

Article

Thermal Threats to Freshwater Mussels: An Empirical Stream Assessment

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Abstract: Freshwater mussels fulfill an important ecological role in aquatic ecosystems, but they currently face many threats, including thermal regime alteration. Thermal transformation of the aquatic environment is associated with climate change, land use alteration, and other pervasive anthropogenic global changes. To enhance our understanding of ecological thermal impacts, we combined extensive field measurements of temperature in the stream water column and substrate depths (5 and 15 cm) at sites where mussels occur, measures of abundance and species richness for mussels and fish, and thermal tolerance knowledge for mussels and fish to generate a comprehensive assessment of the potential threats mussels face as temperatures continue to rise as a result of global change. Mean summer (June–August 2010–2012) temperatures at mussel-occupied sites in the upper Tar River basin of North Carolina, USA, ranged from 16.2 to 34.7 °C. The mean temperature from the hottest 96 h at each site ranged from 23.5 to 31.5 °C. At 80% of sites, a period of moderate drought coincided with the hottest 96 h period. Temperature threshold exceedance durations indicated that chronic, combined chronic/acute, and acute freshwater mussel thermal tolerance thresholds (i.e., 28 °C, 30 °C, and 33 °C, respectively) based on laboratory exposures of glochidia (larvae) and juveniles were commonly exceeded. Water temperatures exceeded 28 °C for at least 24 h at 55% of sites and for at least 96 h at 35% of sites, and they exceeded 30 °C for at least 24 h at 15% of sites. We quantified a thermal buffering effect of the substrate that may be protective of mussels. There was a mean difference of 0.5 °C between the water column and the upper substrate (5 cm) and a mean difference of 0.9 °C between the water column and the lower substrate (15 cm). Maximum differences of up to 5.5 °C between the water column and the upper substrate and 11.5 °C between the water column and the lower substrate were observed. Our models estimating the relation between the water column and substrate temperatures more realistically characterize ambient temperature exposures and have widespread implications for mussel conservation and climate change risk assessment in similar streams. Freshwater mussels currently exist on the edge of their thermal limits, but their abundance and species richness cannot be explained by temperature patterns alone. Fish species richness was related to the thermal regime, indicating that species interactions may be an important driver of freshwater mussel responses to global change.

Keywords: Unionidae; climate change; global change; thermal buffer; fish; temperature; substrate; exceedance; species richness



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1. Introduction

Global change has altered species interactions and caused shifts in species distributions [1–3]. Freshwater ecosystems are particularly threatened by present and future environmental change [3–6], and the temperature and hydrology of rivers and streams are

being affected by human impacts [4,7]. Flood frequency and magnitude are expected to increase, while droughts may also become more frequent [3,4]. These hydrologic changes, along with increasing temperature, can alter nutrient cycling, decrease habitat availability, degrade water quality, and potentially introduce parasites and pathogens to freshwater ecosystems [1,5,6]. These changes in turn alter species interactions and affect the structure and services of freshwater ecosystems [1,3,8–10].

The impacts of changing thermal regimes on freshwater ecosystem services have been demonstrated [9,11]. Guilds of thermally tolerant and thermally sensitive freshwater mussels have differing rates of resource assimilation and subsequent ecosystem services; when temperatures change, species composition can shift and ecosystem services can be altered [9,11,12]. Freshwater mussels fulfill a critical ecological role in aquatic ecosystems. They filter water, process nutrients, serve as ecosystem engineers, and impact assemblages of other macroinvertebrates [13–17]. These key components of freshwater systems are also among the most imperiled taxa globally. For example, of the approximately 300 species in North America, over 75% are considered endangered, threatened, or vulnerable or have already become extinct [18].

Freshwater mussels are threatened in part due to their unique life history, which involves an obligate parasitic larval life stage (glochidia) that requires a suitable, and often a specific, host fish to transform into a juvenile mussel [19]. Because of the obligate relationship between mussels and their host fishes, freshwater mussels are not only limited by their own responses to global change but also by those of their hosts [20]. Their sessile nature, unique life history strategies, and imperiled conservation statuses indicate that freshwater mussels are particularly at risk from climate change [21,22], as well as other factors such as habitat destruction and alteration, pollution and water quality degradation, and invasive species, that influence their abundance and distribution [23,24].

These considerations have spurred recent interest in the effects of temperature and flow changes on freshwater mussels [25–29]. Laboratory experiments have generated basic thermal tolerance thresholds for mussels [25,29–33], and mussel assemblage changes in relation to temperature and flow have been empirically observed [11,34,35]. Information relating the current thermal environment of mussels to their thermal tolerances is expanding, however, little empirical field temperature research has been conducted in mussel habitats [36,37]. In this study, we aimed to combine extensive field measurements of temperature in the water column and substrate at stream sites where mussels occur, measures of abundance and species richness for mussels and fish, and current thermal tolerance knowledge for mussels and fish to generate a comprehensive assessment of potential threats that mussels face as temperatures rise as a result of global change and other human activities.

2. Methods

2.1. Site Selection

Twenty sites were selected within the upper Tar River basin of North Carolina from 3 subbasins with similar drainage areas: the Upper Tar subbasin, Swift Creek subbasin, and Fishing Creek subbasin (Figure 1A). Seven sites were selected within the Swift Creek and Fishing Creek subbasins, and 6 sites were in the Upper Tar subbasins. The sites were selected to represent a longitudinal gradient spanning a range of environmental conditions. The watershed area was calculated by delineating the catchment area upstream of each site and determining the area (km²) using ArcGIS (9.3.1, ESRI, Redlands, CA, USA). Drought status for the Tar River basin throughout the study period, determined by an area-weighted average of the 7-d average streamflow, was derived from existing data (North Carolina Department of Environmental Quality, Division of Water Resources; http://www.ncwater.org/Drought_Monitoring/) accessed on 8 January 2014.

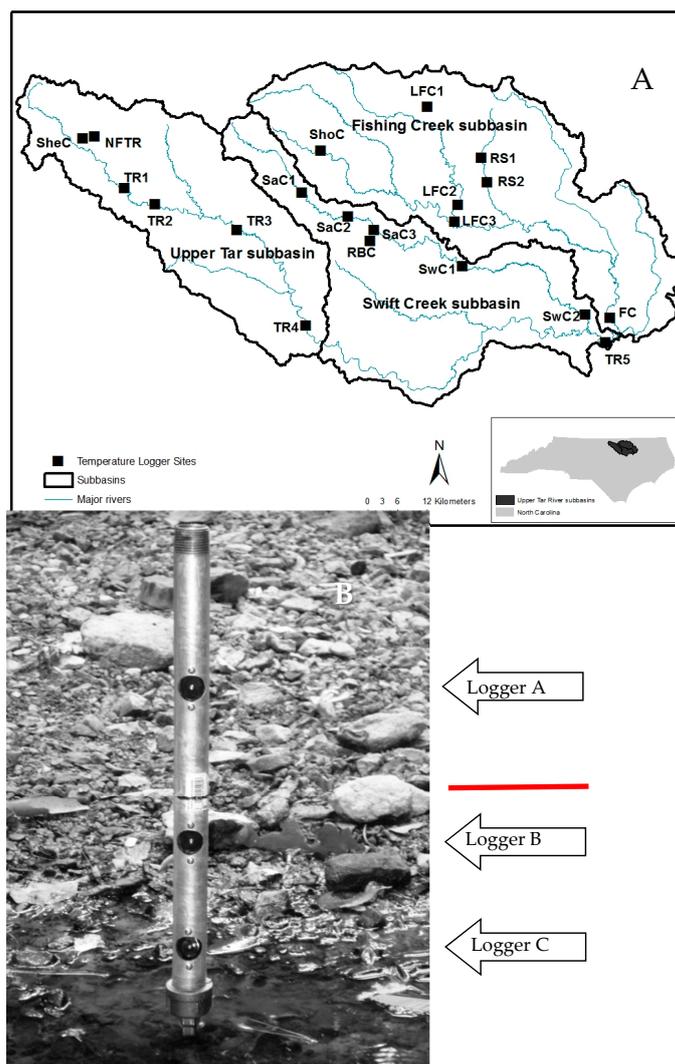


Figure 1. Location of temperature loggers at 20 sites in the upper Tar River basin, North Carolina (A), and photograph showing temperature logger deployment design (B). Logger A is 10 cm above the water/substrate interface in the water column, the red line denotes the location of the water/substrate interface when deployed, Logger B is 5 cm below the interface in the substrate, and Logger C is 15 cm below the interface in the substrate.

2.2. Freshwater Mussel and Fish Surveys

Semi-quantitative snorkel and tactile search freshwater mussel surveys were conducted with the assistance of the North Carolina Wildlife Resources Commission, according to Pandolfo et al. [38]. A minimum of 6 person-hours of effort was expended surveying for mussels at each site. Fish surveys were conducted at each site with 2 backpack electrofishers and 2 dipnetters within a 200 m reach, sampling upstream along the banks followed by a return pass down the center of the stream [39]. Mussel and fish surveys were both conducted in the same stream reach at a given site during the summer of 2010. The stream width among sites ranged from 10 to 30 m, with most stream widths on the order of 10 m.

2.3. Temperature Logger Unit Design and Deployment

Continuous temperature-monitoring iBCod 22L data loggers from Alpha Mach, Inc. (Mont-St-Hilaire, QC, Canada) were deployed at stream sites. Before use, logger temperature measurements were validated in the laboratory with a National Institute of Standards and Technology-certified thermometer (Fisher Scientific, Inc., Waltham, MA, USA). The sensitivity of the loggers was 0.5 °C or less. A temperature logger unit (Figure 1B) was

assembled with 3 iBCod 22L data loggers attached by screws to a drilled galvanized steel pipe at heights that corresponded to stream depths of 10 cm above the substrate/water interface (logger A), 5 cm below the interface (logger B), and 15 cm below the interface (logger C). With this design, 1 data logger (A) recorded temperatures in the water column, and the other 2 recorded substrate temperatures. Each temperature logger unit was fitted with a sharpened bolt at the end to facilitate driving the unit into the substrate. Two temperature logger units were deployed at each site, for a total of 120 iBCod 22L data loggers recording temperature throughout the Tar River basin. Logger placement within a site was based on water depth, substrate type (i.e., no bedrock or large cobble that would prohibit driving the unit into the stream bottom), and proximity to mussel and fish survey reaches. Each logger unit was marked with flagging tape and spray paint on triangulated vegetation on the stream bank, and GPS coordinates were taken to facilitate recovery. Temperature logger units were set to record hourly temperatures throughout the summers (June, July, and August) of 2010, 2011, and 2012. Units were retrieved and redeployed several times to capture data periodically throughout the deployment period. Several loggers malfunctioned, and in some instances, complete units were lost; therefore, the amount and dates of collected thermal data are variable among or within sites.

2.4. Data Analysis

Prior to statistical analysis, data were trimmed to remove the 24 h period surrounding retrieval and deployments to ensure data accuracy (i.e., air temperatures were not recorded). Data were compiled and queried to determine sample size, mean, minimum, and maximum temperatures, and temperature differences among loggers A, B, and C. A series of queries was performed to determine the maximum continuous duration that each site exceeded 1 of 3 critical thermal threshold temperatures (28 °C, 30 °C, and 33 °C) for common and imperiled mussel species. These threshold temperatures were defined as 28 °C = chronic thermal threshold, 30 °C = combined chronic and acute thermal threshold, and 33 °C = acute thermal threshold, based on laboratory exposures of glochidia and juvenile mussels (Fogelman 2023). Thus, the data queries were based on 28 °C, 30 °C, and 33 °C for loggers A, B, and C, and the mean temperature from each site's hottest 96-h period for loggers A, B, and C was identified via moving average. Linear regression was performed to quantify the relationships between water column temperature (logger A) and temperatures at two substrate depths (loggers B and C) for all available data (i.e., corresponding sites, dates, and times).

Three temperature statistics were selected for further analysis: mean water temperature from the hottest 96 h period, maximum site temperature recorded, and maximum duration of 28 °C threshold exceedance of water temperatures. Linear regressions modeled relationships between these three parameters in the water column (logger A) and watershed area: percent of nonnative fish in the total fish sample, percent of tolerant fish species, and percent of intolerant fish species at each site [39]. Three measures of biotic integrity [40], freshwater mussel species richness, fish species richness, and catch-per-unit-effort (CPUE) for *Elliptio complanata*, a relatively common and stable species that was collected at every site, were also regressed with the watershed area. Abundance (CPUE) data were used exclusively for *E. complanata* because no other species occurred at all 20 sites.

Mean temperature from the hottest 96-h period, maximum temperature, and maximum 28 °C threshold exceedance of temperatures in the water column and both substrate depths (loggers A, B, and C) were then examined for relationships with the same three biotic measures mentioned previously. Linear regression was performed to identify untransformed relationships in the original data, as well as logarithmic, square root, and inverse transformations of the response variables. The distribution of these data warranted the testing of non-linear relationships using higher-order polynomial regression fits and penalized splines. When these relationships were not informative, each temperature predictor was then stratified into three equal groups to statistically test for an ideal temperature range [41]. We avoided bias by dividing temperatures into groups of equal range. An

ANOVA was performed to test for the significance of any biologically optimal thermal range. Freshwater mussel and fish species richness were then mapped with mean water temperature from the hottest 96-h period at each study site using ArcGIS (9.3.1, ESRI, Redlands, CA, USA).

3. Results

A total of 16,607 mussels of 16 species were sampled at the 20 study sites. *Elliptio complanata* accounted for most individuals (>14,000) and represented almost 85% of the mussel fauna sampled. Other species that were represented by 20 or more individuals were *Alasmidonta heterodon*, *E. congaraea*, *E. fisheriana*, *E. icterina*, *E. roanokensis*, *Fusconaia masoni*, and *Villosa constricta*. A total of 8240 fish belonging to 49 species were sampled (Table 1). The most abundant fish was *Notropis procne* (1668), and the most ubiquitous was *Lepomis auritus*, the only species to occur at all 20 sites. There were 35 fish species sampled that are documented hosts for Tar River basin mussels [42], Freshwater Mussel Host Database, Illinois Natural History Survey & Ohio State University Museum of Biological Diversity, <https://mollusk.inhs.illinois.edu/57-2/> (accessed 3 January 2024), Christopher B. Eads, NC State University mussel propagation specialist, personal communication).

Table 1. Species and numbers of fish sampled at 20 sites in the Tar River basin, North Carolina. Fish species with an asterisk (*) denote a known mussel host.

Species	Site																			
	SheC	NFTR	TR1	TR2	TR3	TR4	SaC1	SaC2	SaC3	RBC	SwC1	SwC2	ShoC	LFC1	RS1	RS2	LFC2	LFC3	FC	TR5
<i>Acantharchus pomotis</i>	–	–	–	1	–	–	–	1	–	–	–	–	3	–	1	–	–	–	–	–
<i>Ambloplites cavifrons</i> *	–	–	1	30	–	1	1	–	–	–	1	–	–	–	–	–	1	2	–	–
<i>Ameiurus natalis</i>	–	–	–	–	–	–	3	–	–	2	–	1	–	–	1	–	–	–	–	–
<i>Anguilla rostrata</i> *	–	–	–	–	–	–	6	14	17	5	53	47	11	–	1	11	12	67	82	191
<i>Aphredoderus sayanus</i> *	11	6	1	–	1	6	6	2	–	–	5	12	16	169	2	2	3	14	15	1
<i>Centrarchus macropterus</i>	–	–	–	–	–	–	–	2	1	2	–	–	6	1	–	–	–	–	–	–
<i>Clinostomus funduloides</i> *	–	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–
<i>Cyprinella analostana</i> *	–	–	2	48	171	35	10	7	5	–	28	120	–	1	–	–	–	5	58	594
<i>Enneacanthus gloriosus</i>	–	–	–	–	–	–	9	4	3	–	–	4	35	9	23	34	–	1	2	–
<i>Erimyzon oblongus</i>	19	5	–	6	3	–	7	3	–	1	–	–	3	–	4	–	–	1	–	–
<i>Esox americanus</i> *	–	–	–	–	–	–	1	2	1	3	–	–	3	–	3	–	–	–	–	–
<i>Esox niger</i>	–	9	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–
<i>Etheostoma nigrum/olmstedii</i> *	22	26	14	17	63	35	14	28	32	3	65	26	24	25	4	–	59	25	73	32
<i>Etheostoma vitreum</i> *	–	–	3	3	54	–	–	1	5	–	3	–	–	–	–	–	–	2	1	6
<i>Gambusia holbrooki</i> *	13	5	–	7	37	37	3	10	6	2	19	159	49	13	7	62	39	94	248	25
<i>Hybognathus regius</i>	–	–	–	–	–	–	–	–	–	–	1	6	–	–	–	–	–	–	–	17
<i>Hypentelium nigricans</i>	–	–	–	10	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–
<i>Ictalurus punctatus</i> *	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	6
<i>Lampetra aepyptera</i>	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
<i>Lepisosteus osseus</i> *	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	1	–
<i>Lepomis auratus</i> *	12	2	22	43	50	30	42	28	24	14	54	141	17	5	31	6	19	33	255	34
<i>Lepomis cyanellus</i> *	12	12	14	51	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	–
<i>Lepomis gibbosus</i> *	–	–	–	–	–	–	–	1	–	–	1	–	–	1	–	–	–	–	–	–
<i>Lepomis gulosus</i> *	–	6	2	–	–	–	–	1	1	2	3	–	1	–	12	13	–	–	–	–
<i>Lepomis macrochirus</i> *	20	8	8	4	4	10	1	4	–	8	23	26	–	–	11	6	–	–	3	7
<i>Lepomis microlophus</i> *	3	2	–	–	–	–	2	–	–	–	1	3	6	–	8	2	–	–	1	–
<i>Luxilus albeolus</i> *	22	–	–	151	47	–	10	24	2	5	16	1	–	30	–	–	7	29	–	–
<i>Lythrurus matutinus</i> *	4	1	–	36	12	–	1	45	13	–	5	–	–	1	–	–	2	6	4	–
<i>Micropterus salmoides</i> *	–	2	1	18	6	1	–	–	–	2	1	3	–	–	1	1	–	–	1	1
<i>Moxostoma collapsum</i> *	–	–	1	–	12	–	–	5	1	–	6	–	–	–	–	–	1	1	1	–
<i>Moxostoma macrolepidotum</i> *	–	–	–	–	1	–	–	–	–	–	4	–	–	–	–	–	–	–	1	–
<i>Moxostoma pappilosum</i>	–	–	–	2	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Nocomis leptocephalus</i> *	69	–	–	52	5	–	1	4	–	–	5	4	1	36	–	–	–	2	–	–
<i>Nocomis raneyi</i> *	–	–	–	85	15	–	–	–	1	–	10	–	–	–	–	–	–	–	–	–
<i>Notemigonus crysoleucas</i> *	–	–	–	–	–	–	1	–	–	1	–	–	–	–	4	–	–	–	–	–
<i>Notropis altipinnis</i> *	–	–	–	–	–	–	–	–	–	–	–	–	16	3	–	6	–	–	–	–
<i>Notropis amoenus</i>	–	–	–	5	20	18	–	1	–	–	4	10	–	–	–	–	–	–	–	1
<i>Notropis hudsonius</i> *	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
<i>Notropis procne</i> *	16	4	4	45	176	20	8	4	6	–	122	95	–	–	–	–	5	13	72	1078
<i>Notropis volucellus</i> *	–	–	–	128	–	–	–	–	–	–	40	–	–	–	–	–	–	–	–	–
<i>Noturus furiosus</i>	–	–	–	–	8	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–
<i>Noturus insignis</i> *	5	2	2	91	26	–	9	1	12	–	1	1	3	6	2	–	2	15	–	–
<i>Percina nevisense</i> *	–	–	2	22	20	7	2	7	2	–	26	25	1	–	–	–	5	4	–	1
<i>Percina roanoka</i> *	4	–	2	86	14	–	1	1	8	–	31	4	–	4	–	–	11	20	1	1
<i>Petromyzon marinus</i>	–	–	–	–	–	–	–	–	–	–	–	2	1	–	–	–	–	–	–	–
<i>Pomoxis nigromaculatus</i> *	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	3
<i>Scartomyzon cervinus</i>	–	–	–	47	18	–	–	–	1	–	11	–	–	–	–	–	1	2	–	–
<i>Semotilus atromaculatus</i> *	–	–	–	–	–	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–
<i>Umbra pygmaea</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	1	5	–

The number of data points, i.e., hourly temperature readings, that were collected from the water column and 2 substrate depths (5 cm and 15 cm) at each of 20 sites in the upper Tar River basin ranged from 2064 to 23,638 with a mean of 16,887 (Tables 2 and 3). Overall mean summer temperature ranges varied minimally among river subbasins: 16.2–34.2 °C in the Tar River subbasin, 18.2–34.7 °C in the Swift Creek subbasin, and 17.1–31.7 °C in the Fishing Creek subbasin. The overall maximum temperature reached at each site was a water column reading (logger A), with the exception of Sandy Creek 1, where the logger 15 cm in substrate (logger C) recorded the highest temperature (a likely anomaly with no plausible explanation). Fluctuation in hourly temperatures (standard deviation) was lower for the loggers in the substrate (loggers B, C) than for the loggers in the water column (logger A) for all sites, with the exception of Tar River 5, where logger C had the greatest variation. This buffering effect that maintains substrate temperature more constant than in the water column generally increases with substrate depth.

Table 2. Site information and overall mean, minimum, maximum, and standard deviation from temperature loggers that recorded hourly measurements at 20 sites in the Tar River basin, North Carolina, during the summers (June, July, and August) of 2010–2012.

Site	Watershed	Temperature					
		Area (km ²)	N	Mean	Min	Max	SD
Tar River subbasin							
NFTR	North Fork Tar River	40.1	23,638	22.9	16.2	28.7	1.6
SheC	Shelton Creek	58.5	19,834	23.6	17.7	33.7	1.4
TR1	Tar River 1	359.0	12,048	27.6	23.7	34.2	1.5
TR2	Tar River 2	557.1	22,392	26.3	19.6	33.2	2.0
TR3	Tar River 3	948.0	21,648	26.3	19.1	32.6	1.9
TR4	Tar River 4	1428.7	21,648	27.4	21.7	32.6	1.6
Swift Creek subbasin							
RBC	Red Bud Creek	36.2	16,488	24.9	18.2	31.7	1.9
SaC1	Sandy Creek 1	138.9	7200	27.2	22.6	33.1	1.6
SaC2	Sandy Creek 2	252.1	20,736	24.5	18.1	30.2	1.8
SaC3	Sandy Creek 3	287.4	18,072	25.0	19.7	30.7	1.6
SwC1	Swift Creek 1	471.6	15,456	24.5	19.2	30.7	1.9
SwC2	Swift Creek 2	693.5	14,688	26.3	20.6	31.1	1.7
TR5	Tar River 5	3364.1	11,112	27.0	18.6	34.7	2.7
Fishing Creek subbasin							
RS1	Rocky Swamp 1	27.1	11,376	23.3	20.2	29.7	1.5
RS2	Rocky Swamp 2	51.1	22,176	23.4	17.1	29.1	1.6
ShoC	Shocco Creek	62.9	20,664	23.1	17.1	29.1	1.8
LFC1	Little Fishing Creek 1	73.6	17,616	22.1	19.0	26.6	1.3
LFC2	Little Fishing Creek 2	460.7	18,984	24.6	19.7	28.7	1.4
LFC3	Little Fishing Creek 3	485.7	19,896	24.5	18.6	30.7	1.8
FC	Fishing Creek	2035.5	2064	28.5	25.7	31.7	1.3

Table 3. Logger-specific hourly temperature measurements at 20 sites in the Tar River basin, North Carolina, during the summers of 2010–2012. Logger A was 10 cm above the water/substrate interface in the water column, Logger B was 5 cm below the interface in the substrate, and Logger C was 15 cm below the interface in the substrate; *N* = number of temperature measurements.

Site	Logger A				Logger B				Logger C						
	<i>N</i>	Mean	Min	Max	SD	<i>N</i>	Mean	Min	Max	SD	<i>N</i>	Mean	Min	Max	SD
Tar River subbasin															
NFTR	9095	23.3	16.2	28.7	1.8	7512	22.6	17.2	25.7	1.6	7031	22.6	19.6	25.2	1.2
SheC	7913	23.9	17.7	33.7	1.5	6312	23.4	20.2	33.7	1.3	5609	23.2	18.6	26.2	1.2
TR1	4752	28.0	23.7	34.2	1.8	4752	27.4	23.7	31.2	1.3	2544	27.2	24.7	29.6	1.1
TR2	9000	26.8	19.6	33.2	2.1	9000	26.2	20.7	31.2	1.7	4392	25.4	20.6	28.6	1.6
TR3	7416	26.6	19.1	32.6	2.1	6816	26.7	22.2	32.2	1.7	7416	25.7	19.7	29.2	1.7
TR4	7704	27.6	21.7	32.6	1.8	6240	27.5	22.2	31.7	1.6	7704	27.0	22.2	30.2	1.4
Swift Creek subbasin															
RBC	5496	24.8	18.2	31.7	2.1	5496	24.9	19.2	29.7	1.9	5496	24.9	19.7	28.7	1.8
SaC1	2400	27.3	22.6	32.6	1.8	2400	27.3	23.2	32.7	1.6	2400	27.1	23.7	33.1	1.5
SaC2	6912	24.8	18.1	30.2	2.0	6912	24.6	19.1	29.2	1.8	6912	24.2	19.2	28.2	1.7
SaC3	6024	25.7	19.7	30.7	1.7	6024	24.9	20.2	28.7	1.4	6024	24.3	19.7	27.2	1.3
SwC1	5136	25.8	19.6	30.7	1.8	5160	24.3	20.0	28.1	1.5	5160	23.3	19.2	26.7	1.5
SwC2	3552	26.3	20.7	31.1	2.0	5736	26.2	20.6	30.7	1.7	5400	26.4	21.1	29.6	1.5
TR5	4320	28.1	21.7	34.7	2.4	2472	27.3	21.2	32.1	2.4	4320	25.7	18.6	30.6	2.6
Fishing Creek subbasin															
RS1	3792	24.0	20.2	29.7	1.6	3792	23.1	20.2	27.6	1.4	3792	23.0	20.6	26.7	1.3
RS2	7392	23.8	17.1	29.1	1.8	7392	23.3	18.6	27.6	1.5	7392	23.1	19.2	26.7	1.4
ShoC	6888	23.8	17.1	29.1	2.0	6888	23.1	18.1	27.2	1.7	6888	22.5	18.2	25.7	1.5
LFC1	6864	22.5	19.1	26.6	1.3	4656	22.4	19.0	25.6	1.2	6096	21.4	19.2	23.7	0.9
LFC2	6888	24.7	19.7	28.7	1.6	6888	24.7	20.2	28.2	1.4	5208	24.5	21.7	27.2	1.2
LFC3	7368	25.2	18.6	30.7	2.0	5160	24.4	19.1	27.7	1.7	7368	23.9	19.6	27.1	1.5
FC	696	28.6	25.7	31.7	1.4	696	28.8	26.1	31.6	1.3	672	28.2	26.1	30.1	1.0

The substrate thermal buffering effect was also demonstrated by mean differences among water and substrate temperatures (Figure 2). The mean temperature difference among loggers at each site was positive in the majority of sites, i.e., water column temperatures were higher than 5 cm (67%) or 15 cm (76%) substrate temperatures, and 5 cm substrate temperatures were higher than 15 cm (77%) substrate temperatures. Red Bud Creek and Fishing Creek sites did not consistently follow this trend. The linear relationships between water column temperature and temperature at both substrate depths were highly significant (Table 4). Water column temperature accounted for 89% of the variation in temperature at the 5 cm substrate depth and 73% of the variation at the 15 cm substrate depth. These significant relationships resulted in a predictive model that estimates substrate temperatures based on water column temperatures, which provides an additional measure of mussel thermal exposure when burrowed (Table 4).

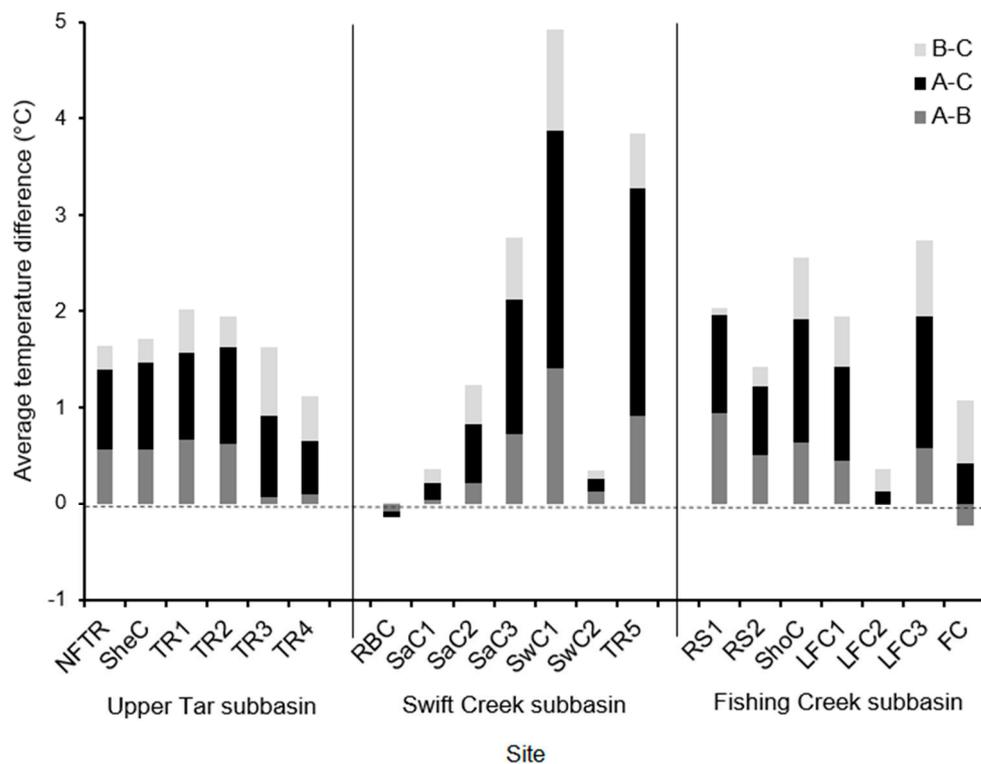


Figure 2. Mean difference between temperatures in the water column and 5 cm in the substrate (A,B), temperatures in the water column and 15 cm in the substrate (A–C), and temperatures 5 cm and 15 cm in the substrate (B,C) at 20 sites in the Tar River basin, North Carolina. Vertical lines separate subbasins. See Table 2 for site abbreviations.

Table 4. Predictive linear models and associated statistics between temperatures in the water column (logger A) and temperatures 5 cm in the substrate (logger B) and 15 cm in the substrate (logger C). Hourly temperature data were pooled across 20 sites; *N* = number of temperature measurements.

Temperature Relationship	<i>N</i>	<i>R</i> ²	<i>F</i>	<i>p</i> -Value
Water column and upper substrate (5 cm)				
Logger B = 2.231 + 0.894(Logger A)	108,096	0.8879	856,000	<0.001
Water column and lower substrate (15 cm)				
Logger C = 4.624 + 0.780(Logger A)	105,729	0.7312	287,600	<0.001

The maximum number of hours for which three mussel threshold temperatures (28, 30, and 33 °C) were exceeded varied greatly among sites (Table 5). In the Swift Creek and Fishing Creek subbasins, the longest 28 °C exceedance durations for each logger occurred at the site with the greatest watershed area. In the Tar River subbasin, exceedance durations were similar among sites, with the exception of the two most upstream sites with the smallest watershed areas. Temperature exceeded 28 °C for at least 24 h at 11, 9, and 7 sites in the water column, 5 cm substrate, and 15 cm substrate, respectively. Temperatures exceeded 28 °C in the water column and 5 cm in the substrate for at least 96 h at 7 sites, and 15 cm in substrate at 6 sites. Temperatures exceeded 28 °C only in the water column (logger A) at 5 sites. Water at only 1 site, Little Fishing Creek 1, never exceeded 28 °C. Temperatures exceeded 30 °C for at least 24 h at 3, 2, and 1 sites for the water column, 5 cm substrate, and 15 cm substrate, respectively. There were 6 sites that never exceeded 30 °C, and 5 of these were in the Fishing Creek subbasin. No sites had temperatures that exceeded 30 °C for 96 h or longer, and no sites had temperatures that exceeded 33 °C for 24 h or more. Only 5 sites ever exceeded 33 °C: 3 in the Tar River subbasin and 2 in the Swift Creek

subbasin. The maximum 33 °C exceedance duration was 8 h in the water column at Tar River 5.

Table 5. Maximum duration (h) that temperatures exceeded 28 °C, 30 °C, and 33 °C at 20 sites in the Tar River basin, North Carolina, during the summers of 2010–2012. Logger A was 10 cm above the water/substrate interface in the water column, Logger B was 5 cm below the interface in the substrate, and Logger C was 15 cm below the interface in the substrate.

Site	Maximum Exceedance Duration (h)								
	28 °C			30 °C			33 °C		
	A	B	C	A	B	C	A	B	C
Tar River subbasin									
NFTR	5	0	0	0	0	0	0	0	0
SheC	9	9	0	6	6	0	2	2	0
TR1	179	156	156	68	16	0	6	0	0
TR2	140	104	16	17	13	1	4	0	0
TR3	71	127	64	14	12	0	0	0	0
TR4	164	308	266	66	67	15	0	0	0
Swift Creek subbasin									
RBC	17	18	17	7	0	0	0	0	0
SaC1	108	111	112	15	16	17	0	0	2
SaC2	66	65	19	2	0	0	0	0	0
SaC3	116	15	0	12	0	0	0	0	0
SwC1	70	7	1	11	0	1	0	0	0
SwC2	89	91	111	13	10	0	0	0	0
TR5	511	513	469	88	88	43	8	0	0
Fishing Creek subbasin									
RS1	10	0	0	0	0	0	0	0	0
RS2	8	0	0	0	0	0	0	0	0
ShoC	7	0	0	0	0	0	0	0	0
LFC1	0	0	0	0	0	0	0	0	0
LFC2	12	7	0	0	0	0	0	0	0
LFC3	19	0	0	6	0	0	0	0	0
FC	165	380	328	16	19	10	0	0	0

The mean temperature from the hottest 96 h at each site ranged from 25.3 °C to 31.5 °C in the water column, 24.5–31.0 °C for loggers 5 cm in the substrate, and 23.5–30.6 °C for loggers 15 cm in the substrate (Figure 3). The coolest temperatures occurred at Little Fishing Creek 1 where the maximum temperature in the hottest 96 h period was 26.2 °C. In comparison, the maximum temperature in the hottest 96 h period from the site with the highest temperatures, Tar River 5, was 33.2 °C. The number of sites that did not exceed a mean temperature of 28 °C during the hottest 96 h period was 7, 10, and 12 sites for the water column, loggers in 5 cm substrate, and loggers in 15 cm substrate, respectively. Of the 13 sites that exceeded an average temperature of 28 °C in the water column, 6 of those exceeded 30 °C, with a maximum of 31.5 °C at Tar River 5. Temperatures measured by loggers 5 cm in the substrate had a mean temperature that exceeded 28 °C at 10 sites, and 4 of those exceeded 30 °C with a maximum of 31 °C, again at Tar River 5. Of the 8 sites

that exceeded a 96 h average temperature of 28 °C for loggers 15 cm in substrate, 2 of those exceeded 30 °C: Sandy Creek 1 and Tar River 5.

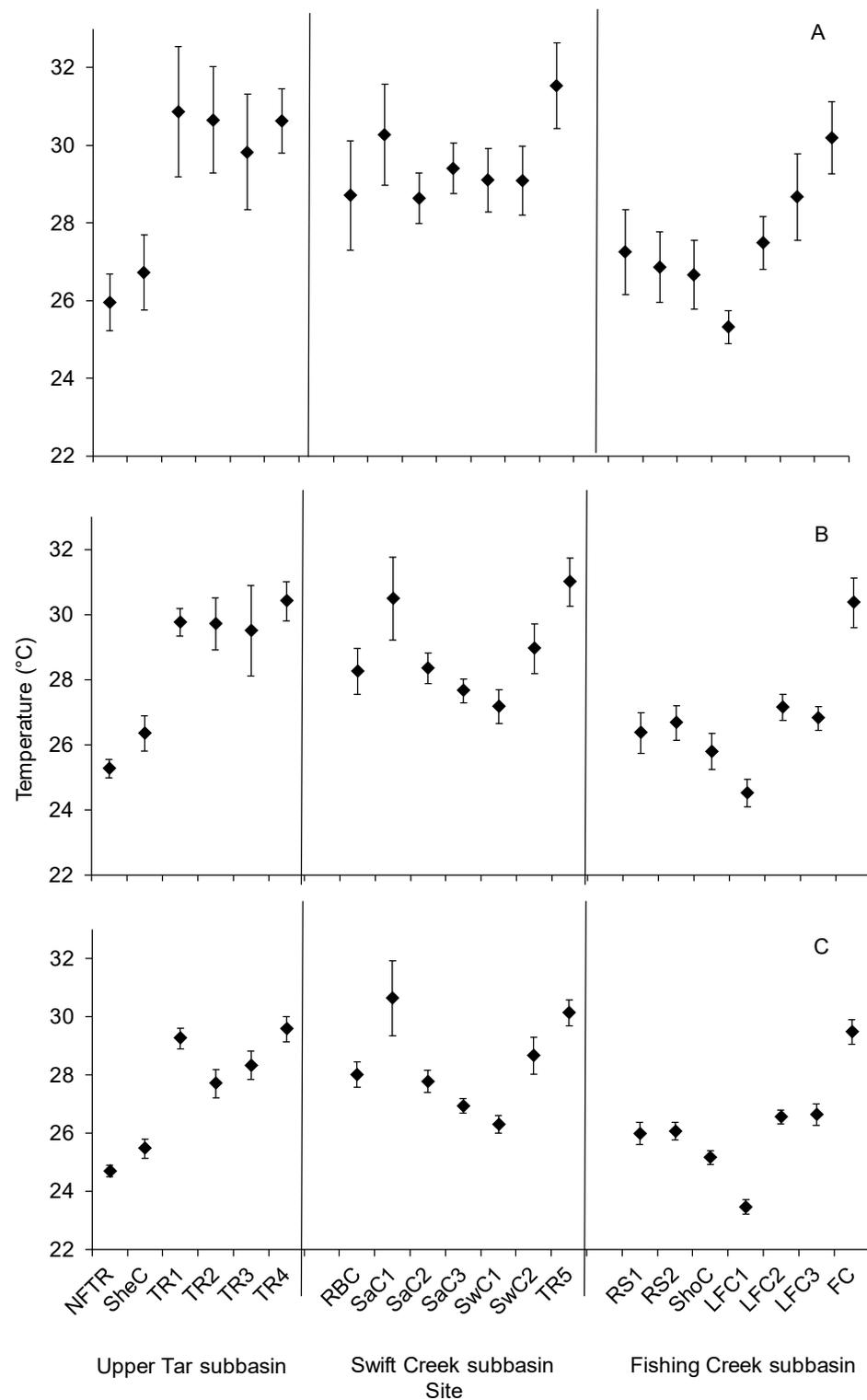


Figure 3. Average temperature (\pm standard deviation) from the hottest 96 h period during the summers of 2010–2012 at 20 sites in the Tar River basin, North Carolina. Temperatures were measured in the water column (Logger A; panel A), 5 cm in the substrate (Logger B; panel B), and 15 cm in the substrate (Logger C; panel C). Vertical lines separate subbasins. See Table 2 for site abbreviations.

The timing of the hottest 96 h period differed among sites and was associated with a drought index for the Tar River basin, but not consistently (Figure 4). The Tar River basin periodically experienced abnormally dry conditions, moderate drought, and severe drought during the summers of 2010, 2011, and 2012. The single period of severe drought did not coincide with the hottest 96 h at any site. Three sites—North Fork Tar River, Tar River 3, and Rocky Swamp 2—did not experience drought conditions during the hottest 96-h period for any logger. The Swift Creek 2 site experienced abnormally dry conditions for all loggers during the hottest 96 h period. At least 1 temperature logger at each of the remaining 16 sites coincided, partially or completely, with moderate drought during the hottest 96 h. Of these 16 sites, 9 experienced moderate drought during the hottest 96 h period recorded at all three loggers.

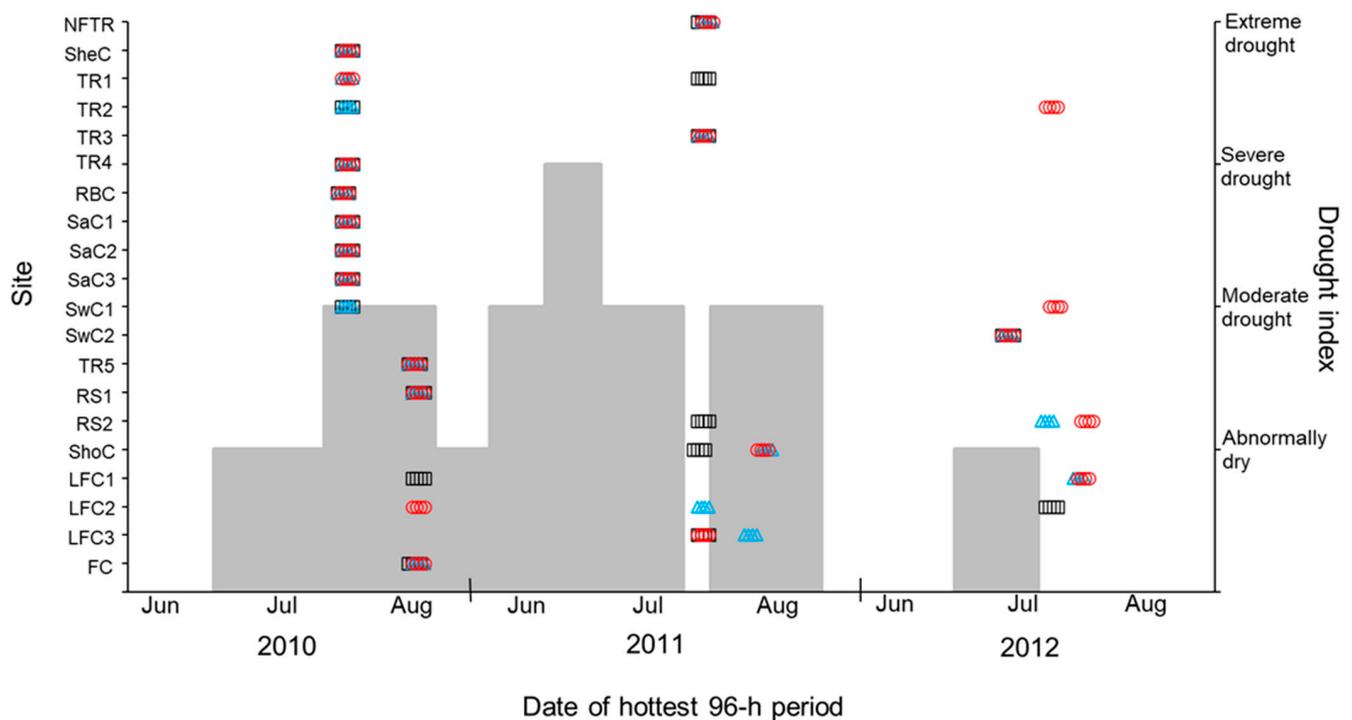


Figure 4. Timing of the hottest 96-h period (symbols) during the summers of 2010–2012 at 20 sites in the Tar River basin, NC, and drought status (shaded area) for the Tar River basin during the same time periods. The timing of the hottest period was measured for the water column (black square; □), 5 cm in the substrate (blue triangle; Δ), and 15 cm in the substrate (red circle; ○). Horizontal lines separate subbasins. See Table 2 for site abbreviations.

Linear regression models indicated a significant relationship between watershed area and mean water temperature from the hottest 96 h period ($p = 0.003$), maximum site temperature ($p = 0.024$), and maximum 28 °C threshold exceedance of water temperatures ($p < 0.001$). All three temperature measures generally increased with watershed area, but the strongest predictive relationship detected was between the maximum 28 °C water temperature threshold exceedance and watershed area ($R^2 = 0.766$).

Linear models generally fail to explain variation in fish and mussel assemblage parameters with thermal or watershed characteristics, but a thermal optimum may influence fish species richness. No significant linear relationship was detected among these 3 temperature measures and fish assemblage biotic metrics (percent of nonnative fish, percent of tolerant fish, and percent of intolerant fish at a site; $p > 0.05$). Linear regression did not indicate a significant relationship between watershed area and mussel species richness, fish species richness, or *E. complanata* CPUE ($p > 0.05$). These 3 biotic metrics also did not exhibit a significant linear relationship with mean temperature from the hottest 96 h period, maximum temperature, and maximum 28 °C threshold exceedance of temperatures in the water

column and both substrate depths (loggers A, B, and C; $p > 0.05$). However, visual inspection of bivariate plots suggested that there may be an ideal temperature range where biotic responses peak, but nonlinear models (e.g., an optimal range of temperature) were typically not significant. An ANOVA that further tested the hypothesis of a biologically optimal thermal range was marginally significant ($\alpha = 0.10$) for fish species richness with mean water temperature from the hottest 96 h period ($p = 0.067$) and maximum site temperature ($p = 0.059$) (Figure 5). In both of these comparisons, fish species richness was significantly lower in the lowest thermal group than in the higher groups. Trends for both indicate an increase in fish richness in the middle grouping and a slight decrease at the highest temperatures. Maps of fish and mussel species richness with the mean water temperature from the hottest 96-h period generally illustrate a closer relationship between fish richness and thermal condition than that demonstrated by freshwater mussel richness (Figure 6).

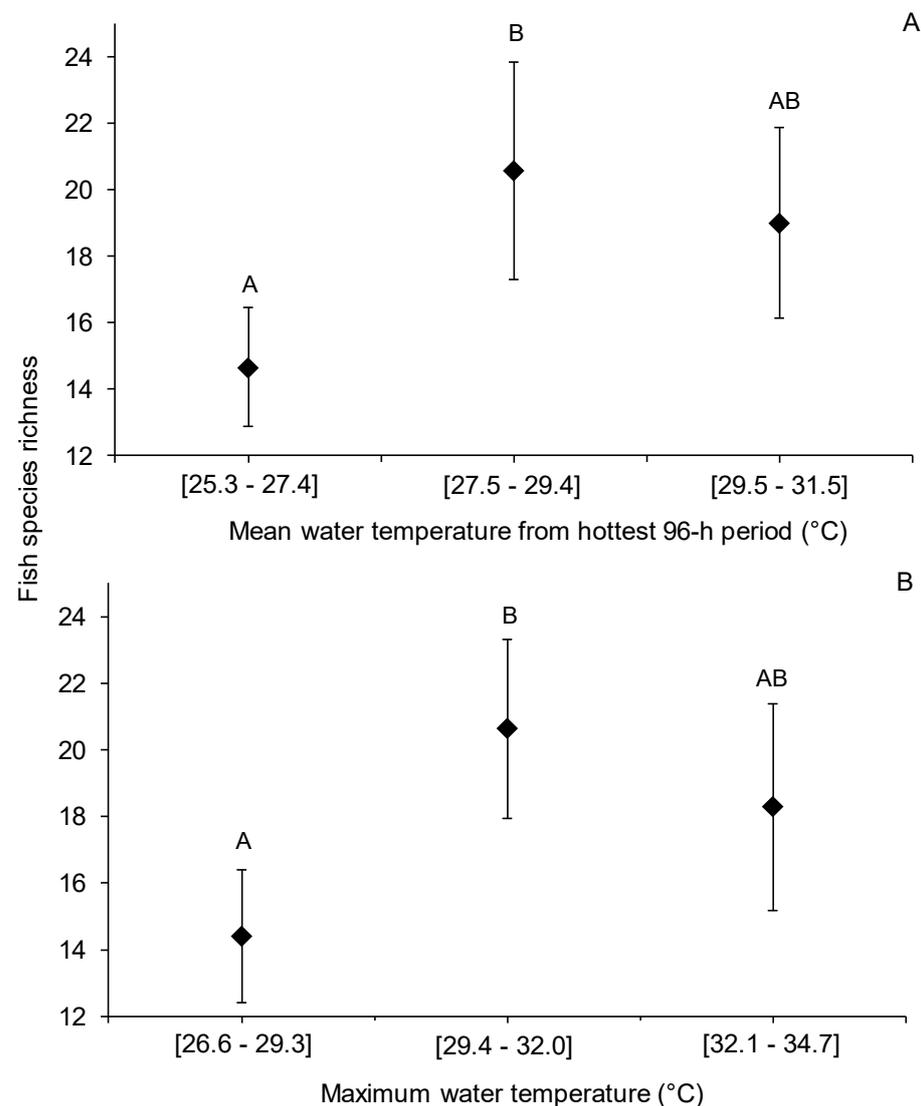


Figure 5. Fish species richness as a function of mean water temperature from the hottest 96-h period (A) and maximum water temperature (B) from 20 sites in the Tar River basin, North Carolina. Different letters indicate statistically significant differences in means among temperature ranges at $\alpha = 0.10$.

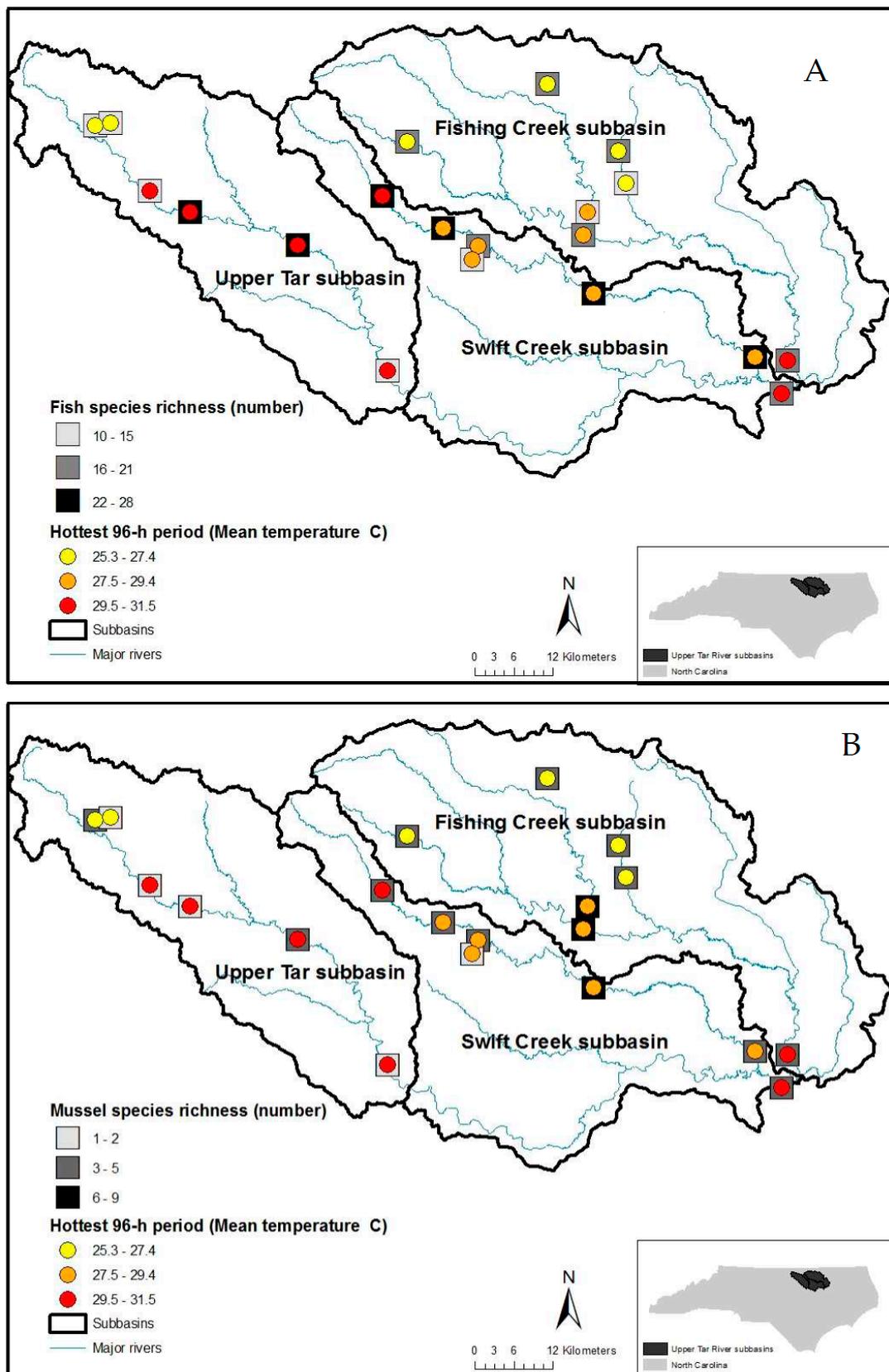


Figure 6. Map of fish (A) and freshwater mussel (B) species richness and the mean temperature from the hottest 96-h period during the summers of 2010–2012 at 20 sites in the Tar River basin, North Carolina.

4. Discussion

We found temperatures at mussel-occupied sites in the Tar River basin of North Carolina have reached thresholds that have been shown to cause harm to mussels in laboratory tests [29,30,33,43]. Both acute and chronic freshwater mussel thermal tolerance thresholds were met or exceeded over the course of three summers in North Carolina. However, the thermal buffering effect of the substrate may be protective of mussels when air and water temperatures are extreme, a concept purported by Archambault et al. [30] in laboratory tests of mussel vertical thermal exposures. Although freshwater mussels currently exist on the edge of their thermal limits, their abundance and species richness cannot be explained by temperature patterns alone. However, fish species richness was related to the thermal regime, indicating that species interactions with fish hosts may be an important driver of freshwater mussel responses to global change [20,37].

The temperatures selected for application in the exceedance duration analysis in this study are representative of several thermal tolerance benchmarks for freshwater mussels. The lowest threshold temperature, 28 °C, represents a chronic thermal limit that would be expected to cause long-term mortality or short-term sublethal effects. It is based on an average 28-d LT50s for 3 species of juvenile mussels [29] and the overall average acute LT05 for glochidia and juveniles of 8 species [25]. The highest temperature, 33 °C, represents an acute thermal threshold towards the maximum of ambient temperatures in the Tar River basin. This threshold is the overall average acute LT50 for glochidia and juveniles of eight species [25] and approximates the 96 h LT50 for juvenile mussels in additional studies [30,43]. The middle temperature, 30 °C, is based on the average of 14-d LT50s for juveniles of three mussel species [29] and the average 96 h LT05 for juveniles of seven mussel species [25], and represents a potential for both chronic and acute exceedances.

The choice of 96 h for the determination of the hottest period at each site is based on the typical duration of acute toxicity tests with juvenile mussels as described in the Standard guide for conducting laboratory toxicity tests with freshwater mussels [44]. The majority of thermal tolerance data that exists for freshwater mussels pertains to exposures of 96 h, so the 96 h duration of the hottest period is easily comparable to existing data. Water-only (i.e., no substrate material present) laboratory-based thermal tolerance criteria for freshwater mussels may not be the most environmentally relevant measure of thermal stress; however, changes in extreme temperatures play a greater role in structuring mussel assemblages than do gradual changes in baseline temperatures [21], and laboratory tests more closely mimic these extreme conditions.

The maximum mean temperature for the hottest 96 h at any site was 31.5 °C. This did not exceed acute (96-h LT50) thermal tolerance thresholds for most juvenile or adult mussels [25,30,35], but it equaled the 96-h LT50 for juvenile *Utterbackia imbecillis*, a species found in the Tar River study area [43]. However, because 31.5 °C was the average temperature of the 96-h period, it does not indicate a 96 h exposure to 31.5 °C but includes diel fluctuations in temperature both above and below 31.5 °C. An additional 5 sites had average temperatures > 30 °C during the hottest 96 h period, and 7 sites had average temperatures > 28 °C.

Exceedance durations for these temperatures are a more informative indicator of potential impact on mussels than thermal maxima because these temperatures are associated with chronic (extended duration or sublethal) effects. Temperature exceeded 28 °C for 96 h at 1 or more loggers at 9 sites. At these sites, 7 loggers in the water column, 7 loggers 5 cm in substrate, and 6 loggers 15 cm in substrate exceeded 28 °C for 96 h or more. The overall average 96-h LT05 for glochidia and juveniles of 8 mussel species was 27.8 °C in laboratory exposures [25]. The longest duration above 28 °C at any site was 513 h for logger B at Tar River 5. This corresponds to ~21 d above 28 °C, and the average laboratory 21-d LT50 for juveniles of 3 species was 28.8 °C (27.0–30.6 °C; [29]). Tar River basin temperatures narrowly missed exceeding the average 21-d LT50 threshold, but they exceeded that of the most sensitive species, *Lampsilis siliquoides* [29]. Fishing Creek exceeded 28 °C at loggers B and C in the substrate for a period of ~14 d. The 14-d LT05 for 3 mussel species was

28.0 °C [29]. No site exceeded 30 °C for 96 h or more, and the maximum duration over 30 °C was 88 h at loggers A and B at Tar River 5. A total of 3 sites exceeded 30 °C for 24 h or more, the established duration of toxicity tests for larval mussels (glochidia; [44]). Acute thresholds for glochidia of some species were exceeded at these 3 sites [25], though glochidia are most likely to be abundant in the spring and these temperatures occurred in the summer months. Loggers at 5 sites exceeded 33 °C for durations lasting 2–8 h. This duration of time at 33 °C is unlikely to harm mussels, but it indicates the potential for mussels to be exposed to extreme temperatures, especially in small streams.

While we formed general conclusions about the exceedance of thermal thresholds for freshwater mussels, species differences are known to exist [29,35]. Specific thermal tolerance data exist only for a few of the mussel species found in our study of an Atlantic Slope river basin. Temperatures in the Tar River basin did not reach the critical thermal maxima of adult *E. complanata* or *Strophitus undulatus* [35]. However, the maximum average temperature from the hottest 96-h period at Tar River 5 met the LC50 for juvenile *U. imbecillis* [43], and *U. imbecillis* was not detected at that site. This may be a spurious association because *U. imbecillis* only occupied 1 of the 20 sites in our study, and that site (Tar River 1) was one with relatively warm water and substrate temperatures. Although Tar River 5 had some of the greatest temperature exceedance durations in our study, the mussel species composition and richness at this site did not differ from other sites with fewer exceedances. Similarly, the site that never exceeded 28 °C, Little Fishing Creek 1, did not support any endangered species, nor was it the site with the highest mussel species richness.

Our empirically derived stream temperature results show that laboratory-derived freshwater mussel thermal tolerance thresholds are being met and exceeded at some sites in the southeastern United States. The actual effect of these temperature exceedances on mussels is difficult to elucidate, but freshwater mussels experience a range of sublethal responses to increasing temperature, including changes in heart rate, lure display, burrowing behavior, and byssus production [28,45–47]. Temperature and watershed area were closely related, as expected. The maximum duration of 28 °C exceedances was the thermal measure most correlated with the watershed area. Though temperature on a broad scale certainly influences animal distributions, none of the stream temperatures measured in our study were related to mussel abundance or richness in any straightforward manner. Nor did mussel abundance or richness relate to the longitudinal position in the river basin, as determined by the use of the watershed area as a proxy for cumulative catchment temperature.

Laboratory-derived thermal thresholds suggest that freshwater mussels are at risk from current environmental temperatures. However, laboratory experiments thus far have failed to replicate the potential for the substrate to act as a thermal buffer in natural conditions. Archambault et al. [30] developed a laboratory method to include substrate in thermal tolerance tests with freshwater mussels and found that the presence of substrate did not alter thermal tolerance. However, due to the nature of laboratory constraints, the substrate was homogenous sand, in which mussels could only burrow to a maximum depth of 2.5 cm [30]. In the field, mussels most often occupy the top 5–10 cm of substrate, with mussels burrowed as deep as 20 cm [48]. Thus, additional laboratory or mesocosm research with vertical thermal gradients in more natural sediment types and textures (i.e., organic content and particle sizes) is warranted with juvenile mussels to understand behavioral and physiological dynamics.

Results from our research and another study of thermal profiles measured in mussel beds in a larger river system [36] both indicate that the thermal buffering capacity of the substrate is substantial. For example, we found a mean difference of 0.5 °C between the water column and the upper substrate (5 cm) and a mean difference of 0.9 °C between the water column and the lower substrate (15 cm). This buffering effect, where the substrate was typically cooler than the water column during the summer, occurred in 67% of cases with a 5 cm substrate and in 76% of cases with a 15 cm substrate. Maximum differences of up to 5.5 °C between the water column and the upper substrate and 11.5 °C between the water column and the lower substrate were observed. This relationship was sufficiently

robust that highly predictive regression models were developed for substrate temperatures using water column temperature with the pooled data from 20 field sites, which may be applied to more realistically characterize ambient temperature exposures. The thermal buffering effect of substrate was also observed in 4 mussel beds in the Upper Mississippi and St. Croix rivers (Minnesota and Wisconsin), where average summer temperatures were 0.5–4.0 °C cooler 5 cm in substrate and 0.6–0.7 °C cooler 15 cm in substrate than in surface water [36]. The substrate was typically within 3 °C of surface water temperatures in their study but varied as high as 7.5 °C cooler than surface water [36]. Differences in substrate buffering capacity among regions, basins, and sites may be influenced by stream size, channel geomorphology, climate and local weather patterns, watershed land use, and other associated physical differences. Our predictive models for water and substrate temperatures will not likely be accurate for large river systems, but in systems that are similar to the Tar River basin, our equations can potentially be used to estimate substrate temperatures given water temperatures. These findings have especially important implications for mussel conservation, restoration, and climate-change risk assessment efforts in the southeastern United States and elsewhere because stream surface water temperature data are widely available but substrate temperatures are not. Thus, our predictive models can be used to estimate water-substrate temperature differentials that may be protective of the mussel fauna.

It is well documented that freshwater mussels burrow into the substrate as a response to increasing temperature [48–50], and some mussels spend the majority of their lives burrowed [50,51]. There is a difference of only a few degrees between temperatures that are lethal to 50% of a mussel population and those that are lethal to only 5% of a population [25,30]. The few degrees of difference between the water column and substrate temperatures may mean the difference between survival and mortality for individual freshwater mussels and ultimately the persistence of a population. This relationship will become increasingly important as global change and other human-mediated impacts on the landscape (e.g., deforestation) continue to increase surface water temperatures and flow rates.

Even if freshwater mussels can survive the physiological stress of increasing temperature, they are especially susceptible to the prevailing effects of global change on species interactions [8,22,26,52]. Because of the obligate nature of their relationship with specific host fishes, freshwater mussels may also be indirectly impacted by environmental stressors that affect fish [20,26]. A meta-analysis of existing freshwater mussel and host fish thermal data indicated that in 62% of species-specific mussel-host fish comparisons, freshwater mussels were more thermally tolerant than their hosts [20]. Of the 49 fish species encountered in our surveys, 35 (71%) of these are known hosts for freshwater mussels. Thermal data are limited for nongame fishes that often serve as mussel hosts, but lethal thresholds for 9 species based on acclimation temperatures close to or equal to those used for mussel thermal tolerance data (20–27 °C, $N = 17$) are similar to those of freshwater mussels with a mean of 32.4 °C (30.0–34.9 °C) [53]. These thermal tolerance data for nine of the host fish species in the Tar River basin indicate that fish, too, are living close to their thermal limits.

We found evidence that fish species richness is related to maximum temperature and the average water temperature for the hottest 96-h period at a site. Mussel species richness did not demonstrate the same relationship. Fish species richness was significantly lowest at sites with the lowest temperatures, and it peaked at a middle range of “optimal” environmental temperatures. The demonstration of fish responses to temperature without evidence of a similar relationship in mussel responses support the assertion that mussels may generally have higher thermal tolerances than fish. We did not find evidence of swiftly changing fish assemblages, as measured by the linear relationships between temperature and the percent of non-native or tolerant fish among sites.

Global change is expected to have a vast array of consequences for fisheries and freshwater ecosystems in general [6,21,54,55]. The expected impacts of global change may be particularly damaging to mussel assemblages in small streams that have less

thermal inertia [21,36]. Hourly temperatures were simulated for the upper Tar River basin under projected climate change scenarios [56], and those projected temperatures from 2021 through 2030 are expected to exceed 30 °C for 96 h or more with a probability of 0.4 and a probability of 0.7 from 2051 through 2060 [56]. Currently, no site in the upper Tar River basin exceeds this temperature duration during the summer. Increasing temperature in a relatively short time period can interfere with mussel recruitment directly [57,58] or indirectly, through a variety of mechanisms, including phenological mismatches with host fish [20,59].

Predicted changes in fish habitat resulting from global change in the United States could affect the availability of host fish for freshwater mussels [54,55]. These predicted habitat impacts are largely expected due to changes in flow, and both reductions in baseline flows and peaks in extreme events are likely due to climate and land use change [3,4]. In the Tar River basin, 16 sites (80%) were under moderate drought during the hottest 96 h period over three summers. This suggests that low flows exacerbate the effects of rising temperatures on freshwater mussels and other aquatic organisms.

We conducted a thorough assessment of the current thermal environment of freshwater mussels over a range of sites in a southeastern U.S. river basin. Our results demonstrate that freshwater mussels and their host fish are currently experiencing temperatures that exceed their upper thermal tolerances. Shifts in mussel assemblages have already been documented as a result of temperature and flow impacts [27,34], and the wide-ranging impact of these changes is experienced at an ecosystem level [9,11]. The buffering capacity of the substrate may provide some protection from thermal stress for freshwater mussels, but they remain limited by the availability of their host fishes and other agents of global change.

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Institutional Review Board Statement: Fish collection and processing procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee under protocol no. 15-042.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to lack of mandate.

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References

1. Lake, P.S.; Palmer, M.A.; Biro, P.; Cole, J.; Covich, A.P.; Dahm, C.; Gibert, J.; Goedkoop, W.; Martens, K.; Verhoeven, J. Global change and the biodiversity of freshwater ecosystems: Impacts on linkages between above-sediment and sediment biota. *BioScience* **2000**, *50*, 1099–1107. [\[CrossRef\]](#)
2. Parmesan, C.; Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **2003**, *421*, 37–42. [\[CrossRef\]](#) [\[PubMed\]](#)
3. IPCC (Intergovernmental Panel on Climate Change). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Core Writing Team, Pachauri, R.K., Meyer, L.A., Eds.; IPCC: Geneva, Switzerland, 2014; 151p.
4. Allan, J.D. Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **2004**, *35*, 257–284. [\[CrossRef\]](#)
5. Mulholland, P.J.; Best, G.R.; Coutant, C.C.; Hornberger, G.M.; Meyer, J.L.; Robinson, P.J.; Stenberg, J.R.; Turner, R.E.; Vera-Herrera, F.; Wetzel, R.G. Effects of climate change on freshwater ecosystems of the south-eastern United States and the Gulf Coast of Mexico. *Hydrol. Process.* **1997**, *11*, 949–970. [\[CrossRef\]](#)
6. Ficke, A.D.; Myrick, C.A.; Hansen, L.J. Potential impacts of global climate change on freshwater fisheries. *Rev. Fish Biol. Fish.* **2007**, *17*, 581–613. [\[CrossRef\]](#)
7. Kaushal, S.S.; Likens, G.E.; Jaworski, N.A.; Pace, M.L.; Sides, A.M.; Seekell, D.; Belt, K.T.; Secor, D.H.; Wingate, R.L. Rising stream and river temperatures in the United States. *Front. Ecol. Environ.* **2010**, *8*, 461–466. [\[CrossRef\]](#)
8. Visser, M.E.; Both, C. Shifts in phenology due to global climate change: The need for a yardstick. *Proc. R. Soc. B* **2005**, *272*, 2561–2569. [\[CrossRef\]](#)
9. Spooner, D.E.; Vaughn, C.C. A trait-based approach to species' roles in stream ecosystems: Climate change, community structure, and material cycling. *Oecologia* **2008**, *158*, 307–317. [\[CrossRef\]](#)
10. Walther, G. Community and ecosystem responses to recent climate change. *Philos. Trans. R. Soc. B* **2010**, *365*, 2019–2024. [\[CrossRef\]](#)
11. Spooner, D.E.; Vaughn, C.C. Species richness and temperature influence mussel biomass: A partitioning approach applied to natural communities. *Ecology* **2009**, *90*, 781–790. [\[CrossRef\]](#)
12. van Ee, B.C.; Johnson, P.D.; Atkinson, C.L. Thermal sensitivity modulates temporal patterns of ecosystem functioning by freshwater mussels. *Freshw. Biol.* **2022**, *67*, 2064–2077. [\[CrossRef\]](#)
13. Gutiérrez, J.L.; Jones, C.G.; Strayer, D.L.; Iribarne, O.O. Mollusks as ecosystem engineers: The role of shell production in aquatic habitats. *Oikos* **2003**, *101*, 79–90. [\[CrossRef\]](#)
14. Howard, J.K.; Cuffey, K.M. The functional role of native freshwater mussels in the fluvial benthic environment. *Freshw. Biol.* **2006**, *51*, 460–474. [\[CrossRef\]](#)
15. Vaughn, C.C.; Spooner, D.E. Unionid mussels influence macroinvertebrate assemblage structure in streams. *J. N. Am. Benthol. Soc.* **2006**, *25*, 691–700. [\[CrossRef\]](#)
16. Vaughn, C.C.; Nichols, S.J.; Spooner, D.E. Community and food web ecology of freshwater mussels. *J. N. Am. Benthol. Soc.* **2008**, *27*, 409–423. [\[CrossRef\]](#)
17. Strayer, D.L. What are freshwater mussels worth? *Freshw. Mollusk Biol. Conserv.* **2017**, *20*, 103–113. [\[CrossRef\]](#)
18. Haag, W.R.; Williams, J.D. Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia* **2014**, *735*, 45–60. [\[CrossRef\]](#)
19. Watters, G.T. A brief look at freshwater mussel (Unionacea) biology. In *Freshwater Bivalve Ecotoxicology*; Farris, J.L., Van Hassel, J.H., Eds.; CRC: Boca Raton, FL, USA; SETAC Press: Pensacola, FL, USA, 2007; pp. 51–64.
20. Pandolfo, T.J.; Kwak, T.J.; Cope, W.G. Thermal tolerances of freshwater mussels and their host fishes: Species interactions in a changing climate. *Walkerana J. Freshw. Mollusk Biol. Conserv.* **2012**, *15*, 69–82. [\[CrossRef\]](#)
21. Hastie, L.C.; Cosgrove, P.J.; Ellis, N.; Gaywood, M.J. The threat of climate change to freshwater pearl mussel populations. *Ambio* **2003**, *32*, 40–46. [\[CrossRef\]](#)
22. Cahill, A.E.; Aiello-Lammens, M.E.; Fisher-Reid, M.C.; Hua, X.; Karanewsky, C.J.; Ryu, H.Y.; Sbeglia, G.C.; Spagnola, F.; Waldron, J.B.; Warsi, O.; et al. How does climate change cause extinction? *Proc. R. Soc. B* **2013**, *280*, 20121890. [\[CrossRef\]](#)
23. Strayer, D.L.; Downing, J.A.; Haag, W.R.; King, T.L.; Layzer, J.B.; Newton, T.J.; Nichols, S.J. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* **2004**, *54*, 429–439. [\[CrossRef\]](#)
24. Cope, W.G.; Bringolf, R.B.; Buchwalter, D.B.; Newton, T.J.; Ingersoll, C.G.; Wang, N.; Augspurger, T.; Dwyer, F.J.; Barnhart, M.C.; Neves, R.J.; et al. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. *J. N. Am. Benthol. Soc.* **2008**, *27*, 451–462. [\[CrossRef\]](#)
25. Pandolfo, T.J.; Cope, W.G.; Arellano, C.; Bringolf, R.B.; Barnhart, M.C.; Hammer, E. Upper thermal tolerances of early life stages of freshwater mussels. *J. N. Am. Benthol. Soc.* **2010**, *29*, 959–969. [\[CrossRef\]](#)
26. Spooner, D.E.; Xenopoulos, M.A.; Schneider, C.; Woolnough, D.A. Coextirpation of host-affiliate relationships in rivers: The role of climate change, water withdrawal, and host-specificity. *Glob. Chang. Biol.* **2011**, *17*, 1720–1732. [\[CrossRef\]](#)
27. Galbraith, H.S.; Spooner, D.E.; Vaughn, C.C. Synergistic effects of regional climate patterns and local water management on freshwater mussel communities. *Biol. Conserv.* **2010**, *143*, 1175–1183. [\[CrossRef\]](#)
28. Archambault, J.M.; Cope, W.G.; Kwak, T.J. Burrowing, byssus, and biomarkers: Behavioral and physiological indicators of sublethal thermal stress in freshwater mussels (Unionidae). *Mar. Freshw. Behav. Physiol.* **2013**, *46*, 229–250. [\[CrossRef\]](#)

29. Ganser, A.M.; Newton, T.J.; Haro, R.J. The effects of elevated water temperature on native juvenile mussels: Implications for climate change. *Freshw. Sci.* **2013**, *32*, 1168–1177. [[CrossRef](#)]
30. Archambault, J.M.; Cope, W.G.; Kwak, T.J. Influence of sediment presence on freshwater mussel thermal tolerance. *Freshw. Sci.* **2014**, *33*, 56–65. [[CrossRef](#)]
31. Khan, J.M.; Hart, M.; Dudding, J.; Robertson, C.R.; Lopez, R.; Randklev, C.R. 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. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2019**, *29*, 1202–1215. [[CrossRef](#)]
32. Goldsmith, A.; Khan, J.M.; Robertson, C.R.; Lopez, R.; Randklev, C.R. Using upper thermal limits of *Lampsilis bracteata* (Texas fatmucket) from the North Llano and San Saba rivers, Texas to inform water management practices in the Edwards Plateau. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2022**, *32*, 85–97. [[CrossRef](#)]
33. Fogelman, K.J.; Archambault, J.M.; Irwin, E.; Walsh, M.; Brewer, S.; Stoeckel, J.A. A review of lethal thermal tolerance among freshwater mussels (Bivalvia:Unionida) within the North American faunal region. *Environ. Rev.* **2023**, *31*, 278–297. [[CrossRef](#)]
34. Haag, W.R.; Warren, M.L., Jr. Effects of severe drought on freshwater mussel assemblages. *Trans. Am. Fish. Soc.* **2008**, *137*, 1165–1178. [[CrossRef](#)]
35. Galbraith, H.S.; Blakeslee, C.J.; Lellis, W.A. Recent thermal history influences thermal tolerance in freshwater mussel species (Bivalvia:Unionida). *Freshw. Sci.* **2012**, *31*, 83–92. [[CrossRef](#)]
36. Newton, T.; Sauer, J.; Karns, B. Water and sediment temperatures at mussel beds in the upper Mississippi River basin. *Walkerana J. Freshw. Mollusk Biol. Conserv.* **2013**, *16*, 53–62. [[CrossRef](#)]
37. Rangaswami, X.L.; Goldsmith, A.M.; Khan, J.M.; Robertson, C.R.; Lopez, R.R.; Randklev, C.R. Thermal tolerances of *Popenaias popeii* (Texas hornshell) and its host fish from the Rio Grande Basin, Texas. *Sci. Rep.* **2023**, *13*, 4603. [[CrossRef](#)] [[PubMed](#)]
38. Pandolfo, T.J.; Kwak, T.J.; Cope, W.G.; Heise, R.J.; Nichols, R.B.; Pacifici, K. Species traits and catchment-scale habitat factors influence the occurrence of freshwater mussel populations and assemblages. *Freshw. Biol.* **2016**, *61*, 1671–1684. [[CrossRef](#)]
39. NCDENR (North Carolina Department of Environment and Natural Resources). *Standard Operating Procedure: Biological Monitoring; Stream Fish Community Assessment Program*, NCDENR, Division of Water Quality: Raleigh, NC, USA, 2006.
40. Kwak, T.J.; Freeman, M.C. Assessment and management of ecological integrity. In *Inland Fisheries Management in North America*, 3rd ed.; Hubert, W.A., Quist, M.C., Eds.; American Fisheries Society: Bethesda, MD, USA, 2010; pp. 353–394.
41. Jobling, M. Temperature tolerance and the final preferendum—Rapid methods for the assessment of optimum growth temperatures. *J. Fish Biol.* **1981**, *19*, 439–455. [[CrossRef](#)]
42. Bogan, A.E. *Workbook and Key to the Freshwater Bivalves of North Carolina*; North Carolina Museum of Natural Sciences: Raleigh, NC, USA, 2002.
43. Dimock, R.V.; Wright, A.H. Sensitivity of juvenile freshwater mussels to hypoxic, thermal, and acid stress. *J. Elisha Mitchell Sci. Soc.* **1993**, *109*, 183–192.
44. ASTM (American Society of Testing and Materials). *Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels*; E2455-06; ASTM International: West Conshohocken, PA, USA, 2006.
45. Pandolfo, T.J.; Cope, W.G.; Arellano, C. Heart rate as a sublethal indicator of thermal stress in juvenile freshwater mussels. *Comp. Biochem. Physiol. Part A* **2009**, *154*, 347–352. [[CrossRef](#)]
46. Landis, A.M.G.; Mosley, T.L.; Haag, W.R.; Stoeckel, J.A. Effects of temperature and photoperiod on lure display and glochidial release in a freshwater mussel. *Freshw. Sci.* **2012**, *31*, 775–786. [[CrossRef](#)]
47. Block, J.E.; Gerald, G.W.; Levine, T.D. Temperature effects on burrowing behaviors and performance in a freshwater mussel. *J. Freshw. Ecol.* **2013**, *28*, 375–384. [[CrossRef](#)]
48. Schwalb, A.N.; Pusch, M.T. Horizontal and vertical movements of unionid mussels in a lowland river. *J. N. Am. Benthol. Soc.* **2007**, *26*, 261–272. [[CrossRef](#)]
49. Amyot, J.; Downing, J.A. Seasonal variation in vertical and horizontal movement of the freshwater bivalve *Elliptio complanata* (Mollusca:Unionidae). *Freshw. Biol.* **1997**, *37*, 345–354. [[CrossRef](#)]
50. Watters, G.T.; O’Dee, S.H.; Chordas, S., III. Patterns of vertical migration in freshwater mussels (Bivalvia:Unionida). *J. Freshw. Ecol.* **2001**, *16*, 541–549. [[CrossRef](#)]
51. Eads, C.B.; Levine, J.F. Vertical migration and reproductive patterns of a long-term brooding freshwater mussel, *Villosa constricta* (Bivalvia: Unionidae) in a small piedmont stream. *Walkerana J. Freshw. Mollusk Biol. Conserv.* **2013**, *16*, 29–41. [[CrossRef](#)]
52. Warren, R.J., II; Bradford, M.A. Mutualism fails when climate response differs between interacting species. *Glob. Chang. Biol.* **2014**, *20*, 466–474. [[CrossRef](#)]
53. Wismer, D.A.; Christie, A.E. *Temperature Relationships of Great Lakes Fishes: A Data Compilation*; Special Publication 87-3; Great Lakes Fishery Commission: Ann Arbor, MI, USA, 1987.
54. Eaton, J.G.; Scheller, R.M. Effects of climate warming on fish thermal habitat in streams of the United States. *Limnol. Oceanogr.* **1996**, *41*, 1109–1115. [[CrossRef](#)]
55. Mohseni, O.; Stefan, H.G.; Eaton, J.G. Global warming and potential changes in fish habitat in U.S. streams. *Clim. Chang.* **2003**, *59*, 389–409. [[CrossRef](#)]
56. Daraio, J.A.; Bales, J.D.; Pandolfo, T.J. Forecasting the effects of land-use and climate change on stream temperature II: Hourly simulations and exceedance duration. *J. Am. Water Resour. Assoc.* **2014**, *50*, 1177–1190. [[CrossRef](#)]

57. Roberts, A.D.; Barnhart, M.C. Effects of temperature, pH, and CO₂ on transformation of the glochidia of *Anodonta suborbiculata* on fish hosts and in vitro. *J. N. Am. Benthol. Soc.* **1999**, *18*, 477–487. [[CrossRef](#)]
58. Taeubert, J.; El-Nobi, G.; Geist, J. Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2014**, *24*, 231–237. [[CrossRef](#)]
59. Philippart, C.J.M.; van Aken, H.M.; Beukema, J.J.; Bos, O.G.; Cadée, G.C.; Dekker, R. Climate-related changes in recruitment of the bivalve *Macoma balthica*. *Limnol. Oceanogr.* **2003**, *48*, 2171–2185. [[CrossRef](#)]

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