking for the vast majority of Unionida mussels, we have organized the data synthesized in this review by taxa and by life stage so that existing data can be extrapolated to conspecifics, congeners, or taxa within the same tribe when data are lacking on a specific species of interest. Table 1, organized by taxonomic tribe, summarizes all lethal studies examined in this review.

5.4.1a Glochidia

Static Acute Exposure – Lethal tolerances of glochidia have been evaluated for 19 species (Table 2; Pandolfo et al., 2010a; Archambault et al., 2014a,b; Khan et al., 2019). All studies reported the LT50, and most studies also reported the LT05. LT50 values from all tests (duration 12 to 24 h; ASTM, 2013) ranged from 21.4°C to 42.6°C (Pandolfo et al., 2010), with an overall mean of 32.8°C (n = 36). The LT50 values showed a left skewed distribution, with 19% of LT50s <30°C, 46% of LT50s between 30°C and 35°C, and the remaining 35% of LT50 data >35°C. Glochidia in all studies were acclimated to one of two acclimation temperatures (22°C or 27°C) for 24 hours. Among those acclimated to 22°C, LT50 values ranged from 24.2°C to 42.6°C (Pandolfo et al., 2010a). In tests where glochidia were acclimated to 27°C, LT50s ranged from 21.4°C to 37.2°C (Pandolfo et al., 2010a; Archambault et al. 2014; Table 2). LT50 values were lower for animals acclimated to 27°C compared to 22°C for glochidia of nine species (Figure 4), but the differences were statistically significant for only two of the nine species studied (*L. cariosa* and *L. fasciola*; Figure 2; Archambault et al., 2014a). Thermal tolerance of glochidia was not sensitive to changes in acclimation temperature for most mussel

species but when differences did occur, higher acclimation temperatures appeared to reduce thermal tolerance.

LT05 values from all tests (duration 12 to 24 h) ranged from 15.6°C to 36.7°C (Pandolfo et al., 2010a, Archambault et al., 2014), with an overall mean of 26.8°C (*n* = 28). The LT05 values for glochidia ranged from 15.6°C to 31.1°C when acclimated to 22°C (Pandolfo et al., 2010; Archambault et al., 2014) and ranged from 20.6°C to 36.7°C when acclimated to 27°C (Khan et al., 2019; Archambault et al., 2014). Based upon mean tribe LT50 values, Quadrilini appeared to be the most thermally sensitive tribe and Anodontini appeared to be the most thermally tolerant tribe for glochidia. However, this comparison requires caution because only two Anodontine (n = 4 LT50 values) and two Quadrilini (*n* = 4 LT50 values) species were tested at the glochidial life stage.

Static Medium, Static Chronic, and Dynamic Exposures – We found no static medium, static chronic, or dynamic exposure studies for glochidia.

5.4.1b Juveniles

Static Acute Exposure - We found 13 species (family Unionidae) for which juvenile acute thermal tolerance was evaluated (Table 3; Dimock & Wright 1993; Pandolfo et al., 2010b; Archambault et al., 2014a,b). All studies reported LT50s and most reported LT05s. LT50 values from all tests (duration 48 to 96 h) ranged from 29.9 to 44.4°C (Archambault et al., 2014; Pandolfo et al., 2010b), with an overall mean of 35.0°C (*n* = 54). All juveniles were held at their respective acclimation temperature (20, 22, or 27°C) for a minimum of 24 hours before being transferred and then exposed to the targeted experimental temperature for ≤ 96 h (ASTM 2013). Depending on the experiment, juveniles were exposed to experimental temperatures in water

only, water plus sediment, or sediment only. There was no significant effect of exposure duration (48, 96 h) on LT50s for any species exposed for different durations within the same study.

Acclimation temperatures rarely had a significant effect on LT50s, with LT50 values fairly evenly distributed on either side of a 1:1 line (Figure 4). When mussels were exposed to experimental temperatures in water-only or in sediment-only (i.e. drought simulation), acclimation temperature did not significantly effect LT50s for any of the 11 species tested (Figure 2). For tests containing water plus sediment, acclimation temperature affected the LT50 for one of three species tested (Figure 2; Archambault et al., 2014b).

In contrast to glochidia, Anodontini appeared to be the most thermally sensitive tribe and Lampsilini appeared to be the most thermally tolerant tribe for juveniles. However, this comparison requires caution because only one Anodontine species was tested at the juvenile life stage, compared to eight Lampsilines.

Static Medium and Chronic Exposure – Juveniles of three species from two tribes in the family Unionidae had medium and chronic thermal tolerance (>5 days exposure) evaluated using static exposure (Figure 2; Table 3; Ganser et al., 2013); all were acclimated to 20°C for ≥ 24 hours prior to the start of experiments, and the studies reported both the LT50 and LT05. LT50 values from all tests (duration 7 to 28 d) ranged from 30.5 to 35.6°C, with an overall mean of 30.1°C, (n = 12). In general, LT50s decreased with increasing exposure time for the three species tested (*L. siliquoidea*, *L. abrupta*, and *M. nervosa*). Across species, the 7-day LT50s ranged from 32.5–35.6°C, the 14-day LT50s from 30.1-30.8°C, the 21-day LT50s from 28.0-29.6°C, and the 28-day LT50s from 26.2-30.3°C. The patter of decreasing LT50 values with longer exposure duration also held true when considering the acute thermal tolerances described in the previous section. Medium to chronic LT50s were 4.9°C lower on average than the acute

LT50s for the three species tested. Across exposure durations, *L. siliqoidea* (Lampsilini) was the least tolerant while *M. nervosa* (Quadrulini) was the most tolerant.

Dynamic Exposures – We found no dynamic exposure studies for juveniles.

5.4.1c Adults

Static Acute Exposures - Acute lethal tolerance was evaluated for adults of four species in the family Unionidae (Figure 2; Table 4; Archambault et al., 2013; Khan et al., 2020). LT50s and LT05s were calculated for all species. All adults were held at one or more acclimation temperatures (22, 23, 27, or 30℃) for a minimum of 24 h before being exposed to a given experimental temperature for 24, 48, or 96 h (ASTM, 2013) with or without water. LT50s ranged from 33.7–38.7℃ (Archambault et al., 2013; Khan et al., 2020), with an overall mean of 36.4℃ (n = 22) across all treatments and studies. There was not a significant effect of exposure duration on LT50s for any species tested at all three durations (24, 48, and 96 h). Acclimation temperatures did not significantly affect LT50s for the two species (*L. fasciola* and *A. plicata*) tested at multiple acclimation temperatures, nor did the presence/absence of water affect LT50s for the single species (*L. fasciola*) tested with and without water. Of the three species that could be directly compared, *Fusconaia mitchelli* LT50 was significantly lower than for *C. necki* or *A. plicata*.

Static Medium and Chronic Exposures - No chronic (≥ 21 days) LT50s for adults of any species were found. Medium-duration thermal tests (10 days) were evaluated for adults of three species in the family Unionidae (Figure 2; Table 4; Khan et al., 2020). LT50s and LT05s were calculated for all studies. Two species were acclimated to a single acclimation temperature (27℃) and one species was acclimated to 23, 27, and 30℃ for 96 h prior to the start of experiments. LT50s ranged from 32.4-37.5℃ (Khan et al., 2020), with an overall mean of

35.9℃ (*n* = 6), and the average LT50 for medium-duration exposures was 0.4℃ lower than the adult acute LT50s reported in acute-duration exposures. There was no significant effect of acclimation temperature on LT50s of the single species (*A. plicata*) that was acclimated to more than one temperature.

Dynamic Studies - Dynamic studies evaluating critical thermal maximum (CTM) were conducted for four species of adult mussels representing two tribes (Figure 3; Table 5; Galbraith et al., 2012, 2020). Across all studies, the CTM ranged from 32.1℃ to 42.7℃ (Galbraith et al. 2012, 2020), with an overall mean of 39.5℃ (n = 15). Mussels were maintained at acclimation temperatures of 10, 15, 20, or 25℃ for a minimum of three days prior to the start of experiments. Mussels were then placed in aerated or non-aerated treatments, and temperature increased at a rate of 0.35℃ or 0.46℃ per minute until they reached their CTM. Acclimation temperature had a significant effect on CTM for three species (*A. varicosa, S. undulata*, and *A. heterodonta*) tested, with CTM increasing with increasing acclimation temperature. Increasing thermal tolerance with increasing acclimation temperature was the opposite pattern of what was observed for glochidial static acute studies, where higher acclimation temperatures generally resulted in lower LT50s. Acclimation temperature had no effect for a third species (*E. complanata*). The presence/absence of aeration had no effect on CTM except for one species (*E. complanata*) where CTM was significantly higher with aeration when acclimated to 15℃.

An innovative, in-situ experiment was conducted on *Leaunio lienosus* and *Cambarunio nebulosus* where animals in a mesocosm were exposed to an ambient seasonal temperature regimes as well as a regime that was elevated 3℃ above ambient water temperatures (Payton et al., 2016). Both species exhibited negligible mortality (< 2%) under ambient temperatures. Under the warmer temperature regime, mortality of *L. lienosus* remained negligible, but mortality of *C.*

nebulosus increased to 32% by day 70 – supporting the idea that some mussel populations are already living close to their lethal thermal limits during the hot summer months.

5.4.2 Ecological and Global Change Context

5.4.2a Comparisons across life stages

For acute exposures, glochidia of most species tested were generally less thermally tolerant than juvenile conspecifics (*Megalonaias nervosa*, *Villosa delumbis, Potamilus alatus, Lampsilis siliquoidea, L. cariosa*, *Ellipsaria lineolata*, and *Amblema plicata*). However, the glochidia of three species were more thermally tolerant than juveniles (*Lampsilis fasciola, L. abrupta*, and *Alasmidonta varicosa*). This could be due to a life history adaptation, where the more thermally tolerant juveniles are likely to experience higher temperatures due to their spatial distribution or biology of their host species and deserves further study. There were few comparative studies between glochidia and adults and no clear trend. Glochidia of two species (*Cyclonaias necki* and *Amblema plicata*) were less thermally tolerant than adults, but two other species (*Fusconia mitchelli* and *Lampsilis fasciola*) exhibited similar LT_{50}s across the two life stages. There is currently only one species (*A. plicata*) which has had the juvenile and adult life stages compared for thermal tolerance, and these two life stages had similar acute and chronic upper lethal limits. Due to the limited number of species with data for multiple life-history stages, trends in thermal tolerance among life stages should be viewed with caution. Additional research will be required to accurately assess the degree and consistency of differences in thermal tolerance across life-stages, and the factors driving these differences -when they occur.

5.4.2b Comparisons across tribes

Across all life stages combined, Amblemini had the highest acute thermal tolerance with an average LT50 of 36.5°C (*n* = 17) over 1–4 d. Amblemini was followed by Anodontini (35.5°C; n = 17 over 1–4 d), Pleurobemini (35.1°C; n=4 over 12 h–4 d), Lampsilini, (34.1°C; n = 74 over 12 h–4 d) and Quadrilini (33.6°C; n = 9 over 1–4 d). Chronic thermal tolerance followed a similar trend, with Amblemini having the highest mean chronic LT50 of 36.7°C (n = 4 at 10 d). Amblemini was followed by Quadrilini (36.2°C; n = 1 at 10 d), Pleurobemini (32.4°C; n = 1 at 10 d), and Lampsilini (30.1°C; n = 6 over 7–28 d). The chronic tribal thermal tolerance should be interpreted with caution, with Amblemini and Quadrilini having the greatest chronic tolerance. Within Amblemini, *Amblema plicata* is the only species that has been studied in this tribe, and it's chronic thermal tolerance is represented by one study within one system despite this species being widespread in North America (Khan et al., 2020). Similarly, *Cyclonaias* necki was the only species to have chronic thermal tolerance assessed within Quadrilini within one study in one system (Khan et al., 2020). Accurately assessing patterns in chronic thermal tolerance trends across tribes will require greater sampling of subpopulations across geographic gradients and greater species representation.

5.4.2c Climate and global change context

Anthropogenic drivers of extreme heat events include low flow, thermal pollution, and atmospheric warming. Currently many lotic systems in North America are facing low flow events due to human abstraction for agriculture and human consumption (Suren & Riis; 2010; Strayer & Dudgeon, 2010; Rolls et al., 2012). Systems with low flow are more vulnerable to warming due to their lower thermal capacity and experience more extreme thermal dynamics, particularly in smaller systems (Pletterbauer et al., 2018). Climate change will continue to

produce changes to hydrological regimes with altered precipitation and surface run off as well as additional diversion of water for anthropogenic use as water scarcity increases. Thermal pollution can cause increased ambient water temperatures through inputs of heated industrial effluents or reductions in riparian cover that increases surface heat flux from solar radiation inputs (Speight, 2020). Discharge of heated industrial effluents can cause increases in recipient water by 4–8°C (Wellborn & Robinson, 1996; Cooke et al., 2004; Encina et al., 2008). Land use for development, infrastructure and agriculture has reduced riparian vegetation, and the importance of riparian cover in mitigating stream temperatures is correlated with channel width and velocity, with riparian cover being more important for systems with low flow (Woltemade & Hawkins, 2016; Durfee et al., 2021).

Maximum stream temperatures in the southern regions of North America can already reach 34–40°C (Wellborn & Robinson, 1996; Dyar & Alhadeff, 1997; Wright et al., 1999; Spooner & Vaughn, 2008) which is at or above the acute LT50 grand mean of 34.6°C and the chronic LT50 grand mean of 32.0°C across all life stages. Empirical modeling of stream warning shows that riverine temperatures increase 1°C with every 1°C increase in air temperature (Leach & Moore, 2019). The Intergovernmental Panel on Climate Change (IPCC) has modeled climate scenarios projecting 1.5°C (near term; 2021–2040) to 4°C (long term; 2081-2100) increases in global temperatures, with stream temperatures increasing in tandem (IPCC, 2021). Additional models reveal that stream temperatures are predicted to increase an average of 2.2°C in North America by 2100, or an average of 0.6°C increase and stream temperature for every 1°C increase in air temperature (Hill et al., 2014). Regions that have the greatest predicted summer stream temperature warming include the Pacific Northwest and the Appalachian mountains (Hill et al., 2014). The Southeastern Rocky and Appalachian Mountains are predicted to have moderate

warming of 2.3°C, while the Southeastern USA was predicted to have the least responsive stream temperature increases compared to air temperature increases (Hill et al., 2014). The Pacific region has five species of mussels, all of which are endemic, and the Northerm Atlantic Atlantic faunal region, where the Appalachian mountains extend through, have 37 endemic species (Haag, 2012). Seven of 52 total species from the Atlantic faunal region had thermal tolerance data included within this review (14%) and no Pacific mussels have had lethal thermal tolerance evaluated. Despite the south being predicted as the least likely to experience warming, the species reviewed in this publication are already living in habitats that experience temperatures at or near the upper lethal limits where 50% of individuals would experience mortality. The thermal tolerance data synthesized here suggests that climate change will be particularly detrimental for mussel populations in the biodiverse, southern United States. However, as mentioned previously, the current paucity of data limits our ability to predict which taxa and life-stages will be most strongly affected, and which taxa and life-stages may already be adapted to tolerate high temperatures.

Researchers also have investigated sublethal effects of thermal stress, thermal guilds, and the effects of thermal stress coupled with other stressors (e.g., contaminants). Although beyond the scope of this review, they provide additional valuable insight into mussel thermal ecology and the risks associated with thermal inputs from land use and climate changes. Sublethal effects investigated included effects on O:N ratios (Ganser et al., 2015), clearance and filtration rates (Galbraith et al., 2020), ammonium excretion rates and resource acquisition and assimilation which can effect ecosystem services (Spooner & Vaughn, 2008). Additionally, temperature can effect behavior including burrowing (Archambault et al., 2013, 2014b), byssus production (Archambault et al., 2013, 2014b), movement, righting behavior, and valve closure.

Reproduction can also be impacted, including the timing and structure of glochidia release, glochidia maturation, lure display, and the gametogenic cycle. Finally, temperature can impact the structure, development, encystment, metamorphosis, and viability of glochidia. While outside the scope of this review, sublethal effects of temperature are important to consider because they can be an early sign of stress.

5.4.3 Future Directions

The effect of acclimation temperature on metabolic adaptation has been an important topic of research for ectotherms such as reptiles and fish. Acclimation to warm temperatures (both short term and long term) has been found to reduce the metabolic effects of high temperature (Beitinger & Lutterschmidt, 2011; Jutfelt 2020) and studies have evaluated zones of lethal tolerance across a variety of acclimation temperatures and exposures to upper and lower temperatures (see review by Beitinger & Lutterschmidt, 2011). Many unionid studies have tested thermal tolerance of individuals at a variety of acclimation temperatures (Pandolfo et al., 2010b; Archambault et al. 2014a,b; Ganser et al., 2013; Martin, 2016; Khan et al., 2020; Galbraith et al., 2012, 2020) but this review revealed that >50% of studies found no difference in LT50 or CTM endpoints across varying acclimation temperatures. Because acclimation temperature appears to be of less important for freshwater mussels than it is for other ectotherms, evaluating the thermal tolerance for a larger number of species and subpopulations may be more important than evaluating thermal tolerance across a variety of acclimation temperatures for a smaller number of species.

As of this review, upper thermal limits have been published for ~10% of unionid species for at least one life stage. We know even less about how thermal tolerance varies among

subpopulations within a species across geographical gradients. Some represented species have wide latitudinal distribution (*A. plicata*, *C. pustulosa*) but the thermal tolerance endpoints represented here represent only a few subpopulations at similar latitudes. Additionally, studies that compare effects of long-term chronic exposure under ambient seasonal thermal regimes to effects under modified seasonal regimes are extremely rare (but see Payton et al., 2016). Future studies examining latitudinal gradients and/or effects of projected increases in ambient seasonal thermal regimes are much needed. As some geographic regions are predicted to see increased rates of warming, such as the Appalachian and Pacific Northwest, it is important to evaluate the thermal tolerances of mussels within these faunal regions as they have high degrees of endemism and are understudied.

Mussel conservation ecology would benefit from future work on modeling the frequency and duration of lethal upper limit exceedance for unionids in North American systems. Additionally, modeling of stream temperatures in North America, combined with mussel distribution maps, could be used to evaluate what proportion of mussel populations are living in environments that regularly approach their upper thermal limits. These types of models would also be extremely useful for relocation programs that have been proposed as an important method for freshwater organism conservation (Strayer & Dudgeon, 2010). Identifying systems that have suitable thermal regimes will require additional research on general patterns in mussel thermal ecology, as well as species-specific upper and lower thermal tolerance limits.

5.5 References

Aceves, A. K., P. Johnson, S. A. Bullard, S. Lafrentz, and C. R. Arias. 2018. Description and characterization of the digestive gland microbiome in the freshwater mussel Villosa nebulosa (Bivalvia: Unionidae). Journal of Molluscan Studies 84:240–246.

Archambault, J. M., W. G. Cope, and T. J. Kwak. 2013. Burrowing, byssus, and biomarkers: Behavioral and physiological indicators of sublethal thermal stress in freshwater mussels (Unionidae). Marine and Freshwater Behaviour and Physiology 46:229–250.

Archambault, J. M., W. G. Cope, and T. J. Kwak. 2014a. Survival and behaviour of juvenile unionid mussels exposed to thermal stress and dewatering in the presence of a sediment temperature gradient. Freshwater Biology 59:601–613.

Archambault, J. M., W. G. Cope, and T. J. Kwak. 2014b. Influence of Sediment Presence on Freshwater Mussel Thermal Tolerance. Freshwater Science 33:56–65.

ASTM. 2013. Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels. E2455-06(2013), West Conshohocken, PA.

Atkinson, C. L., J. P. Julian, and C. C. Vaughn. 2014. Species and function lost: Role of drought in structuring stream communities. Biological Conservation 176:30–38.

Baker, S. M., and D. J. Hornbach. 2001. Seasonal metabolism and biochemical composition of two unioid mussels, *Actinonaias ligamentina* and *Amblema plicata*. Journal of Molluscan Studies 67:407–416.

Becker, C. D., and R. G. Genoway. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environmental Biology of Fishes 4:245.

Beitinger, T. L. and W. I. Lutterschmidt. 2011. Measures of thermal tolerance. In A. P. Farrell (Ed.), Encyclopedia of fish physiology: From genome to environment pp. 1695-1702. Academic Press, San Diego, CA.

Bennett, W. A., and F. W. Judd. 1992. Comparison of Methods for Determining Low Temperature Tolerance: Experiments with Pinfish, Lagodon rhomboides. Copeia 1992:1059–1065.

Brett, J. R. 1952. Temperature Tolerance in Young Pacific Salmon, Genus Oncorhynchus. Journal of the Fisheries Research Board of Canada 9:265–323.

Černá, M., O. P. Simon, M. Bílý, K. Douda, B. Dort, M. Galová, and M. Volfová. 2018. Within-river variation in growth and survival of juvenile freshwater pearl mussels assessed by in situ exposure methods. Hydrobiologia 810:393–414.

Cooke, S. J., C. M. Bunt, J. F. Schreer. 2004. Understanding fish behavior, distribution, and survival in thermal effluents using fixed telemetry arrays: a case study of smallmouth bass in a discharge canal during winter. Environmental Management 33:140-150.

Cowles, R. B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bulletin of the AMNH ; v. 83, article 5.

Dimock, R. V., and A. H. Wright. 1993. Sensitivity of juvenile freshwater mussels to hyoxic, thermal and acid stress. Journal of the Elisha Mitchell Scientific Society 109:183–192.

Dyar, T. R. and S. J. Alhadeff. 1977. Stream-temperature characteristics in Georgia. U.S. Geological Survey Water-Resources Investigations Report 96-4203.

Encina, L., A. Rodriguez-Ruiz, C. Granado-Lorencio. 2008. Distribution of common carp in a Spanish reservoir in relation to thermal loading from a nuclear power plant. Journal of Thermal Biology 33:444-450.

Farris, J. L., and J. H. Van Hassell. 2006. Freshwater Bivalve Ecotoxicology. CRC Press.

Fritts, A., J. Peterson, P. Hazelton, and R. Bringolf. 2015. Evaluation of methods for assessing physiological biomarkers of stress in freshwater mussels. Canadian Journal of Fisheries and Aquatic Sciences 150311143313000.

Fry, F. E. J. 1947. Effects of the environment on animal activity. Publications of the Ontario Fisheries Research Laboratory 68:1-62.

Galbraith, H. S., C. J. Blakeslee, and W. A. Lellis. 2012. Recent thermal history influences thermal tolerance in freshwater mussel species (Bivalvia:Unionoida). Freshwater Science 31:83–92.

Galbraith, H. S., C. J. Blakeslee, D. E. Spooner, and W. A. Lellis. 2020. A weight-of-evidence approach for defining thermal sensitivity in a federally endangered species. Aquatic Conservation: Marine and Freshwater Ecosystems 30:540–553.

Galbraith, H. S., D. E. Spooner, and C. C. Vaughn. 2010. Synergistic effects of regional climate patterns and local water management on freshwater mussel communities. Biological Conservation 143:1175–1183.

Gangloff, M. M., and J. W. Feminella. 2007. Stream channel geomorphology influences mussel abundance in southern Appalachian streams, U.S.A. Freshwater Biology 52:64–74.

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

Ganser, A., T. Newton, and R. Haro. 2013. The effects of elevated water temperature on native juvenile mussels: Implications for climate change. Freshwater Science 32:1168–1177.

Gates, K. K., C. C. Vaughn, and J. P. Julian. 2015. Developing environmental flow recommendations for freshwater mussels using the biological traits of species guilds. Freshwater Biology 60:620–635.

Gough, H. M., A. M. G. Landis, and J. A. Stoeckel. 2012. Behaviour and physiology are linked in the responses of freshwater mussels to drought. Freshwater Biology 57:2356–2366.

Graf, D. L., and K. S. Cummings. 2021. A 'big data' approach to global freshwater mussel diversity (Bivalvia: Unionoida), with an updated checklist of genera and species | Journal of Molluscan Studies | Oxford Academic. Journal of Molluscan Studies 87.

Haag, W. R., and J. D. Williams. 2014. Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. Hydrobiologia 735:45–60.

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

Heath, W. G. 1967. Ecological Significance of Temperature Tolerance in Gulf of California Shore Fishes. Journal of the Arizona Academy of Science 4:172–178.

Hill, R. A., C. P. Hawkins, and J. Jin. 2014. Predicting thermal vulnerability of stream and river ecosystems to climate change. Climatic Change 125:399–412.

Howard, J. K., and K. M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460–474.

IPCC. 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press.

Jones, J., R. Mair, and R. Neves. 2005. Factors Affecting Survival and Growth of Juvenile Freshwater Mussels Cultured in Recirculating Aquaculture Systems. North American Journal of Aquaculture 67:210–220.

Jutfelt, F. 2020. Metabolic adaptation to warm water in fish. Functional Ecology. 34:1138-1141.

Khan, J., J. Dudding, M. Hart, C. Robertson, R. Lopez, and C. Randklev. 2020. Linking flow and upper thermal limits of freshwater mussels to inform environmental flow benchmarks. Freshwater Biology 1–16.

Khan, J. M., M. Hart, J. F. Dudding, C. R. Robertson, R. Lopez, and C. R. Randklev. 2019. Evaluating the upper thermal limits of glochidia for selected freshwater mussel species (Bivalvia: Unionidae) in central and east Texas, and the implications for their conservation. Aquatic Conservation: Marine and Freshwater Ecosystems 29:1202–1215.

Leach, J. A., and R. D. Moore. 2019. Empirical Stream Thermal Sensitivities May Underestimate Stream Temperature Response to Climate Warming. Water Resources Research 55:5453–5467.

Lowe, C. H., and W. G. Heath. 1969. Behavioral and Physiological Responses to Temperature in the Desert Pupfish Cyprinodon macularius. Physiological Zoology 42:53–59.

Lutterschmidt, W., and V. Hutchison. 1997. The critical thermal maximum: History and critique. Canadian Journal of Zoology-revue Canadienne De Zoologie—CAN J ZOOL 75:1561–1574.

Martin, K. 2016. Upper Thermal Limits Of Freshwater Mussels (Bivalvia, Unionoida) In Ramped Temperature Exposures. MSU Graduate Theses.

McFarlane, R. W., B. C. Moore, and S. E. Williams. 1976. Thermal tolerance of stream cyprinid minnows. United States.

NOAA National Centers for Environmental Information. 2021. Climate at a Glance: Regional Time Series.

Pandolfo, T. J., T. J. Kwak, W. G. Cope. 2012. Thermal tolerances of freshwater mussels and their host fishes: species interactions in a changing climate. Freshwater Mollusk Biology and Conservation 15:69-82.

Pandolfo, T. J., W. G. Cope, and C. Arellano. 2010a. Thermal tolerance of juvenile freshwater mussels (Unionidae) under the added stress of copper. Environmental Toxicology and Chemistry 29:691–699.

Pandolfo, T. J., W. G. Cope, C. Arellano, R. B. Bringolf, M. C. Barnhart, and E. Hammer. 2010b. Upper thermal tolerances of early life stages of freshwater mussels. Journal of the North American Benthological Society 29:959–969.

Payton, S., P. Johnson, and M. Jenny. 2016. Comparative physiological, biochemical, and molecular thermal stress response profiles for two Unionid freshwater mussel species. The Journal of experimental biology 219.

Pletterbauer, F., A. Melcher, and W. Graf. 2018. Climate Change Impacts in Riverine Ecosystems. Pages 203–223 *in* S. Schmutz and J. Sendzimir (editors). Riverine Ecosystem Management: Science for Governing Towards a Sustainable Future. Springer International Publishing, Cham.

Reynolds, W. W., and M. E. Casterlain. 1979. Behavioral Thermoregulation and the “Final Preferendum” Paradigm. American Zoologist 19:211–224.

Rolls, R. J., C. Leigh, F. Sheldon. 2012. Mechanistic effects of low-flow hydrology on riverine ecosystems: ecological principles and consequences of alteration. Freshwater Science 31:1163-1186.

Seagroves, L. A., M. C. Barnhart, T. Hardy, and A. N. Schwalb. 2019. Reproductive ecology of the threatened and endemic freshwater mussel *Lampsilis bracteata*. Aquatic Conservation: Marine and Freshwater Ecosystems 29:1216–1226.

Speight, J. G. 2020. Natural Water Remediation: Chemistry and Technology, Butterworth-Heinemann.

Spooner, D. E., and C. C. Vaughn. 2008. A trait-based approach to species' roles in stream ecosystems: Climate change, community structure, and material cycling. Oecologia 158:307–317.

Strayer, D. L., and D. Dudgeon. 2010. Freshwater biodiversity conservation: Recent progress and future challenges. Freshwater Science 29:344–358.

Durfee, N., C. G. Ochoa, G. Jones. 2021. Stream temperature and environmental relationships in a semiarid riparian corridor. Land. 10:519.

Surn, A. M. and T. Riis. 2010. The effects of plant growth on stream invertebrate communities during low flow: a conceptual model. Journal of the North American Benthological Society 29:711-724.

Sylvester, J. R. 1975. Critical thermal maxima of three species of Hawaiian estuarine fish: A comparative study. Journal of Fish Biology 7114:257–262.

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

Vaughn, C. C., K. B. Gido, and D. E. Spooner. 2004. Ecosystem Processes Performed by Unionid Mussels in Stream Mesocosms: Species Roles and Effects of Abundance. Hydrobiologia 527:35–47.

Wellborn, G. A. and J. V. Robinson. 1996. Effects of thermal effluent on macroinvertebrates in a Central Texas Reservoir. The American Midland Naturalist 136:110-120.

Westhoff, J. T., and A. E. Rosenberger. 2016. A global review of freshwater crayfish temperature tolerance, preference, and optimal growth. Reviews in Fish Biology and Fisheries 26:329–349.

Wright, S. A., F. M. Holly Jr., A. A. Bradley, and W. Krajewski. 1999. Long-term simulation of thermal regime of Missouri River. Journal of Hydraulic Engineering. 125:242-252.

Woltemade, C. J. and T. W. Hawkins. 2016. Stream temperature impacts because of changes in air temperature, land cover and stream discharge: Navarro River Watershed, California, USA. River Research and Application 32:2020-2031.

5.6 Tables

Table 1 Summary of all lethal tolerance studies completed – organized by taxa, life stage evaluated (G = glochidia, J = juvenile, A = adult), whether the study was acute (A) or chronic (C) and whether the study was static (S) or dynamic (D). Includes data type reported (lethal temperature or percent survival at a specific temperature).

Tribe	***Taxa evaluated***	***NatureServe Status***	***IUCN Status***	***Life stages evaluated***	***Acute or Chronic***	***Test Type***	***Endpoint***	***Publication***
Amblemini	*Amblema plicata*	Secure (G5)	Least concern (stable)	G, J, A	A, C	S	Lethal temperature	Archambault, Cope & Kwak, 2014a; Khan *et al.*, 2020
Anodontini	*Alasmidonta heterodon*	Critically imperiled (G1)	Vulnerable (decreasing)	A	A	D	Lethal temperature	Galbraith *et al.*, 2020
	Alasmidonta varicosa	Vulnerable (G3)	Vulnerable (decreasing)	G, J, A	A	D, S	Lethal temperature	Pandolfo *et al.*, 2010b; Ganser *et al.*, 2013
	Lasmigona complanata	Secure (G5)	Least concern (stable)	G	A	S	Lethal temperature	Pandolfo *et al.*, 2010b
	Anodonta digonata	Secure (G5)	Least concern (stable)	J	A	S	Lethal temperature	Dimock & Wright, 1993
	Strophitus undulatus	Secure (G5)	N/D	A	A	D	Lethal temperature	Galbraith *et al.*, 2012
	Utterbackia imbecilis	Secure (G5)	Least concern (stable)	J	A	S	Lethal temperature	Dimock & Wright, 1993
Lampsilini	*Ellipsaria lineolata*	Apparently secure (G4)	Near threatened	G, J	A	S	Lethal temperature	Pandolfo *et al.*, 2010b
	Lampsilis abrupta	Critically imperiled (G1)	Vulnerable (decreasing)	G, J	A, C	S	Lethal temperature	Ganser *et al.*, 2013; Archambault *et al.*, 2014a; Archambault, Cope & Kwak, 2014b
	Lampsilis bergmanni	Critically imperiled (G1)	N/D	G	A	S	Lethal temperature	Khan *et al.*, 2019
	Lampsilis bracteata	Critically imperiled (G1)	Near threatened	G	A	S	Lethal temperature	Khan *et al.*, 2019
	Lampsilis cariosa	Vulnerable (G3)	Vulnerable (decreasing)	G, J	A	S	Lethal temperature	Archambault *et al.*, 2014a
	Lampsilis fasciola	Secure (G5)	Least concern (stable)	G, J, A	A	S	Lethal temperature	Archambault, Gregory Cope & Kwak, 2013

	Lampsilis hydiana	Apparently secure (G4)	Least concern (stable)	G	A	S	Lethal temperature	Khan *et al.*, 2019
	Lampsilis radiata	Secure (G5)	N/D	J	A	S	Lethal temperature	Archambault *et al.*, 2014b
	Lampsilis satura	Imperiled (G2)	Near threatened	G	A	S	Lethal temperature	Khan *et al.*, 2019
	Lampsilis siliquoidea	Secure (G5)	N/D	G, J	A, C	D, S	Lethal temperature	Pandolfo, Cope & Arellano, 2010a; Pandolfo *et al.*, 2010b; Ganser *et al.*, 2013; Archambault *et al.*, 2014b; Martin, 2016
	Lampsilis teres	Secure (G5)	Least concern (stable)	G	A	S	Lethal temperature	Khan *et al.*, 2019
	Ligumia recta	Apparently secure (G4)	Near threatened (decreasing)	G, J	A	S	Lethal temperature, % survival	Pandolfo, Cope & Arellano, 2010a; Pandolfo *et al.*, 2010b
	Obovaria arkansasensis	No status rank	N/D	G	A	S	Lethal temperature	Khan *et al.*, 2019
	Potamilis alatus	Secure (G5)	N/D	G, J	A	S	Lethal temperature	Pandolfo *et al.*, 2010ab
	Villosa delumbis	Apparently secure (G4)	Least concern	G, J	A	S	Lethal temperature	Pandolfo *et al.*, 2010b
	Leaunio lienosus	Secure (G5)	N/D	A	C	D	% Survival	Payton *et al.*, 2016
	Cambarunio nebulosus	Vulnerable (G3)	N/D	A	C	D	% Survival	Payton *et al.*, 2016
Pleurobemini	*Elliptio complanata*	Secure (G5)	Least concern (increasing)	A	A	D	Lethal temperature	Galbraith *et al.*, 2012
	Fusconaia mitchelli	Unranked (GNR)	Critically endangered (decreasing)	G, A	A, C	S	Lethal temperature	Khan *et al.*, 2019, 2020
Quadrilini	*Cyclonaias necki*	Unranked (GNR)	N/D	G, A	A, C	S	Lethal temperature	Khan *et al.*, 2020
	Megalonaias nervosa	Secure (G5)	Least concern (stable)	G, J	A, C	D, S	Lethal temperature	Pandolfo *et al.*, 2010b; Ganser *et al.*, 2013; Archambault *et al.*, 2014b

Table 2 Summary of all lethal temperature studies conducted on glochidia and the experimental parameters. All species were acclimated at a rate of 1°C/h and maintained at the target acclimation temperature for a period of 2 hours prior to the start of experiments. ND = value could not be determined in the original study. Data denoted with an asterisk (*) indicates that acclimation temperature endpoints were averaged for LT50 and LT05 values, upper and lower 95% confidence intervals between the two acclimation temperatures because there was no significant difference between LT50 and LT05 values determined by overlapping confidence intervals. Data denoted with a cross (†) indicates that LT values for that species were collected in the same study across multiple regional sampling sites and there were significant differences of endpoints collected from different locations so values were not combined (significant differences determined by non-overlapping 95% confidence intervals).

Tribe	Taxa evaluated	LT50 (°C) (95% CI)	LT05 (°C) (95% CI)	LT time (h)	Acclimation Temp (°C)	Publication
Amblemini	*Amblema plicata*	28.3 (27.3–29.3)	22.2 (20.2–24.2)	24	27	Khan *et al.*, 2019
Anodontini	*Alasmidonta varicosa*	37.1 (30.9–43.2)	28.3 (20.2–36.3)	24	22 & 27*	Pandolfo *et al.*, 2010b
	Lasmigona complanata	37.5 (31.7–43.3)	29.1 (26.5–36.8)	24	22 & 27*	Pandolfo *et al.*, 2010b
Lampsilini	*Ellipsaria lineolata*	28.4 (21.0–35.7)	ND	24	22 & 27*	Pandolfo *et al.*, 2010b
	Lampsilis abrupta	37.2 (37.0–37.4)	36.7 (ND)	24	27	Archambault, Cope & Kwak, 2014b
	Lampsilis bergmanni	33.1 (32.7–33.4)	27.2 (26.4–28.0)	24	27	Khan *et al.*, 2019
		33.9 (33.6–34.2)	28.5 (28.1–28.9)	12	27	
	Lampsilis bracteata†	32.4 (32.1–32.7)	25.5 (24.7–26.3)	24	27	Khan *et al.*, 2019
		34.7 (34.4–35.0)	27.9 (27.2–28.6)	24	27	

	33.8 (33.6–34.0)	29.7 (29.3–30.1)	24	27	
	33.9 (33.7–34.1)	30.0 (28.8–30.2)	12	27	
Lampsilis cariosa	35.8 (35.3-36.2)	31.1 (25.1-32.8)	24	22	Archambault *et al*. 2014a
	33.3 (32.7-33.8)	23.8 (13.4-27.8)	24	27	
Lampsilis fasciola	36.3 (36.2–36.4)	ND	24	22	Archambault *et al*. 2014a
	35.5 (35.3–35.7)	35 (ND)	24	27	
Lampsilis satura	32.5 (32.0–33.0)	24.2 (23.1–25.3)	24	27	Khan *et al.*, 2019
Lampsilis siliquoidea†	34.4 (34.1–34.7)	29.1 (28.5–29.7)	24	27	Khan *et al.*, 2019; Pandolfo *et al*. 2010a; Archambault *et al*. 2014a
	33.7 (33.5–33.9)	28.7 (28.1–29.3)			
	34.1 (33.9–34.3)	29.9 (29.7–30.1)	12	27	
	32.8 (26.7–38.8)	ND	24	27	
Lampsilis teres	31.1 (30.6–31.6)	22.1 (20.9–20.9)	24	27	Khan *et al.*, 2019
Ligumia recta	37.8 (24.8–50.9)	24.2 (11.5–36.9)	24	22 & 27*	Pandolfo et al. 2010b
Obovaria arkansasensis	33.2	24.2	24	27	Khan *et al.*, 2019

		(32.7–33.7)	(23.1–25.3)			
	Potamilis alatus	22.8 (16.6–29.1)	ND	24	22 & 27*	Pandolfo et al. 2010b
	Villosa delumbis	27.6 (21.1–34.1)	ND	24	22 & 27*	Pandolfo et al. 2010b
Pleurobemini	*Fusconaia mitchelli*	36.1 (35.7–36.5)	27.9 (27.4–28.4)	12	27	Khan *et al.*, 2019
Quadrilini	*Cyclonaias necki*	36.4 (36.0–36.8)	27.4 (26.5–28.3)	24	27	Khan *et al.*, 2019
		26.9 (25.5–28.3)	20.6 (18.1–23.1)	24	27	
	Megalonaias nervosa	29.9 (24.8–34.9)	18.3 (6.4–30.2)	24	22 & 27*	Pandolfo et al. 2010b

Table 3 Summary of all acute, medium, and chronic lethal temperature studies conducted on juveniles and the experimental parameters. All species were acclimated at a rate of 2.5°C/d and maintained at the target acclimation temperature for a period of ≥24 hours prior to the start of experiments, with the exception of the Dimock &Wright 1993 studies which did not report their acclimation rate or duration of acclimation at target temperature. ND = value could not be determined in the original study. Data denoted with an asterisk (*) indicates that acclimation temperature endpoints were averaged for LT50, LT05 values, and upper and lower 95% confidence intervals between acclimation temperatures and/or LT time because there was no significant difference between LT50 and LT05 values determined by overlapping confidence intervals. Data denoted with a dagger (†) indicates that LT values for differing treatment types (watered + sediment versus sediment only) were not significant, with significant differences determined by non-overlapping 95% confidence intervals. Medium-term exposure durations are denoted with a double dagger (‡) to differentiate from chronic exposures (no double dagger).

	Tribe	Taxa evaluated	LT50 (C)	LT05 (C)	LT time (h)	Acclimation temp	Treatment Notes	Publication
ACUTE	Amblemini	*Amblema plicata*	36.4 (35.8–37.0)	ND	96	22 & 27*	Water only	Archambault et al. 2014a
			35.3 (34.6–36.1)	34.8 (30.1–35.6)	96	27	Water + Sediment	Archambault et al. 2014a
			37.2 (36.7–37.7)	ND	96	27	Sediment only	Archambault et al. 2014a
	Anodontini	*Alasmidonta varicosa*	35.05 (32.4–37.7)	30.45 (25.5–35.5)	96	22 & 27*	Water only	Pandolfo et al. 2010b
		Anodonta digonata	33 NR	ND	96	20	Water only	Dimock & Wright 1993
		Utterbackia imbecilis	31.5 NR	ND	96	20	Water only	Dimock & Wright 1993
	Lampsilini	*Ellipsaria lineolata*	36.0 (30.3–41.8)	30.25 (23.6–36.9)	96	22 & 27*	Water only	Pandolfo et al. 2010b
		Lampsilis abrupta	34.9 (34.0–35.8)	34.7 ND	96	22 & 27*	Water only	Archambault et al. 2014a
			32.6	26.1	96	22 & 27*		

		(31.4–34.0)	(17.3–29.8)			Water + Sediment	Archambault et al. 2014b
		36.5 (35.8–37.2)	31.2 (6.4–33.9)	96	27	Water + Sediment	Archambault et al. 2014b
		35.2 (34.4–36.1)	30.7 (14.7–33.2)	96	22 & 27*	Sediment only	Archambault et al. 2014ab
	Lampsilis cariosa	36.2 (35.3–37.0)	22.2 ND	96	22 & 27*	Water only	Archambault et al. 2014a
		35.7 (34.9–36.5)	26.7 (0.2–30.4)	96	22 & 27*	Water + Sediment; Sediment only†	
	Lampsilis radiata	30.5 (29.5–31.5)	26.4 (18.3–28.8)	96	22 & 27*	Water + Sediment	Archambault et al. 2014b
		34.8 (34.1–35.6)	ND		22	Sediment only	
	Lampsilis siliquoidea	35.5 (33.9–37.1)	32.1 (28.5–35.6)	48 & 96*	22 & 27*	Water only	Pandolfo et al. 2010ab; Archambault et al. 2014a
		33.3 (32.4–34.2)	28.7 (17.0–31.1)	96	22	Water + Sediment	
		36.0 (35.4–36.5)	ND		27		
		35.4 (34.5–36.2)	32.2 (23.9–33.8)		22 & 27*	Sediment only	
	Ligumia recta	37.2 (21.2–47.3)	25.1 (17.1–33.0)	48 & 96*	22 & 27*	Water only	Pandolfo et al. 2010ab
	Potamilis alatus	35.1 (33.1–37.0)	30.0 (25.1–35.0)	48 & 96*	22 & 27*	Water only	Pandolfo et al. 2010ab
	Villosa delumbis	34.4 (31.7–37.1)	29.4 (24.2–34.7)	96	22 & 27*	Water only	Pandolfo et al. 2010b
Quadrilini	*Megalonaias nervosa*	34.1	28.6	96	22 & 27*	Water only	

			(31.3–36.9)	(23.0–34.3)			Pandolfo et al. 2010b
MEDIUM AND CHRONIC	Lampsilini	*Lampsilis abrupta*	33.6 (32.5–34.6)	30.2 (27.4–31.5)	7‡		
			30.5	29.5	14‡		
			ND	ND			
			28 (26.9–29.0)	24.3 (22.0–25.6)	21 & 28*		
		Lampsilis siliquoidea	32.5 (31.5–33.5)	30.2 (28.4–31.2)	7‡		
			30.1	24.7	14‡	20	Ganser et al. 2013
			ND	ND			
			26.15 (24.7–27.8)	20.9 (17.1–22.8)	21 & 28*		
	Quadrilini	*Megalonaias nervosa*	35.6	34.3	7‡		
			ND	ND			
			30.8	29.9	14‡		
			ND	ND			
			29.6	30.6	21		
			ND	ND			
			30.3 (28.4–32.5)	25.6 (20.0–27.7)	28		

Table 4 Summary of all acute and medium lethal temperature studies conducted on adults and the experimental parameters. Data denoted with an asterisk (*) indicates that acclimation temperature endpoints were averaged for LT50, LT05 values, and upper and lower 95% confidence intervals between acclimation temperatures and/or LT time because there was no significant difference between LT50 and LT05 values determined by overlapping confidence intervals.

	Tribe	Taxa Evaluated	LT_{50} (°C) (95% CI)	LT_{05} (°C) (95% CI)	LT Time (h)	Acc. Temp. (°C)	Acc. Rate (°C/ day)	Acc. Period (h)	Publication
ACUTE	Lampsilini	*Lampsilis fasciola*	34.1 (33.0-35.4)	27.5 (19.8-30.0)	96	22 & 27*	2.5	≥ 24	Archambault et al. 2013
	Amblemini	*Amblema plicata*	37.3 (35.6–38.9)	36.9 (35.3–39.1)	24, 48 & 96*	23, 27 & 30*	>3	96	Khan et al. 2020
	Quadrulini	*Cyclonaias necki*	37.1 (35.3–39.0)	36.7 (35.2–38.6)	24, 48 & 96*	27	>3	96	Khan et al. 2020
	Pleurobemini	*Fusconaia mitchelli*	37.8 (33.4–36.2)	33.4 (30.7)	24, 48 & 96*	27	>3	96	Khan et al. 2020
MEDIUM	Amblemini	*Amblema plicata*	36.7 (34.7–38.7)	36.2 (34.6–38.1)	240	23, 27 & 30*	>3	96	Khan et al. 2020
	Quadrulini	*Cyclonaias necki*	36.2 (34.5–37.9)	35.4 (30.0-40.9)	240	27	>3	96	Khan et al. 2020
	Pleurobemini	*Fusconaia mitchelli*	32.4 (31.1–33.6)	28.4 (26.0–30.9)	240	27	>3	96	Khan et al. 2020

Table 5 Summary of all dynamic lethal temperature studies conducted on adults and the experimental parameters. Data denoted with an asterisk (*) indicates that acclimation temperature or treatment endpoints were averaged for critical thermal maximum (CTMax) values and upper and lower 95% confidence intervals between acclimation temperatures and/or treatment because there was no significant difference between CTM values determined by overlapping confidence intervals.

Tribe	Taxa evaluated	CTMax (°C) (95% CI)	Acclimation Temp (°C)	CTM Rate of Change	Acclimation Rate	Acclimation Period (d)	Treatment Notes	Publication
Anodontini	*Alasmidonta varicosa*	39.5 (39.15–39.85)	15	0.35°C/ min	3°C/ day	≥ 7	Aerated & Not Aerated	Galbraith et al. 2012
		41.1 (40.8–41.3)	25				Aerated & Not Aerated	
	Alasmidonta heterodonta	32.1 (31.6–32.6)	10	0.46°C/ min	2°C/ day	8		Galbraith et al. 2020
		35.1 (34.3–35.9)	15 & 20			3–8		
	Strophitus undulatus	40 (39.7–40.3)	15	0.35°C/ min	3°C/ day	≥ 7	Aerated	Galbraith et al. 2012
		39.1 (38.7–39.5)	15				Not Aerated	
		42.3 (42.1–42.5)	25				Aerated & Not Aerated	
Pleurobemini	*Elliptio complanata*	42.7 (42.1–43.3)	15	0.35°C/ min	3°C/ day	≥ 7	Aerated	Galbraith et al. 2012
		40.3 (39.9–40.7)	15				Not Aerated	
		41.5 (41.1–41.9)	25				Aerated & Not Aerated	

5.7 Figures

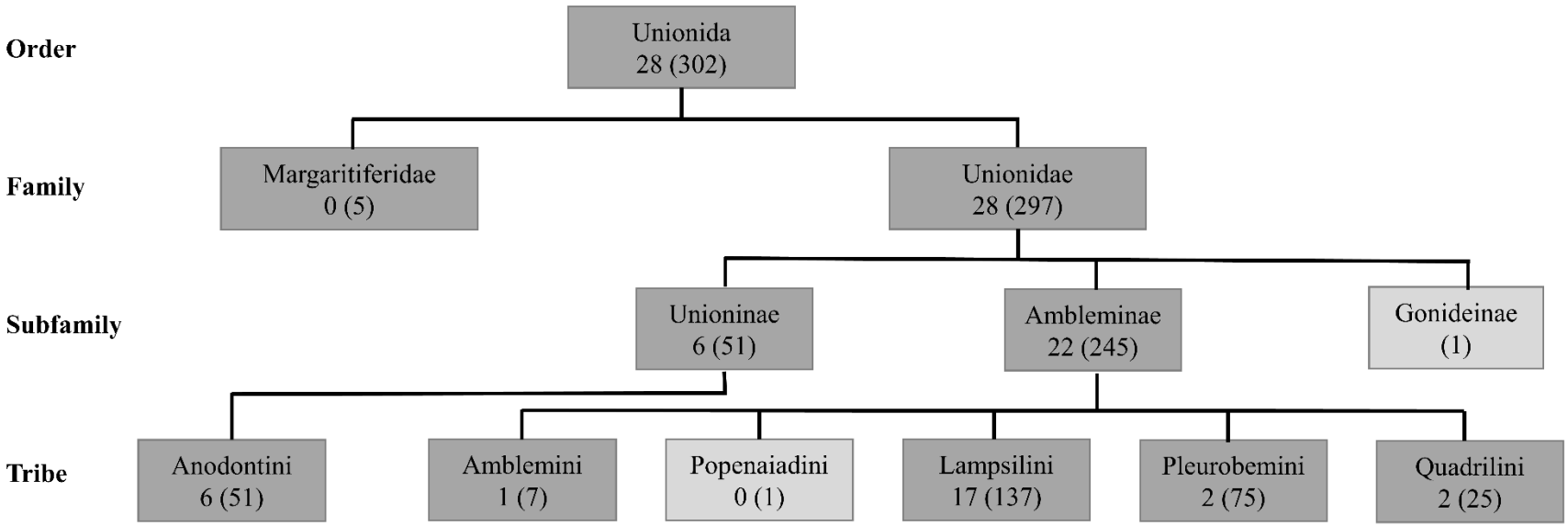

Figure 1 Twenty-eight Unionida species were represented in the lethal thermal literature, shown here by taxonomic group, compared to species richness in the North American faunal region, listed in parentheses.

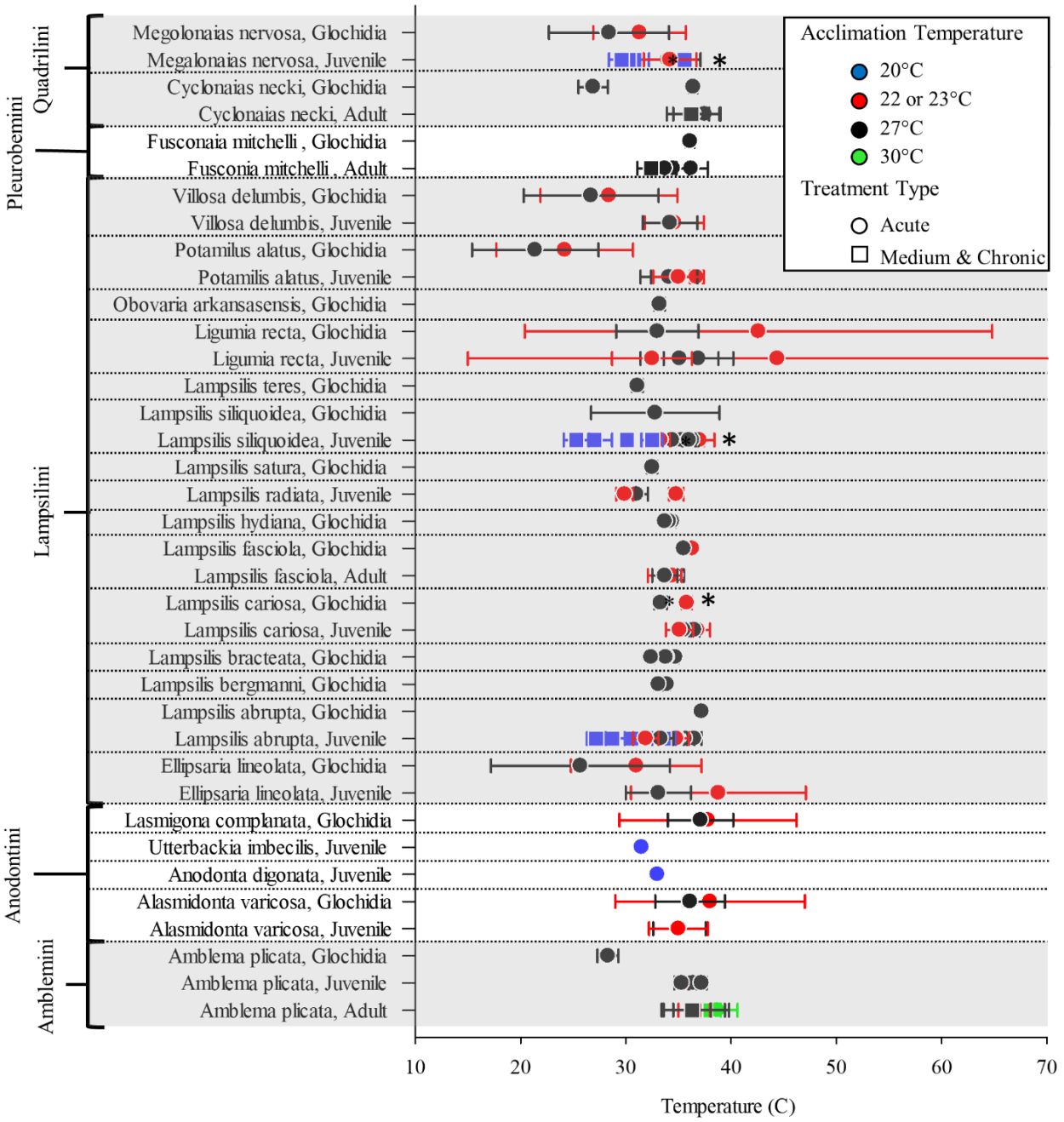

Figure 2 Median lethal temperatures (LT50) and corresponding 95% confidence intervals from static thermal exposures conducted on glochidia, juvenile, and adult mussels under a range of conditions [i.e., exposure duration (acute/chronic), acclimation temperature (20-30°C), or exposure type (water-only or with sediment)]. LT50s ranged from 21.4–44.4°C (mean = 34.2°C) among all species, life stages, and test conditions (n = 130). Species are arranged by decreasing LT50 values within tribes. Acclimation temperature is signified by color (20°C = blue, 22 or 23°C = red, 27°C = black, 30°C = green). Treatments are signified by symbol shape (● = acute water-only exposure and ■ = chronic water-only exposure. Asterisk (*) denotes when there were significant differences in mean LT50s across acclimation temperatures (determined by 95% CI's not overlapping).

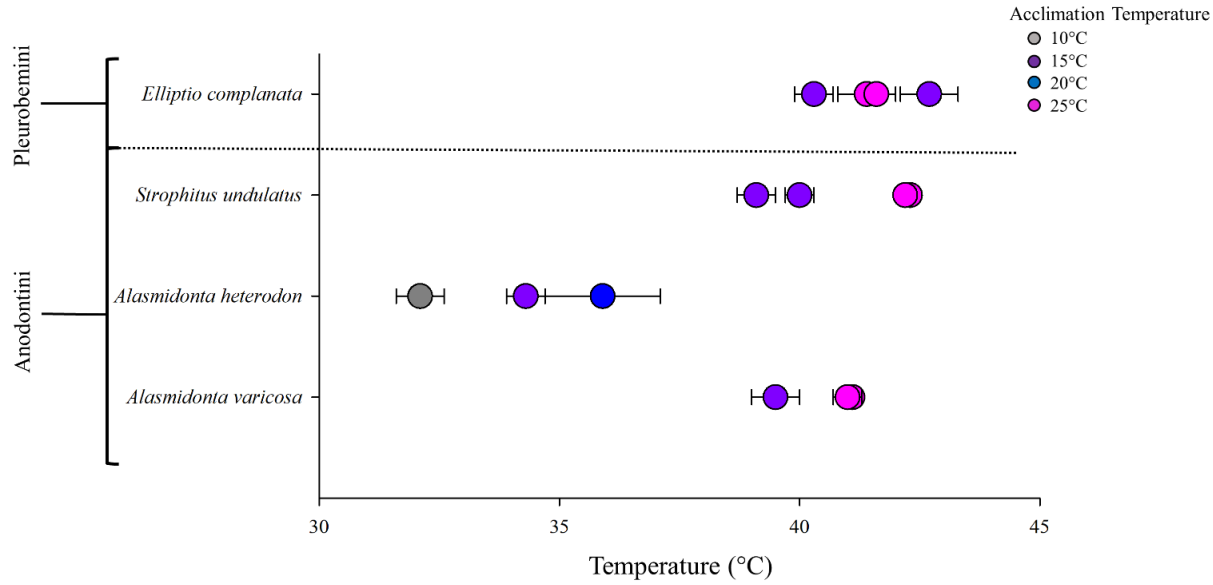

Figure 3 Adult dynamic critical thermal maximum (CTM) data organized by taxa. CTM values ranged from 32.1–42.7°C (mean = 39.5°C) among all species and test conditions (n = 15). Acclimation temperature (10°C = grey, 15°C = purple, 20°C = blue, 25°C = pink) and tribe for each species denoted. Error bars represent 95% confidence intervals.

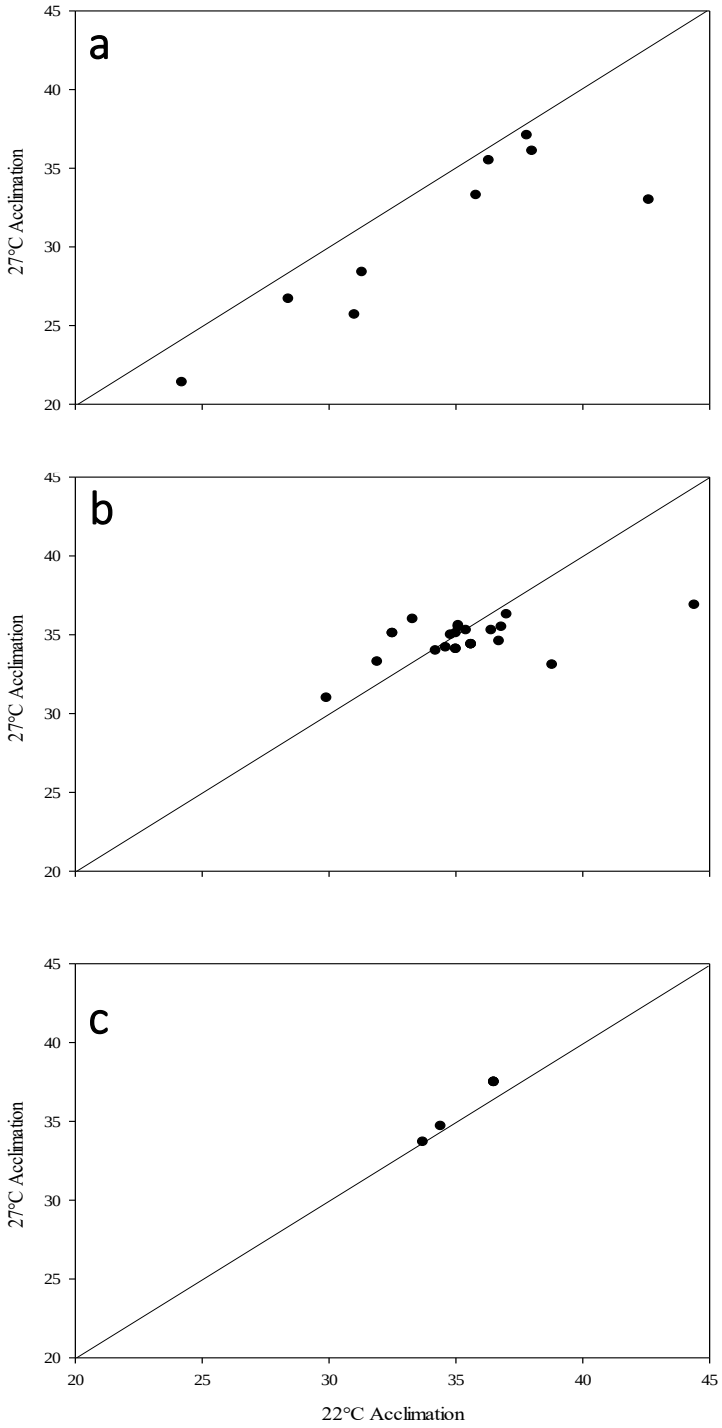

Figure 4 Mean median lethal temperature (LT50) endpoints for 22 vs 27°C acclimation for glochidia (a), juveniles (b) and adults (c). The line represents no difference in LT50 values across acclimation temperatures.

CHAPTER 6
CONCLUSION

This research evaluated the feeding ecology and lethal thermal tolerances of North American unionid mussels to assist in conservation of these imperiled species. The feeding ecology of five species of freshwater mussels within four lotic systems was quantified seasonally using stable isotope and fatty acid analysis. Additionally, the feeding ecology of three species of unionids in a lentic system was quantified using stable isotope analysis. Finally, the lethal thermal tolerances of unionids in North America was synthesized and reviewed.

In Texas lotic systems, unionid mussels primarily utilizing coarse particulate organic matter associated with decaying leaf litter, with secondary contributions from suspended particulate organic matter. In these systems we found evidence that all five species were experienced poor food quality and thermal stress during the summer – evidenced by high C:N ratios and enriched stable nitrogen signatures. Fatty acid analysis of these same mussels within the Texas systems suggested that the dietary items being consumed from bulk coarse particulate organic material and suspended particulate organic material pools were of algal, rather than bacterial, origins. Specific fatty acid markers suggested that mussels were utilizing both suspension and deposit feeding modes of feeding, which corresponded with food resources revealed in the stable isotope analysis. Both stable isotope and fatty acid analyses revealed that there was minimal variation across species, but signatures and profiles varied predominately across drainages.

In an Alabama lentic system, the dietary contributions for three species of unionids came from limnetic fine particulate organic material associated with benthic sediments. Littoral fine particulate organic material, littoral and limnetic suspended particulate organic material and

coarse particulate organic material collectively contributed <1%. Additionally in this system the isotopic signature of a species of mussels that was recently immersed versus individuals that had been emersed for eight weeks was compared. The eight week emersed mussels had significantly greater nitrogen signatures than those individuals that were collected and sampled after being recently emersed.

Mussels in both lotic and lentic systems relied predominantly on benthic food sources. This provides further evidence that we must manage benthos as more than just habitat for mussels, but instead as an important source of nutrition. Because there was minimal variation in stable isotope signatures and food resource utilization across species, this provides evidence that the species sampled feed nonselectively within a system, and we can potentially utilize community wide management approaches when addressing food resources. Finally, mussels in both the lotic and lentic systems exhibited signs of self-catabolism, or relying on internal energy stores, to survive temperature and emersion stress.

When synthesizing the known thermal tolerance data for North American unionids, it was found that there are estimates for 28 of 302 (~10) North American species in the families Unionidae and Margaritiferidae for one or more life stages. Lethal thermal tolerance data is thus lacking for 90% of unionids. Median lethal temperatures across acute and chronic exposures for all life stages ranged from 21.4–44.4°C with a mean of 34.2°C. The faunal regions that are predicted to have the greatest rates of stream temperature increases also have high degrees of endemism and little thermal tolerance data evaluated for species. Lethal tolerance data has only been evaluated for more than one life stage (i.e., glochidia, juvenile, and adult). Mussel conservation would benefit from continued evaluations of lethal thermal tolerances of unrepresented but vulnerable taxa, studies evaluating effects of latitudinal distribution on thermal

tolerance, studies that reflect thermal exposures under ambient regimes, and the effects of sublethal stressors – which can often be the first indicators of stress.

Appendix A Nominal classifications for all taxa with published literature on temperature tolerance and their current valid classification as of July 2021. Valid taxonomy for all species included in this study was confirmed through the MUSSEL Project MUSSELp database developed and maintained by Dan Graf and Kevin Cummings.

Valid classification	**Nominal classification**
Lasmigona complanata	*Symphynota complanata*
Anodonta digonata	*Pyganodon cataracta*
Lampsilis siliquoidea	*Lampsilis hydiana*
Leaunio lienosus	*Villosa lienosa*
Cambarunio nebulosus	*Villosa nebuosa*

Appendix B Summary of all studies with their included focal taxa, life stage, and test type.

Publication	**Taxa**	**Life Stage**	**Test**
Archambault et al. 2013	*Lampsilis fasciola*	Adult	Static, Acute
Archambault et al. 2014a	*Amblema plicata* *Lampsilis abrupta* *Lampsilis cariosa* *Lampsilis fasciola* *Lampsilis siliquoidea*	Glochidia, Juvenile	Static, Acute
Archambault et al. 2014b	*Lampsilis abrupta* *Lampsilis radiata*	Juvenile	Static, Acute
Dimock & Wright 1993	*Utterbackia imbecilis* *Pyganodon cataracta*	Juvenile	Static, Acute
Galbraith et al. 2012	*Elliptio complanata* *Strophitus undulatus* *Alasmidonta varicosa*	Adult	Dynamic
Galbraith et al. 2020	*Alasmidonta heterodon*	Adult	Dynamic
Ganser et al. 2013	*Lampsilis abrupta* *Lampsilis siliquoidea* *Megalonaias nervosa*	Juvenile	Static, Chronic
Khan et al. 2019	*Amblema plicata* *Cyclonaias necki* *Fusconaia mitchelli* *Lampsilis bracteata* *Lampsilis bergmanni* *Lampsilis hydiana* *Lampsilis satura* *Lampsilis satura* *Lampsilis teres* *Obovaria arkansasensis*	Glochidia, Juvenile	Static, Acute
Khan et al. 2020	*Amblema plicata* *Cyclonaias necki* *Fusconaia mitchelli*	Adult	Static, Acute and Chronic

Martin 2016	*Lampsilis siliquoidea* *Megalonaias nervosa*	Juvenile	Dynamic
Pandolfo et al. 2010a	*Lampsilis siliquoidea* *Potamilus alatus* *Ligumia recta*	Juvenile	Static, Acute
Pandolfo et al. 2010b	*Potamilus alatus* *Ligumia recta* *Ellipsaria lineolata* *Lasmigona complanata* *Megalonaias nervosa* *Alasmidonta varicosa* *Villosa delumbis*	Glochidia, Juvenile	Static, Acute
Payton et al. 2016	*Villosa lienosa* *Villosa nebulosa*	Adult	Static, Chronic