

ABSTRACT

PANDOLFO, TAMARA JANE. Biotic and Abiotic Influences on Common and Imperiled Freshwater Mussels at Multiple Spatial and Temporal Scales with Inferences to Global Change. (Under the direction of Thomas J. Kwak and W. Gregory Cope).

Freshwater mussels are among the most imperiled faunal groups globally, and relevant ecological information is urgently needed to guide their management and conservation in the face of global change. We explored the influence of species traits, host fishes, and habitat at three spatial scales (micro-, reach-, and catchment-scale) on the detection and occupancy of 14 species of freshwater mussels in the Tar River basin, North Carolina. Detection probability for all species was 0.42 (95% CI, 0.36 – 0.47) with no species- or site-specific detection effects identified. Mean occupancy probability among species ranged from 0.04 (95% CI, 0.01 – 0.16) for *Alasmidonta undulata*, an undescribed *Lampsilis* sp., and *Strophitus undulatus* to 0.67 (95% CI, 0.42 – 0.86) for *Elliptio icterina*. Median occupancy probability among sites was < 0.30 for all species with the exception of *E. icterina*, and the maximum predicted occupancy probability for 71% of the species did not exceed 0.50. Catchment-scale abiotic variables (stream power, agricultural land use) and species traits (brood time, host specificity, tribe) influenced the occupancy of mussel assemblages more than reach- or microhabitat-scale features.

To further investigate mussel demographics in the Tar River basin, we integrated existing survey records from 1986 – 2011 in a dynamic occupancy model. Results indicated that occupancy probability for 14 species decreased over the study period. Occupancy probability in 1986 ranged from 0.19 for *Utterbackia imbecillis* to 0.60 for *Fusconaia masoni*. Occupancy probability at the end of the 26-year study period ranged from 0.10 for *Lampsilis radiata* to 0.40 for *F. masoni*. The maximum difference between occupancy

probability in 1986 and 2011 was 0.30 for *Alasmidonta undulata*. Mean survival for all species was high (0.97, 95% CI = 0.95 – 0.99). However, the mean colonization probability was very low (< 0.01, 95% CI = < 0.01 – 0.01). These results indicate that mussels persist at sites that they have already occupied, but that they are not colonizing sites where they had not been previously.

One particular threat that mussels face with global change is thermal regime alteration. We combined extensive field measurements of temperature in the stream water column and substrate at 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 mussels face as temperatures continue to rise with global change. Average summer (June – August, 2010 – 2012) temperatures at mussel-occupied sites in the upper Tar River basin ranged 16.2 – 34.7 °C. The mean temperature from the hottest 96 h at each site ranged 23.5 – 31.5 °C. Temperature threshold exceedance durations indicated that both acute and chronic freshwater mussel thermal tolerance thresholds were met or exceeded.

Another primary threat from global change is habitat alteration and degradation. We empirically measured habitat availability and microhabitat use for 10 freshwater mussel species to determine habitat suitability distributions for six microhabitat parameters. Among species, mean water depth of occupied habitats ranged 0.23 – 0.54 m, mean bottom velocity ranged 0.001 – 0.055 m/s, average mean-column velocity ranged 0 – 0.055 m/s, and mean substrate penetrability ranged 0.11 – 11.67 on an index scale. The most commonly measured dominant substrate materials were silt, sand, very coarse sand, pea gravel, and coarse gravel. The most commonly associated cover types were coarse woody debris and fine woody

debris. We found a relationship between niche breadth and conservation status of a species. Federally endangered *A. heterodon* consistently showed a narrower suite of suitable microhabitats than the common mussel *E. complanata*. These findings will enhance the understanding of freshwater mussel ecology and will guide conservation strategies in the face of global change.

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Biotic and Abiotic Influences on Common and Imperiled Freshwater Mussels at Multiple
Spatial and Temporal Scales with Inferences to Global Change

by
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DEDICATION

To Mom, your limitless support means more to me than I can ever say.

BIOGRAPHY

Tamara Pandolfo grew up in Greensboro, North Carolina. She attended the University of North Carolina at Asheville and earned her B.S. in Environmental Studies, with a concentration in Pollution Control, in 2004. Following graduation, she worked as an analyst at the Environmental Quality Institute in Asheville, where she previously worked as an undergraduate researcher. She joined the Department of Environmental and Molecular Toxicology at North Carolina State University in 2006, and earned her M.S. in Toxicology under the mentorship of Dr. Greg Cope in 2008. In 2009, she continued her graduate education as a doctoral student under Drs. Tom Kwak and Greg Cope in the Applied Ecology Department at North Carolina State University.

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CHAPTER 1

Biotic and abiotic influences on the occurrence and detection of freshwater mussels at multiple scales: an assemblage modeling approach

Abstract

Freshwater mussels are multifaceted organisms due to their unique life cycle, cryptic behavior, benthic occurrence, and imperiled status, and relevant ecological information is urgently needed to guide their management and conservation in the face of global change. We adopted a modeling approach that enhances inference on rare species by borrowing data among species in a hierarchical framework to conduct the most comprehensive occupancy analysis for freshwater mussels to date. We incorporated detection probability to more accurately examine effects of biotic and abiotic factors at multiple scales on the occurrence of 14 mussel species and the entire assemblage of the Tar River basin of North Carolina. The single assemblage estimate of detection probability for all species was 0.42 (95% CI, 0.36 – 0.47) with no species- or site-specific detection effects identified. Mean occupancy probability among species ranged from 0.04 (95% CI, 0.01 – 0.16) for *Alasmidonta undulata*, an undescribed *Lampsilis* sp., and *Strophitus undulatus* to 0.67 (95% CI, 0.42 – 0.86) for *Elliptio icterina*. Median occupancy probability among sites was < 0.30 for all species with the exception of *E. icterina*, and the maximum predicted occupancy probability for 71% of the species did not exceed 0.50. Site occupancy probability generally related to mussel conservation status, with reduced occupancy for endangered and threatened species. Catchment-scale abiotic variables (stream power, agricultural land use) and species traits

(brood time, host specificity, tribe) influenced the occupancy of mussel assemblages more than reach- or microhabitat-scale features. We empirically observed 15 mussel species in the basin, but estimated total species richness at 20 (95% CI, 16-24) when accounting for imperfect detection. Our robust findings reflect the complexity of mussel biology and ecology and indicate that habitat restoration alone may not be adequate for mussel conservation.

Keywords: unionid, Bayesian, species richness, rare species, land use, global change

Introduction

Freshwater mussels are an integral part of aquatic ecosystems. They provide vital services to the freshwater food web by converting food from the water column into organic matter that is available to other organisms (Howard and Cuffey 2006). They also contribute to nutrient cycling (Vaughn et al. 2004, Spooner and Vaughn 2006), filter large quantities of water (Strayer et al. 1994), influence assemblages of macroinvertebrates (Vaughn and Spooner 2006), and act as ecosystem engineers (Gutierrez et al. 2003). Their vast ecological contribution is at risk, as freshwater mussels are among the most threatened taxa globally. There are nearly 300 species of freshwater mussels in North America, and 69% of these are at risk; 37 species are already extinct or presumed so (Williams et al. 1993, Master et al. 2000). Freshwater mussels are particularly susceptible to environmental stressors, in part because of their reliance upon a host fish to complete their life cycle. The larval life stage

(glochidia) is an obligate parasite that requires encystment on a host fish to transform into the juvenile life stage (e.g., Kat 1984).

Habitat alteration or destruction has been identified as a leading cause of mussel decline (Downing et al. 2010), and many studies have attempted to clarify the influence of habitat on the distribution of freshwater mussels at multiple spatial, temporal, and organizational scales (e.g., Holland-Bartels 1990, McRae et al. 2004, Steuer et al. 2008). However, mussel distribution has also been connected to biotic factors such as species traits and the distribution and habitat affinity of host fishes (Haag and Warren 1998, Schwalb et al. 2013).

Another consideration when attempting to describe the distribution of mussels is their detection during sampling events. Detection probability is an important component of any animal survey because subjects are rarely always detected (i.e., detection probability is < 1 ; MacKenzie et al. 2002). Estimates of freshwater mussel distribution and abundance are particularly affected by detection probability because they often burrow and have patchy distributions (Strayer and Smith 2003). Only very recently have predictive freshwater mussel models begun to incorporate detection probability in attempts to assess impacts of various environmental factors (Shea et al. 2013, Wisniewski et al. 2013).

Assessing detection probability for freshwater mussels is inherently difficult, because many species are imperiled and rare, but methods for estimating detection are data intensive requiring large sample sizes. Some suggested techniques for addressing this dilemma include borrowing information from related species or sites and determining occupancy probability or species richness rather than abundance (MacKenzie et al. 2005). A recently

developed community modeling approach utilizes both of these approaches and allows for the inclusion of rare species with limited data by borrowing data among species in a hierarchical framework while producing estimates of occupancy and species richness (Kery and Royle 2009, Zipkin et al. 2009). Species richness, a commonly used measure of biodiversity, is an indicator of community structure and the integrity of ecosystem processes (Naeem et al. 1995, Chapin et al. 2000, Kwak and Peterson 2007). Ecological processes are specifically affected by the abundance and species composition of mussel assemblages (Vaughn et al. 2004, Vaughn et al. 2008). Thus, the estimation of freshwater mussel species richness allows for an assemblage-level assessment of ecological health.

We adopted this assemblage occupancy modeling approach in a Bayesian framework to investigate the effects of mussel species traits, host fish, and stream ecosystem habitat variables at the microhabitat, reach, and watershed scale on the detection and occupancy of freshwater mussels in the Tar River basin of North Carolina. We are also the first investigators using this novel approach to determine mussel species richness basin-wide and for each site while accounting for species detection and occupancy probabilities.

Methods

Freshwater mussel and fish surveys

We studied 20 sites within the upper Tar River basin of North Carolina including three subbasins with similar drainage areas: the upper Tar River, Swift Creek, and Fishing Creek (Figure 1). The sites represented a longitudinal gradient spanning a range of environmental conditions (e.g., watershed area, land cover) within the river basin. Semi-

quantitative snorkel and tactile search freshwater mussel surveys were conducted in collaboration with the North Carolina Wildlife Resources Commission during the summer of 2010. A minimum of six person-hours of effort was expended at each 200–500-m stream reach, more effort than typically allocated to such surveys (Metcalf-Smith et al. 2000).

Fish species richness and Index of Biotic Integrity were calculated for each site from electrofishing surveys conducted in 2010. Surveys consisted of two backpack electrofishers and two dipnetters sampling 200 m upstream along the banks followed by a return pass down the center of the stream. This protocol followed that of the North Carolina Department of Environment and Natural Resources, Division of Water Quality (NCDENR 2006). Our objective was not to measure fish occupancy, but to apply fish parameters as a covariate to potentially explain mussel occurrence due to the obligate mussel-host fish relationship

Habitat covariates

When a mussel was discovered during the survey, we measured microhabitat characteristics at the precise location the mussel resided within mussel survey reaches. Microhabitat use measurements were recorded for all encountered individuals of non-abundant species, and for up to 20 individuals of the ubiquitous *Elliptio complanata*, at each site. Microhabitat measurements included water depth, bottom and mean-column water velocity, embeddedness, roughness, distance to nearest cover, and nearest cover type. Dominant and subdominant substrate types were classified using a modified Wentworth particle size scale (Bovee and Milhous 1978), and substrate heterogeneity was calculated by determining the number of different dominant and subdominant substrate types at a site. Substrate compaction was measured using a Lang penetrometer (Johnson and Brown 2000),

such that low resistance was associated with the least compacted/most penetrable substrates (e.g., silt and sand). All other parameters were measured using established methods for stream habitat assessment (Bain and Stevenson 1999).

At the reach scale, 10 transects were established every two mean-stream-widths apart (Simonson et al. 1994). Stream shading was determined by measuring riparian open canopy with a densiometer at both banks and in the center of each transect (Platts et al. 1987), and bank angle was measured on both banks in each transect. A cross-sectional area survey was conducted once per site using the Tape-Down Method to determine the bankfull width, bankfull cross-sectional area, bankfull mean and maximum depth, and width-to-depth ratio (Harrelson et al. 1994). A pebble count was conducted at the location of the cross-sectional area survey to determine median particle size (D50) as well as D16 and D84 (Potyondy and Hardy 1994). A Bank Erosion Hazard Index survey was conducted on each bank (Rosgen 2001). Continuous hourly temperatures were taken 10 cm above the sediment/water interface in the water column, 5 cm below the interface, and 15 cm below the interface at each site by deploying two replicate monitoring units that utilized iBCod 22L data loggers from Alpha Mach, Inc. (Mont-St-Hilaire, Quebec, CAN; Chapter 3, this dissertation) in July and August of 2010.

Catchment boundaries for each sampling location were delineated in ArcGIS (9.31, Redlands, CA) using GPS coordinates that were taken at each site as the outlet of each catchment. These boundaries were used for all further landscape analyses. Watershed area and the proportions of different categories of watershed land use, riparian land use (15 and 30 m buffers; USGS 2011), ecoregion (USEPA 2010), and geologic rock type (NCGS 2007)

were calculated in ArcGIS using existing data. Stream power was calculated using discharge and slope measurements for each catchment using the mean annual flow from 2010.

Covariate selection

Data on a total of 144 continuous covariates for habitat and fish were collected. We reduced the list to 35 representative covariates after careful review of previous mussel distribution studies (e.g., Hardison and Layzer 2001, McRae et al. 2004, Strayer 2008). Correlation analysis was used to determine the final list of covariates for our analysis ($|r| < 0.60$), with the stipulation that at least one variable from each habitat scale (microhabitat-, reach-, and catchment-scale) and at least one fish covariate were retained. The selected covariates were then stratified into occupancy covariates (i.e., variables expected to influence mussel occupancy) and detection covariates (i.e., variables expected to influence mussel detection). The resulting eight occupancy covariates were substrate compaction, substrate heterogeneity, bankfull maximum depth, 2010 substrate degree days, geologic rock type, watershed agricultural land use, stream power, and fish species richness (Table 1). The three detection covariates were dominant substrate type, D84, and water depth.

Temperature data were not available for all 20 sites due to malfunctioning loggers or complete loss of logger units (Chapter 3, this dissertation), and four sites were missing a value for 2010 substrate degree days. Missing temperature values were generated using a predictive linear regression ($p < 0.001$, $R^2 = 0.8284$) based on related temperature points (2010 substrate degree days were predicted based on 2011 substrate degree days).

The effects of different mussel species traits on occupancy were tested in separate models. Each species' tribe, host specificity (generalist or specialist), brood time (tachytictic

or bradyctictic), and reproductive strategy (attractor or non-attractor) were tested in the absence of other covariates (J. M. Archambault, unpublished database; e.g., Bauer and Wachtler 2000, Barnhart et al. 2008).

Hierarchical occupancy modeling

Hierarchical multispecies models “borrow” information across all species in an assemblage, which leads to more precise species-level inferences (Dorazio and Royle 2005). In these models, each species influences the parameter estimates of all other species in the assemblage. As a result, individual species-level estimates are a combination of data from the single species and the mean estimate of those parameters for the entire assemblage (or group of species). The extent to which estimates are pooled together rather than estimated separately (i.e., pooling or “shrinkage”) is dependent upon the quality and quantity of available data (e.g., number and locations of species detections; Gelman and Hill 2007). A major benefit of shrinkage is the ability to estimate parameters for species that are rarely detected and would otherwise not be estimable or would be too imprecise for meaningful inference (Kery and Royle 2008).

Modeling framework

We modeled the mussel assemblage using the approach of Dorazio and Royle (2005) and Dorazio et al. (2006). In our model, N denotes the unknown number of unique species that occur within the region of interest (here N can represent the total assemblage of species). Surveys are conducted wherein each of the $j=1, 2, \dots, J$ sites is independently observed $k=1, 2, \dots, K$ times, and the identities of all species $i = 1, 2, \dots, n$ are recorded as they are

detected during the sampling event. There is an assumption of community closure (i.e., N remains constant).

Site-specific occurrence for species $i = 1, 2, \dots, n, \dots, N$ at site j , denoted z_{ij} , is a latent random variable where $z_{ij} = 1$ if species i occurs in site j and is zero otherwise. We specify the occurrence model as $z_{ij} \sim \text{Bern}(\psi_{ij})$ where ψ_{ij} is the probability that species i is present at j . True occurrence is only partially observed through the detection/non-detection data, where x_{ijk} (recorded as a one if a species is observed and zero otherwise) for species i at site j for observer k is $x_{ijk} \sim \text{Bern}(p_{ijk} * z_{ij})$. The parameter p_{ijk} is the detection probability of species i at site j for the k th observer. If species i is present ($z_{ij} = 1$) at site j , then the probability of detecting that species is p_{ijk} ; otherwise if $z_{ij} = 0$, then $x_{ijk} = 0$, and we ensure that detection is a fixed zero when a species is not present.

Our intent was to obtain a parsimonious model that explored the effects of important covariates on mussel occurrence and detection. To do so, we incorporated covariate effects into the occurrence and detection models linearly on the logit-probability scale. We modeled occurrence as a function of eight site-level covariates (Table 1) on the logit scale as follows: $\text{logit}(\psi_{ij}) = \mu_i + \alpha 1_i \text{spow}_j + \alpha 2_i \text{wsag}_j + \alpha 3_i \text{geob}_j + \alpha 4_i \text{bmax}_j + \alpha 5_i \text{degdays}_j + \alpha 6_i \text{sedtypes}_j + \alpha 7_i \text{penet}_j + \alpha 8_i \text{fish}_j$. We similarly modeled the detection probability for species i as a function of d84, domsed, depth (Table 1): $\text{logit}(p_{ijk}) = v_i + \beta 1_i \text{d84}_{jk} + \beta 2_i \text{domsed}_{jk} + \beta 3_i \text{depth}_{jk}$. All covariates were centered and normalized (mean = 0, variance = 1), such that the inverse-logit of μ_i , for example, is the occurrence probability for species i in sites with ‘average’ site conditions.

An additional hierarchical component was added to the model, wherein we assumed that the species-level parameters were random effects governed by assemblage hyper-parameters (i.e., parameters of the prior distribution). All model coefficients were assumed to come from a normal distribution (e.g., $\alpha_{1_i} \sim N(\mu_{\alpha_1}, \sigma_{\alpha_1})$) where the mean of the distribution represents the community response to that particular covariate and the standard deviation is the variation among species within the particular group. We followed Dorazio et al. (2006) and used a parameterization of the unconditional likelihood and data augmentation to estimate species richness N for all groups within each of the different classification schemes.

Model selection

We were interested in understanding which covariates were influencing individual mussel species occurrence and the effects on the species assemblage. Therefore, we fit 36 models that varied in complexity (Table 2). The most complex model (i.e., global, or full, model) was constructed with species-specific random effects for the intercept and species-specific random effects for the covariates for both occurrence and detection. We then explored all possible combinations of having species-specific random effects and constant effects for the intercept and covariates influencing occurrence and detection. The simplest model had no covariate effects at all and only included a constant intercept for occurrence and detection (i.e., null model; Table 2). We used the Deviance Information Criterion (DIC; Spiegelhalter et al. 2002) to select the most parsimonious model. DIC is a Bayesian analogue to the more familiar Akaike's Information Criterion (Akaike 1973), which selects the best relative fitting model (lowest AIC score) by balancing model accuracy and precision

(Burnham and Anderson 2002). The most parsimonious model was used to determine detection probability, a mean occupancy probability for each species, site and species-specific occupancy probability, projected occupancy probability over a range of covariate values, basin-wide species richness, and species richness at each site.

We explored species-level effects (host, tribe, brood, strategy) by setting up four separate models with each model containing one of the species-level effects of interest (Table 3). Because we were interested in examining the effects of these individual species-level covariates we used the most parsimonious model from the above assemblage analysis, wherein occurrence was modeled only as a function of each species-level effect and the intercept was held constant, $\text{logit}(\psi_{ij}) = \mu + \alpha 1_{\text{host}_i}$. We modeled detection as a constant intercept across all species.

Model evaluation

We fit each of the models separately using a Bayesian approach in WinBUGS (Spiegelhalter et al. 2003) through R statistical software (R2WinBUGS; Sturtz et al. 2005) by running three parallel chains, each of length > 77,000 with burn-in of at least 25,000 and thinning by 25. We used vague priors (e.g., uniform distribution from zero to one for assemblage- or group-level occurrence and detection covariates; normal distributions with mean zero and variance 1,000 for community or group-level habitat and sampling covariates) for all hyper-parameters in all models. Convergence was confirmed through trace plots, correlation, and the Gelman-Rubin statistic (Gelman and Rubin 1992).

For all analyses, covariate effects were evaluated using the posterior distribution of each covariate parameter estimate. When the 95% credible interval of a parameter estimate

did not include 0, a strong effect of the covariate was evident. If the interval included 0, but the bulk of the parameter's distribution was either positive or negative, a weaker but supported effect of the covariate was evident. For covariates deemed to have an effect on occupancy or detection, we calculated odds ratios (OR) for ease of interpretation. The OR of a covariate represented the associated change in odds given a change of one standard deviation in the covariate.

Results

A total of 16,603 mussels of 15 species were sampled at the 20 study sites in the Tar River basin. Some mussels ($n = 112$) were identified as *Elliptio* spp. because of the difficulty of discriminating between *E. complanata*, *E. congaraea*, and *E. icterina* at several locations with co-occurrences. Only mussels that were confidently identified to species were included in occupancy analyses. *E. complanata* accounted for most individuals (14,057) and was the only species found at all 20 sites. *E. icterina* (1,788) and *E. congaraea* (453) were the other most abundant species, and the remaining 12 species accounted for only 305 individuals. Two federally endangered species, *Alasmidonta heterodon* and *E. steinstansana*, were encountered during surveys. A total of 20 *A. heterodon* were found at three sites, and three *E. steinstansana* were found at two sites. Remaining species and counts were *A. undulata* (1), *E. fisheriana* (23), *E. lanceolata* (2), *E. roanokensis* (69), *Fusconaia masoni* (25), *Lampsilis cariosa* (5), undescribed *Lampsilis* sp. (6), *Strophitus undulatus* (1), *Utterbackia imbecillis* (4), and *Villosa constricta* (34). Preliminary model runs indicated that *E. complanata* could not be included in model estimations because of the lack of any absence

data (i.e., *E. complanata* was always present); therefore, data from the remaining 14 species were modeled.

A total of 36 candidate models were examined to describe the potential impact of habitat and fish covariates on freshwater mussel occupancy and detection. Two models emerged as the most plausible fits for our data, with a very small Δ DIC (0.37) between them (Table 2). The model with the lowest DIC (260.5) had an occupancy intercept that randomly varied among species, a detection intercept that was constant across species, and no covariates added for either occupancy or detection. The other most plausible model (DIC = 260.8) also had an occupancy intercept that varied randomly among species, a detection intercept that was constant across species, and no covariates added for detection. However, this model also included effects of occupancy covariates that were constant across species. Because there was virtually no difference between DIC values for these models, the second model was chosen as the basis of further analysis because our main objective was to examine effects of abiotic and biotic covariates on mussel occupancy probability.

Our primary model of interest indicated that geologic rock type, bankfull maximum depth, substrate degree days, substrate heterogeneity, substrate compaction, and fish species richness did not explain the occupancy of freshwater mussels at our study sites (Table 4). However, there was a strong negative effect of watershed-scale agricultural land use on mussel occupancy probability, and evidence of a positive, but imprecise, effect of stream power on mussel occupancy. Species occupancy responses over a range of proportions of agricultural use in the watersheds were predicted for two representative species, *E. roanokensis* and *V. constricta* (Figure 2A). Results indicated a similar response magnitude

between the species, with an occupancy probability of ~0.85 associated with 13% of the watershed in agricultural use declining to ~0.55 at 23% agricultural land use, the mean percentage among our study sites. As the percentage of agricultural land use in the watershed increased to 33%, occupancy probability dropped to ~0.20 for both species. Odds ratios indicated that predicted mussel occurrence would be 2.33 times lower with every increase of 5.0% in watershed agricultural land use (OR = 0.43, 95% CI = 0.19 – 0.85). The predicted relationship between stream power and mussel occupancy was also explored using *E. congaraea* and *A. heterodon* as representative species (Figure 2B). Again, the magnitude of species responses was largely similar, but *A. heterodon* occupancy probability was always predicted to be ~0.04 less than that of *E. congaraea*. The mean stream power among our study sites (0.06 W/m) resulted in mussel occupancy ~0.55, with occupancy estimated to reach ~0.75 with a stream power of 0.14 W/m. Odds ratios indicated that with every predicted 0.04 W/m increase in stream power, mussel occurrence probability would be 1.68 times higher (OR = 1.68, 95% CI = 0.93 – 3.15).

A separate analysis to investigate the influence of four mussel species life history traits indicated that brood time, host specificity, and tribe had an effect on occupancy probability, but reproductive strategy did not (Figure 3). The estimated occupancy probability for a host generalist (0.08) was less than that of a host specialist (0.24), and bradytic (long-term) brooders had an estimated occupancy probability of 0.11 compared to 0.28 for tachytic (short-term) brooders. Of the three unionid tribes represented in our study, Anodontini had the lowest occupancy probability (0.08), followed by Pleurobemini

(0.15), and *Lampsilini* (0.28). The occupancy probability between host attractors and non-attractors (Barnhart et al. 2008) did not differ.

Detection probability did not vary by species or covariates in our most plausible models. Detection probability did not vary randomly across species in any of the 10 models with the lowest DIC values, so we are confident that although species has influenced detection in other studies (e.g., Smith et al. 2001, Meador et al. 2011), our data did not support these previous findings. Similarly, six of the 10 models with the lowest DIC values did not include any covariate effect on detection probability. A mean detection probability of 0.42 was estimated for all mussel species (Table 5). The occupancy probability predicted for each mussel species in an average habitat ranged from 0.04 for *A. undulata*, *Lampsilis* sp., and *S. undulatus* to 0.67 for *E. icterina* (Table 5). Seven more species had estimated overall occupancy probability ≤ 0.15 , leaving only *E. congaraea* (0.29), *E. icterina* (0.67), *F. masoni* (0.29), and *V. constricta* (0.19) with occupancy probability ≥ 0.15 .

Because our primary model of interest included occupancy covariates, it is appropriate to consider occupancy probability estimates that were calculated with site specific covariate data (Table 6). The median occupancy probability for each species predicted with covariate information at 20 sites was generally low (Figure 4). *E. icterina* was the exception to this trend, with a median occupancy probability of 0.62. All other species had a median occupancy of < 0.30 . Six species had median occupancy probability < 0.10 , including the federally endangered *E. steinstansana*. In addition to the low median values, the maximum occupancy probability for 10 of the species (71%) did not exceed 0.50. The

four species with higher maximum occupancy probability estimates were *E. congaraea* (0.65), *E. icterina* (0.89), *F. masoni* (0.65), and *V. constricta* (0.53).

When compared, site occupancy probability for *E. icterina*, North Carolina endangered *F. masoni*, and federally endangered *A. heterodon* and *E. steinstansana* in the Tar River basin indicate distinct patterns that relate to conservation status (Figure 5). Federally endangered *E. steinstansana*, a North Carolina endemic species, had the lowest site occupancy probability of the four species, with 17 of 20 sites (85%) having an occupancy probability of ≤ 0.20 . The other federally endangered species, *A. heterodon*, also had occupancy probability ≤ 0.20 at the majority of sites (75%). North Carolina endangered species *F. masoni* was predicted to have occupancy probability ≤ 0.20 at four sites (20%). The stable species *E. icterina* was predicted to occur at all sites with a probability > 0.20 , with a minimum occupancy probability of 0.41.

A total of 15 freshwater mussel species were observed during surveys in the Tar River basin, whereas the predicted mean species richness for the basin was 20 with a 95% credible interval of 16 – 24 species. This suggests it is likely that we failed to detect at least one species in our surveys. Predicted estimates of mussel species richness at each site were similar to the observed species richness for all 20 sites (Figure 6). In all cases, the observed value occurred within the 95% credible interval for the site prediction.

Discussion

We have conducted the most inclusive occupancy analysis for freshwater mussels to date. Our study incorporated detection probability, while examining the effects of multi-

scale biotic and abiotic factors on the occurrence of an assemblage of 14 species of freshwater mussels. Our study is novel not only because of its breadth, but also because we were able to estimate the mussel species richness of a river basin based on the detection and occupancy probabilities of the mussels surveyed there. The estimation of species richness for a site is a valuable conservation tool, because it is an indicator of biodiversity, and the value of biodiversity to ecosystem processes has been well-documented (Naeem et al. 1995, Purvis and Hector 2000, Schwartz et al. 2000). Species richness is a measure of community structure and ecological function (Chapin et al. 2000, MacKenzie et al. 2005), and for species that fulfill similar functional roles within a community, a diverse assemblage enhances the likelihood that some of the species, even rare ones, may be able to survive and uphold ecosystem integrity in the face of environmental challenges (Chapin et al. 2000). However, empirical measures of species richness may not be accurate because it is likely that some species will escape detection (MacKenzie et al. 2005, Kery and Royle 2009). Biased measures of species richness will in turn bias estimates of species turnover, local extinction, and colonization (Kery and Royle 2009).

Our results support the application of detection probability in the estimation of species-level occupancy as well as assemblage-level species richness. The single assemblage estimate of detection probability for the freshwater mussels in our study was 0.42, well below 1.0 (i.e., the assumption that every individual is always detected). The importance of detection probability in freshwater mussel surveys has been documented in a number of studies (e.g., Smith et al. 2001, Mynsberge et al. 2009, Meador et al. 2011, Shea et al. 2013). For example, Wisniewski et al. (2013) recently deduced that based on naive occupancy, i.e.,

observed occupancy without detection probability, their surveys indicated that the distribution of some species appeared to have contracted, but this was actually a result of imperfect detection. Similarly, based on empirical abundance estimates, *S. undulatus* was determined to be the 10th most abundant species in the Allegheny River in Pennsylvania, however if the species was perfectly detected, that species would have been the 5th most abundant species (Smith et al. 2001). These examples illustrate the importance of considering detection probability in population- and assemblage-level assessments and the spurious results that may occur if detection is overlooked.

Some of these investigations documented the influence of various mussel, habitat, and survey traits on detection. Mussel detection has been found to vary among species (Smith et al. 2001, Strayer and Smith 2003, Villella et al. 2004, Meador et al. 2011, Wisniewski et al. 2013), burrowing behavior (Strayer and Smith 2003, Villella et al. 2004, Meador et al. 2011, Wisniewski et al. 2013), habitat (Huang et al. 2011, Meador et al. 2011), and survey characteristics (Smith et al. 2001, Shea et al. 2013, Wisniewski et al. 2013). Our data did not support a species or habitat effect on detection probability. Mussel detection probability in other studies was generally low (i.e., < 0.30 ; Villella et al. 2004, Meador et al. 2011, Wisniewski et al. 2013), but ranged up to 0.69 for some species (Wisniewski et al. 2013). Our detection probability estimate of 0.42 may be biased high because of the data structure we used, i.e., species with the most robust data drove the analyses, and those species tended to be common.

Because methods of determining detection probability are relatively data-intensive, the investigation of rare species is often troublesome (MacKenzie et al. 2005). Rare mussel

species are often overlooked, with less than half of rare species found in a typical 1.5 person-h survey (Metcalf-Smith et al. 2000), and a mean of only 61% of species detected in four person-h (Huang et al. 2011). Thus, one approach for estimating detection or other sampling or demographic parameters of interest for a rare species is to intensify the search effort (Metcalf-Smith et al. 2000, MacKenzie et al. 2005, Huang et al. 2011). We designed our mussel surveys to detect rare species by investing a greater effort (minimum of 6 p-h) than is applied in typical mussel surveys (Metcalf-Smith et al. 2000). Our intensive surveys accomplished the goal, with the observed species richness for each site occurring within the 95% credible interval of the estimated species richness based on detection. We suspect that if a lesser effort were expended in our surveys, the disparity between observed and estimated species richness would have been greater.

Another approach for dealing with rare species is to borrow information from other species, and use it to estimate occupancy rather than abundance (MacKenzie et al. 2005). We were able to generate estimates of occupancy for even the rarest species in the Tar River basin because of the hierarchical community-modeling occupancy approach that we adopted. We generated mean occupancy probability among 14 species in average habitat conditions. The most common species that was modeled, *E. icterina*, had the highest mean occupancy probability of 0.67. The species with the lowest mean occupancies, *A. undulata*, *Lampsilis* sp., *S. undulatus*, and *U. imbecillis*, were each encountered at only one site. We were also able to estimate occupancy probability for some species of particular conservation interest, including *A. heterodon*, *E. steinstansana*, and *F. masoni*. There was evidence that occupancy

related to conservation status, and species occupancy probability was directly related to the stability of the species.

Our primary aim was to investigate the effects of a range of multi-scale covariates on mussel occupancy, particularly for species of conservation interest. Studies with similar goals conducted over the past few decades have indicated that spatial scale is an important consideration in these investigations (e.g., Strayer and Ralley 1993, McRae et al. 2004). Measurements of microhabitat characteristics have had mixed success in predicting mussel distributions (e.g., Salmon and Green 1983, Strayer and Ralley 1993), whereas reach and catchment scale variables such as hydraulics and geomorphology have proven to be more reliable (e.g., McRae et al. 2004, Gangloff and Feminella 2007, Newton et al. 2008). We found that two of the eight occupancy covariates tested influenced mussel occupancy, stream power and agricultural land use. Both of these covariates measured habitat characteristics at the catchment scale.

The finding in our study that stream power influenced mussel occupancy was not surprising. The role of complex hydraulic variables in structuring the distribution of freshwater mussels is well-established (e.g., Hardison and Layzer 2001, Gangloff and Feminella 2007, Zigler et al. 2008). Our results supported a positive link between mussel occupancy and stream power. However, the majority of studies have found a negative association between mussel distribution and related hydraulic variables, including Layzer and Madison's (1995) finding that stream power is negatively related to mussel density. The negative effects of shear stress on mussels have been documented in a number of studies (Layzer and Madison 1995, Hardison and Layzer 2001, Gangloff and Feminella 2007, Steuer

et al. 2008, Zigler et al. 2008), and the importance of stability in flow and substrate for mussels is also well-known (Strayer 1999, McRae et al. 2004, Zigler et al. 2008).

However, because stream power is a one-dimensional measurement made at a large scale, stream power is not easily distinguished from discharge. Some investigators have found that mussel distribution is limited by both high and low flows (Layzer and Madison 1995, Steuer et al. 2008, Peterson et al. 2011). Most of the negative relationships among hydraulic variables and mussels pertain to very high flows associated with flooding. However, a positive relationship may exist below a certain threshold (Layzer and Madison 1995, Steuer et al. 2008). This may explain of the positive relationship between stream power and mussel occupancy in our study. Mussel recruitment and mussel species richness have both been positively related to discharge (Peterson et al. 2011, Spooner et al. 2011). Annual flows were used to calculate stream power, and 2010 was a year that reached moderate drought conditions in eastern North Carolina (NCDMAC 2010). The positive relationship may indicate that reduced or absent flow was more detrimental than flooding during the study period. Additionally, stream power more or less determines the total sediment transport of flowing waters (Yang and Stall 1974). Low stream power is associated with low flows and deposition of fine sediments, whereas higher stream power can flush the fines from a system, and diverse mussel assemblages have been linked to areas with fewer fine sediments (McRae et al. 2004).

The strong negative influence of agricultural land use on freshwater mussel occupancy in this study is likely due in part to its impact on hydrologic stability (Gangloff and Feminella 2007). Catchment scale land use influences the hydrology of the entire stream

network (Allen 2004), but flow stability is especially important to mussels in the lotic environment (e.g., Strayer 1999, McRae et al. 2004). Agricultural land use in particular can increase the magnitude, frequency, and duration of high flows, decrease base flows, increase runoff, and contribute to a more flashy flow regime (Allen 2004, Poff et al. 2006). These hydrologic alterations in turn intensify erosion and destabilize banks, alter channel geomorphology, and lead to greater amounts of sediments, nutrients, and pesticides in the system (Allen 2004, Poff et al. 2006, Gangloff and Feminella 2007).

Our findings indicated that with every 5% increase in agricultural land use in a watershed, the probability of freshwater mussel occupancy was 2.33 times lower. Our study sites had 13 – 31% agricultural land use in their catchments, suggesting that a 5% change in agricultural land use is not an extreme benchmark in an agricultural state like North Carolina, the top producer of tobacco and sweet potatoes in the United States (USDA 2013). Other studies have also found a negative relationship between mussel diversity and agricultural land use (e.g., Poole and Downing 2004, Cao et al. 2013, Daniel and Brown 2013), but the effects of contemporary land use can be difficult to isolate from the legacy effects of past land use.

We assessed agricultural land use using contemporary data, but the negative effects that we found were most likely also associated with past agricultural activities in the same catchments. Although many physical and chemical properties of streams strongly relate to current land use, the present composition of macroinvertebrate, fish, and freshwater mussel assemblages have been influenced by past land use (Harding et al. 1998, Poole and Downing 2004, Maloney et al. 2008). Historical land use can alter stream ecosystems for decades,

even after land has been repurposed (Harding et al. 1998, Maloney et al. 2008). The long-lasting effects of agricultural land use have led to an extinction debt in freshwater mussels (Tilman et al. 1994, Poole and Downing 2004), with diversity dwindling generations after the habitat has been altered. Although the amount of agricultural acreage in North Carolina has been declining (USDA 2013, Sleeter et al. 2013), and may continue to do so with climate change (Adams et al. 1990), the legacy effects of current land use practices will continue to affect freshwater mussels into the future. The impacts of various components of global change cannot be easily distinguished, and species loss will most likely result from synergistic effects among them (Brook et al. 2008).

In addition to catchment scale habitat variables, we also found that mussel occurrence is influenced by several biological species traits. Our data provided evidence that mussel occupancy probability is affected by host specificity, tribe, and brood time. The influence of brood time and host specificity on mussel occupancy is not surprising, given that the parasitic nature of the freshwater mussel life cycle results in dispersal costs (Kat 1984). Our results suggest that host generalists have a lower occupancy probability than host specialists, which seems counterintuitive, but host specialists often have adaptation strategies which lead to a higher reproductive success rate (Kat 1984, Barnhart et al. 2008).

Haag and Warren (1998) found that the reproductive strategy (host specificity and attraction method) of mussels helped describe mussel community composition. Though we found an influence of host specificity, we did not detect an effect of attraction strategy on mussel occurrence. However, this distinction often falls along genetic boundaries (Barnhart et al. 2008). Most Unioninae (such as the Anodontini) broadcast glochidia without

specialized attraction strategies (Barnhart et al. 2008), and we found that the Anodontini had the lowest occupancy probability in our study. Pleurobemini and Lampsilini, the tribes that had greater occupancy probability in our study, are members of the Ambleminae subfamily which tend to use host attraction mechanisms to enhance reproductive success (Barnhart et al. 2008). Therefore, it is reasonable to speculate that the apparent influence of tribe on mussel occupancy probability may relate to reproductive strategy, and specifically to host attraction strategy.

The influence of brood time on occupancy is again related to overall reproductive strategy. Our results indicated that bradytictic species (long-term brooders) were expected to have a lower occupancy probability than tachytictic species (short-term brooders). This may be due to the longer period for other stressors to interfere with the reproductive process in long-term brooders, but it is also possible that this is related to detection. Meador et al. (2011) found that burrowing behavior was associated with reproductive activity in mussels. The different brooding strategies may lead to long-term brooders being less available for detection during the typical summer field season. We did not investigate the potential effect of brood time on detection in this study.

Although we did not detect an effect of fish species richness on mussel occupancy, several studies have found that variability or abundance of fish relates to the distribution of mussels (Haag and Warren 1998, Vaughn and Taylor 2000, Cao et al. 2013, Schwalb et al. 2013). There have been indications that the influence may be species-specific (Haag and Warren 1998, Cao et al. 2013), and driven in part by host attraction strategy (Haag and Warren 1998). The community modeling approach that we used averaged effects across

species, so if a few species exhibited a positive relationship with fish richness, but others did not, the result would not be detected or included in plausible candidate models. We are not the only investigators to find little or no support of a link between fish community metrics and mussel distribution (Poole and Downing 2004, Daniel and Brown 2013), but the presence of effects of species traits related to reproduction suggests a connection. Moreover, we may not have measured the most predictive fish assemblage metric. Cao et al. (2013) found that mussel species richness increased with total fish abundance, but not with fish richness or fish IBI, yet others have found a relationship between mussel and fish species richness (Vaughn and Taylor 2000).

The need for a multi-dimensional approach when studying freshwater mussels is widely accepted (e.g., Haag and Warren 1998, Vaughn and Taylor 2000, Strayer 2008, Cao et al 2013, Daniel and Brown 2013), and our results corroborate the importance of an inclusive design that includes abiotic and biotic factors to describe patterns in freshwater mussel distributions. Our approach represented a further advance in the study of mussel ecology and incorporated detection probability to obtain estimates of mussel species richness at the assemblage level. Accurate estimates of mussel populations, including diversity, are necessary for developing successful conservation and management strategies. The conservation of an entire mussel assemblage, rather than populations, is the only means to be certain that species interactions and ecological processes are preserved (Vaughn et al. 2008). Changes in mussel abundance and assemblage composition can lead to altered ecosystem function (Vaughn et al. 2004, Vaughn and Spooner 2006, Vaughn et al. 2008), and only by accurately assessing assemblage dynamics can proper conservation actions be taken.

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Table 1. Descriptions, model abbreviations, mean, standard deviation, and range of covariates used in candidate models for the occupancy and detection probabilities of the freshwater mussel assemblage in the Tar River basin of North Carolina.

Covariate	Abbrev	Description	Mean	SD	Min	Max
<i>Detection</i>						
D84	d84	Particle size which is larger than 84% of other particles (mm)	18.7	26.4	0.5	90
Dominant substrate	domsed	Mode substrate type based on index of Modified Wentworth Scale	2.1	2.4	1	11
Water depth	depth	Median water depth (m)	0.31	0.16	0.10	0.64
<i>Occupancy</i>						
<i>Microhabitat scale</i>						
Substrate compaction	penet	Median penetrability of substrate (psi)	21.0	30.4	0.0	86.9
Substrate heterogeneity	sedtypes	Total number of dominant and subdominant substrate types	8.9	2.96	4	14
<i>Reach scale</i>						
Bankfull max depth	bmax	Depth (m) to bottom of channel from bankfull maximum height	2.23	1.04	0.77	5.00
Degree days	degdays	Cumulative degrees per day from hourly substrate temperatures	2033.5	134.5	1793.5	2312.7
<i>Catchment scale</i>						
Geologic rock type	geob	Proportion of catchment classified as biotite gneiss	0.12	0.14	0.00	0.46
Land use, agriculture	wsag	Proportion of catchment classified as agriculture	0.23	0.05	0.13	0.31
Stream power	spow	Discharge*slope (Watts/m) , based on mean annual 2010 flow	0.06	0.04	0.00	0.12
Fish species richness	fish	Number of fish species encountered during electroshock surveys	18.3	4.85	10	28
<i>Species traits</i>						
Brood time	brood	Tachytictic or bradytictic				categorical
Host specificity	host	Host generalist or host specialist				categorical
Reproductive strategy	strategy	Host attractor or non-attractor				categorical
Tribe	tribe	Anodontini, Pleurobemini, Lamprosilini pairwise comparisons				categorical

Table 2. Candidate models for the occupancy (Ψ) and detection (p) probabilities of a freshwater mussel assemblage, with Deviance Information Criterion (DIC) rankings and DIC difference in successive candidate models (Δ DIC).

Ψ	p	Parameters	DIC	Δ DIC
μ_i	v	60.1	260.46	0.00
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v	60.9	260.83	0.37
μ	v	68.0	266.99	6.53
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v	71.1	271.70	11.24
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	76.7	287.39	26.93
μ_i	$v + \beta_1 + \beta_2 + \beta_3$	84.6	291.41	30.95
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v	89.2	295.06	34.60
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	83.3	296.78	36.32
μ	$v + \beta_1 + \beta_2 + \beta_3$	91.1	298.41	37.95
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v	97.2	303.68	43.22
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	106.0	317.74	57.28
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	126.0	344.69	84.23
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	126.0	344.69	84.23
μ_i	$v + \beta_1 + \beta_2 + \beta_3$	462.3	668.74	408.28
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v_i	452.1	671.54	411.08
μ_i	v_i	473.3	683.44	422.98
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v_i	528.2	741.52	481.06
μ	v_i	569.9	786.15	525.69
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v_i	564.7	794.71	534.25
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	626.1	844.76	584.30
μ_i	$v_i + \beta_1 + \beta_2 + \beta_3$	654.6	877.09	616.63
μ	$v_i + \beta_1 + \beta_2 + \beta_3$	655.9	880.37	619.91
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	709.5	938.63	678.17
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	733.7	945.68	685.22
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	v_i	751.5	981.49	721.03
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	846.2	1071.61	811.15
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	1056.4	1291.91	1031.45
μ_i	$v_i + \beta_1 + \beta_2 + \beta_3$	1526.4	1764.85	1504.39
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	1552.7	1797.58	1537.12
μ	$v + \beta_1 + \beta_2 + \beta_3$	1591.3	1824.48	1564.02
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	1552.7	1979.58	1719.12
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v + \beta_1 + \beta_2 + \beta_3$	1755.8	1991.64	1731.18
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	1953.0	2212.97	1952.51
$\mu_i + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	2120.3	2370.12	2109.66
$\mu + \alpha_1 + \alpha_2 + \dots + \alpha_8$	$v_i + \beta_1 + \beta_2 + \beta_3$	2320.5	2589.61	2329.15
μ	$v_i + \beta_1 + \beta_2 + \beta_3$	2342.0	2607.45	2346.99

Table 3. Occupancy (Ψ) and detection (p) models that tested effects of four biological species traits, tribe, brood time, host specificity, and attraction strategy, on the occupancy of a freshwater mussel assemblage, for each species i at site j . Models include occupancy and detection intercepts (μ and ν , respectively), and species random effects (α).

Occupancy	Detection
$\text{logit}(\Psi_{ij}) = \mu + \alpha_1 \text{host}_i$	$\text{logit}(p_{ij}) = \nu$
$\text{logit}(\Psi_{ij}) = \mu + \alpha_1 \text{tribe}_i$	$\text{logit}(p_{ij}) = \nu$
$\text{logit}(\Psi_{ij}) = \mu + \alpha_1 \text{brood}_i$	$\text{logit}(p_{ij}) = \nu$
$\text{logit}(\Psi_{ij}) = \mu + \alpha_1 \text{tribe}(L)_i + \alpha_2 \text{tribe}(P)_i$	$\text{logit}(p_{ij}) = \nu$

Table 4. Mean effect, standard deviation, and 95% credible interval of the habitat and species trait occupancy covariates from the most plausible occupancy and detection candidate models.

Covariate	Mean effect (SD)	95% CI	
<i>Microhabitat scale</i>			
Substrate compaction	-0.19 (0.29)	-0.77	0.34
Substrate heterogeneity	0.28 (0.29)	-0.26	0.86
<i>Reach scale</i>			
Bankfull max depth	-0.09 (0.36)	-0.80	0.59
Degree days	-0.02 (0.31)	-0.64	0.62
<i>Catchment scale</i>			
Geologic rock type	0.08 (0.27)	-0.46	0.58
Land use, agriculture	-0.85 (0.38)	-1.65	-0.17
Stream power	0.52 (0.31)	-0.07	1.15
Fish species richness	0.36 (0.30)	-0.23	0.97
<i>Species traits</i>			
Brood time	1.13 (0.34)	0.50	1.80
Host specificity	1.30 (0.44)	0.50	2.20
Reproductive strategy	0.35 (0.32)	-0.24	0.99
Tribe			
Lampsilini - Anodontini	0.67 (0.55)	-0.45	1.77
Pleurobemini - Anodontini	1.48 (0.46)	0.64	2.44

Table 5. Mean occupancy (Ψ) and detection (p) probabilities for 14 species of freshwater mussels, with standard deviation and 95% credible intervals.

Species	p (SD)	95% CI		Ψ (SD)	95% CI	
<i>Alasmidonta heterodon</i>	0.42 (0.03)	0.36	0.47	0.11 (0.06)	0.03	0.29
<i>Alasmidonta undulata</i>	0.42 (0.03)	0.36	0.47	0.04 (0.04)	0.01	0.16
<i>Elliptio congaraea</i>	0.42 (0.03)	0.36	0.47	0.29 (0.10)	0.13	0.52
<i>Elliptio fisheriana</i>	0.42 (0.03)	0.36	0.47	0.11 (0.06)	0.03	0.28
<i>Elliptio icterina</i>	0.42 (0.03)	0.36	0.47	0.67 (0.12)	0.42	0.86
<i>Elliptio lanceolata</i>	0.42 (0.03)	0.36	0.47	0.08 (0.05)	0.02	0.25
<i>Elliptio roanokensis</i>	0.42 (0.03)	0.36	0.47	0.11 (0.07)	0.03	0.30
<i>Elliptio steinstansana</i>	0.42 (0.03)	0.36	0.47	0.08 (0.05)	0.02	0.23
<i>Fusconaia masoni</i>	0.42 (0.03)	0.36	0.47	0.29 (0.11)	0.13	0.52
<i>Lampsilis cariosa</i>	0.42 (0.03)	0.36	0.47	0.11 (0.06)	0.03	0.28
<i>Lampsilis</i> sp.	0.42 (0.03)	0.36	0.47	0.04 (0.04)	0.01	0.17
<i>Strophitus undulatus</i>	0.42 (0.03)	0.36	0.47	0.04 (0.03)	0.01	0.17
<i>Utterbackia imbecillis</i>	0.42 (0.03)	0.36	0.47	0.05 (0.04)	0.01	0.16
<i>Villosa constricta</i>	0.42 (0.03)	0.36	0.47	0.19 (0.09)	0.07	0.40

Table 6. Site and species-specific occupancy (Ψ) probability estimates from 20 sites in the Tar River basin, North Carolina.

Species	Site Occupancy																			
	SheC	NFTR	TR1	TR2	TR3	TR4	SaC1	SaC2	SaC3	RBC	SwC1	SwC2	ShoC	LFC1	RS1	RS2	LFC2	LFC3	FC	TR5
<i>Alasmidonta heterodon</i>	0.10	0.06	0.10	0.13	0.26	0.17	0.13	0.13	0.16	0.06	0.30	0.10	0.07	0.24	0.08	0.08	0.40	0.38	0.12	0.09
<i>Alasmidonta undulata</i>	0.05	0.02	0.05	0.06	0.13	0.08	0.06	0.06	0.08	0.03	0.16	0.05	0.03	0.12	0.03	0.04	0.22	0.21	0.05	0.04
<i>Elliptio congaraea</i>	0.25	0.14	0.25	0.29	0.49	0.36	0.29	0.30	0.36	0.16	0.54	0.24	0.18	0.47	0.20	0.21	0.65	0.63	0.27	0.22
<i>Elliptio fisheriana</i>	0.10	0.05	0.10	0.12	0.25	0.16	0.12	0.12	0.16	0.06	0.29	0.10	0.07	0.23	0.07	0.08	0.39	0.37	0.11	0.09
<i>Elliptio icterina</i>	0.58	0.41	0.58	0.62	0.81	0.70	0.63	0.65	0.71	0.44	0.83	0.58	0.47	0.79	0.51	0.53	0.89	0.88	0.62	0.54
<i>Elliptio lanceolata</i>	0.07	0.04	0.08	0.09	0.20	0.12	0.09	0.09	0.12	0.05	0.23	0.07	0.05	0.18	0.06	0.06	0.32	0.30	0.08	0.07
<i>Elliptio roanokensis</i>	0.10	0.06	0.11	0.13	0.26	0.17	0.13	0.13	0.16	0.06	0.30	0.10	0.07	0.24	0.08	0.08	0.40	0.38	0.12	0.09
<i>Elliptio steinstansana</i>	0.07	0.04	0.07	0.09	0.19	0.12	0.09	0.09	0.12	0.04	0.23	0.07	0.05	0.18	0.05	0.06	0.31	0.29	0.08	0.06
<i>Fusconaia masoni</i>	0.25	0.14	0.25	0.29	0.49	0.36	0.29	0.30	0.36	0.16	0.54	0.24	0.18	0.47	0.20	0.21	0.65	0.63	0.27	0.22
<i>Lampsilis cariosa</i>	0.10	0.05	0.10	0.12	0.25	0.16	0.12	0.13	0.16	0.06	0.29	0.10	0.07	0.23	0.08	0.08	0.39	0.37	0.11	0.09
<i>Lampsilis</i> sp.	0.05	0.03	0.05	0.06	0.13	0.08	0.06	0.06	0.08	0.03	0.16	0.05	0.03	0.12	0.04	0.04	0.22	0.21	0.05	0.04
<i>Strophitus undulatus</i>	0.05	0.02	0.05	0.06	0.13	0.08	0.06	0.06	0.08	0.03	0.15	0.05	0.03	0.12	0.03	0.04	0.22	0.21	0.05	0.04
<i>Utterbackia imbecillis</i>	0.05	0.02	0.05	0.06	0.13	0.08	0.06	0.06	0.08	0.03	0.16	0.05	0.03	0.12	0.03	0.04	0.22	0.21	0.05	0.04
<i>Villosa constricta</i>	0.17	0.09	0.17	0.20	0.38	0.26	0.20	0.21	0.25	0.11	0.43	0.17	0.12	0.36	0.13	0.14	0.53	0.51	0.19	0.15

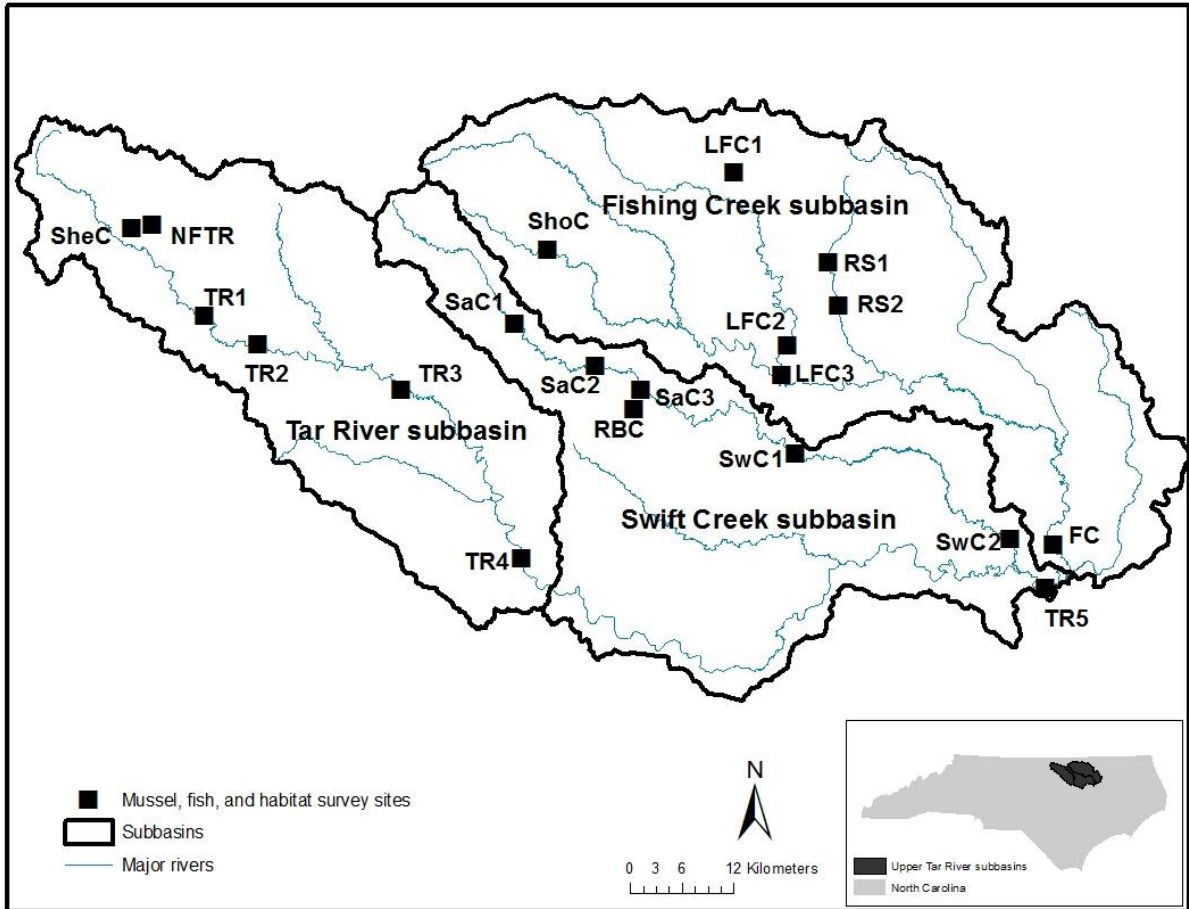


Figure 1. Location of 20 study sites within the upper Tar River basin, North Carolina.

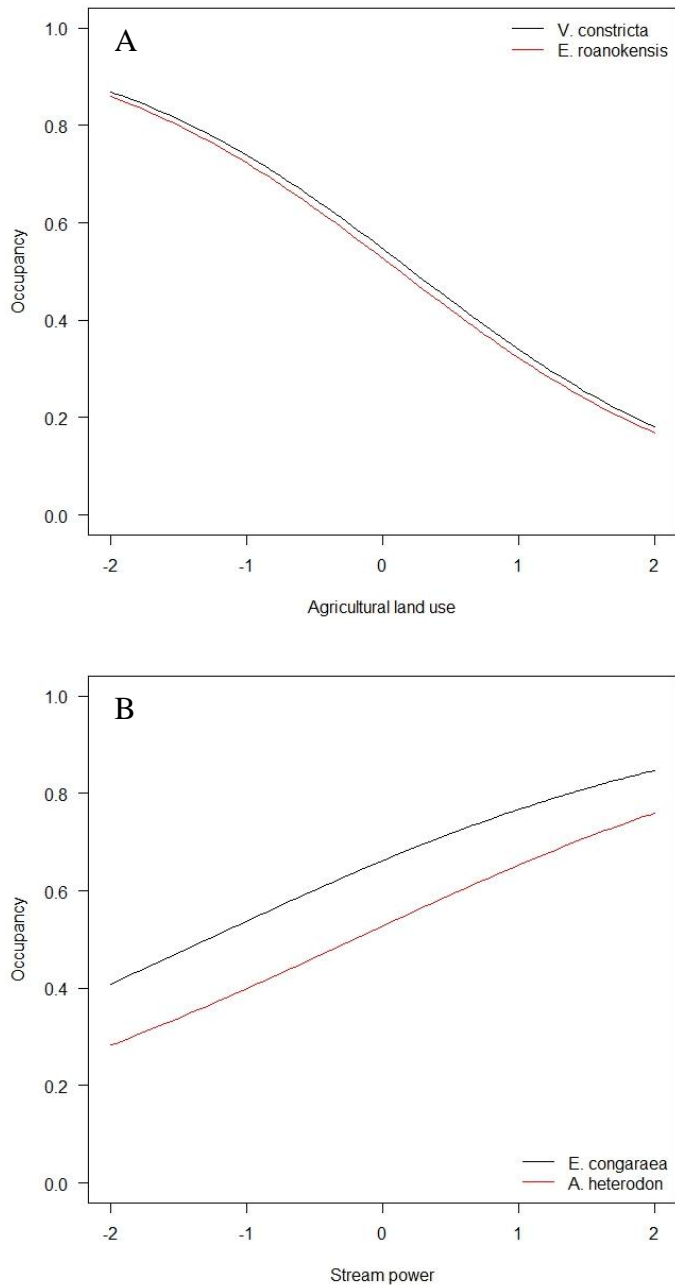


Figure 2. Predicted responses in the occupancy probability of *Villosa constricta* and *Elliptio roanokensis* over a range of agricultural land use values (A) and *E. congaraea* and *A. heterodon* for a range of stream power values (B) in a catchment.

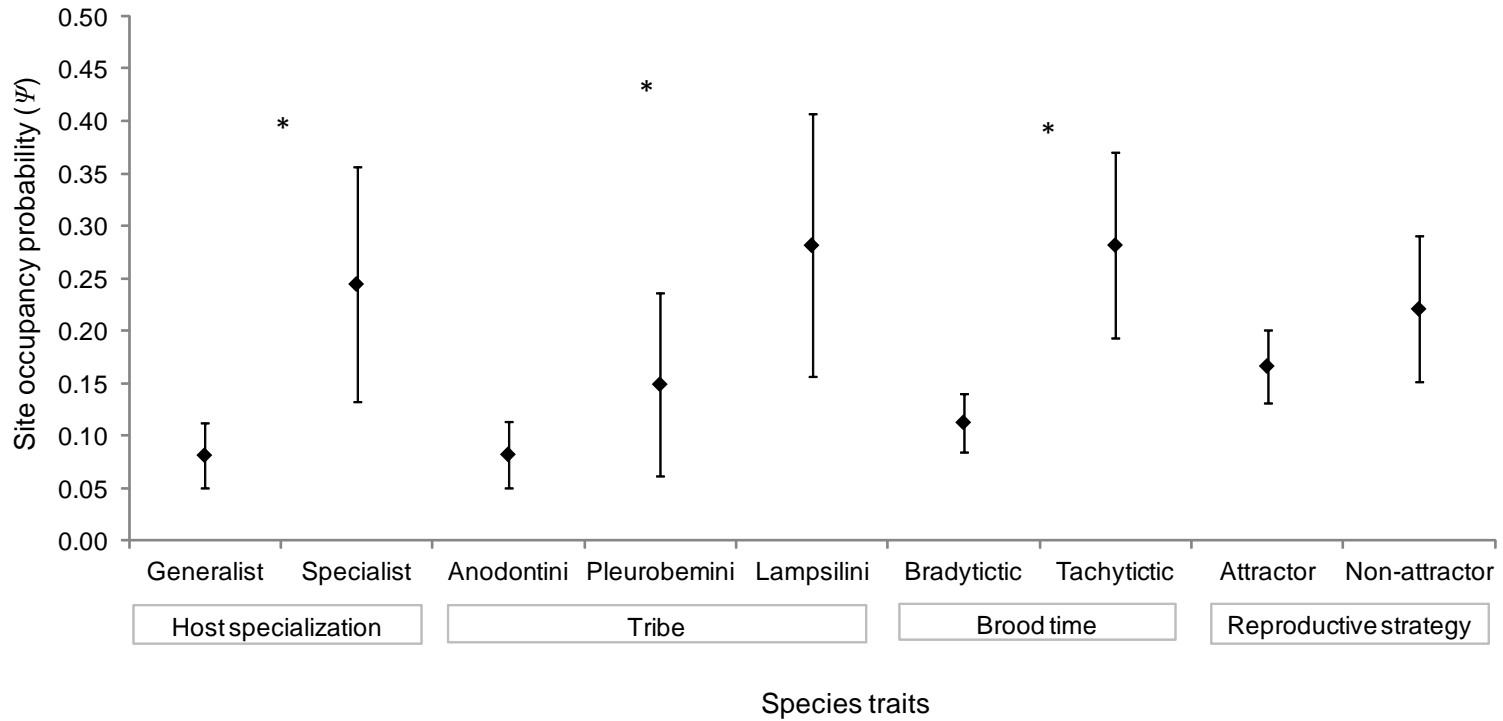


Figure 3. Predicted mean occupancy probability (\pm standard deviation) for four freshwater mussel species traits, host specialization, tribe, brood time, and reproductive strategy. An asterisk indicates that the 95% credible interval for the mean effect of the species trait did not include 0.

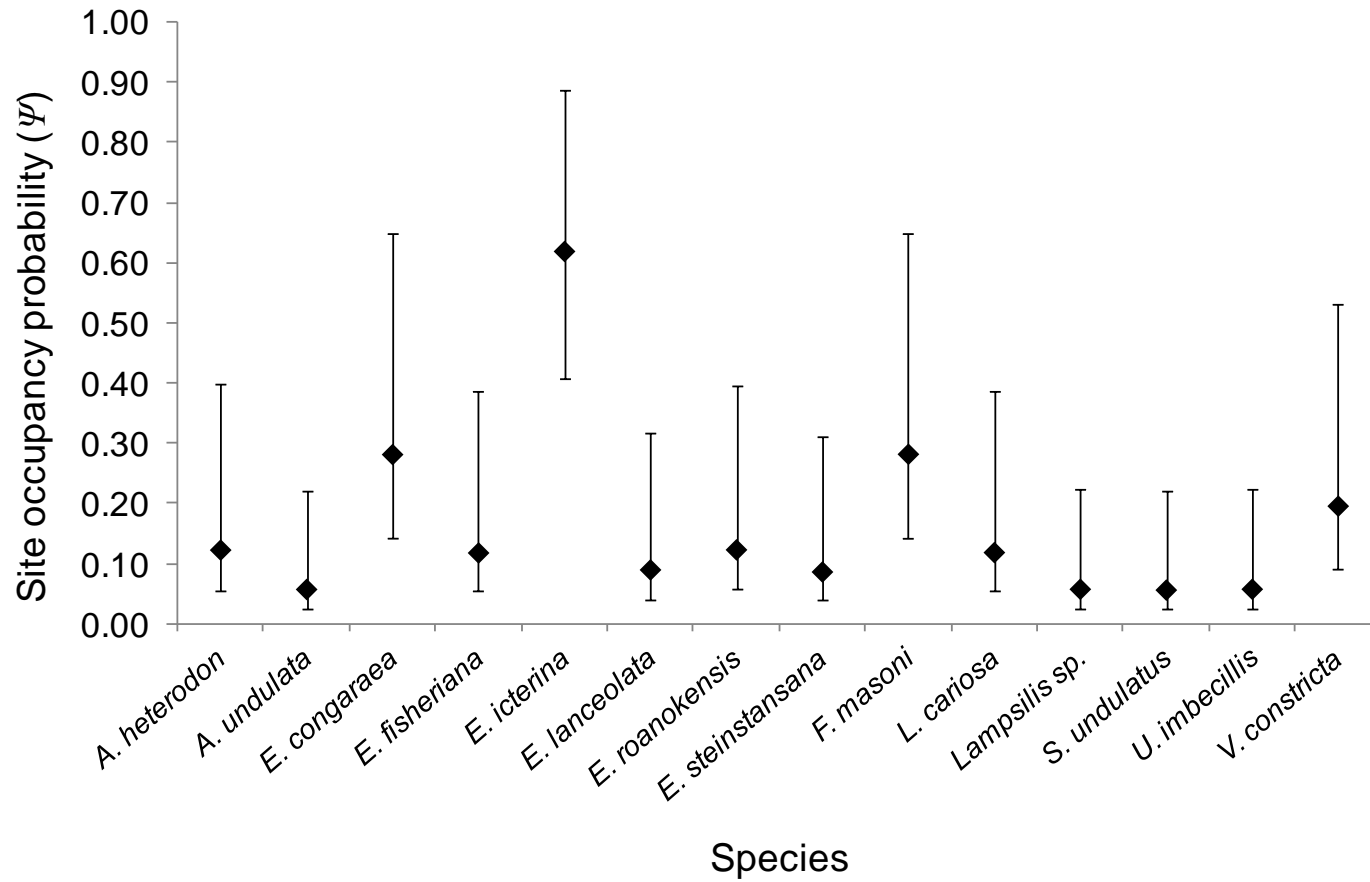


Figure 4. Median occupancy probability of 14 species of freshwater mussels from 20 study sites in the Tar River basin, North Carolina. Error bars indicate range of occupancy probability among sites for a species.

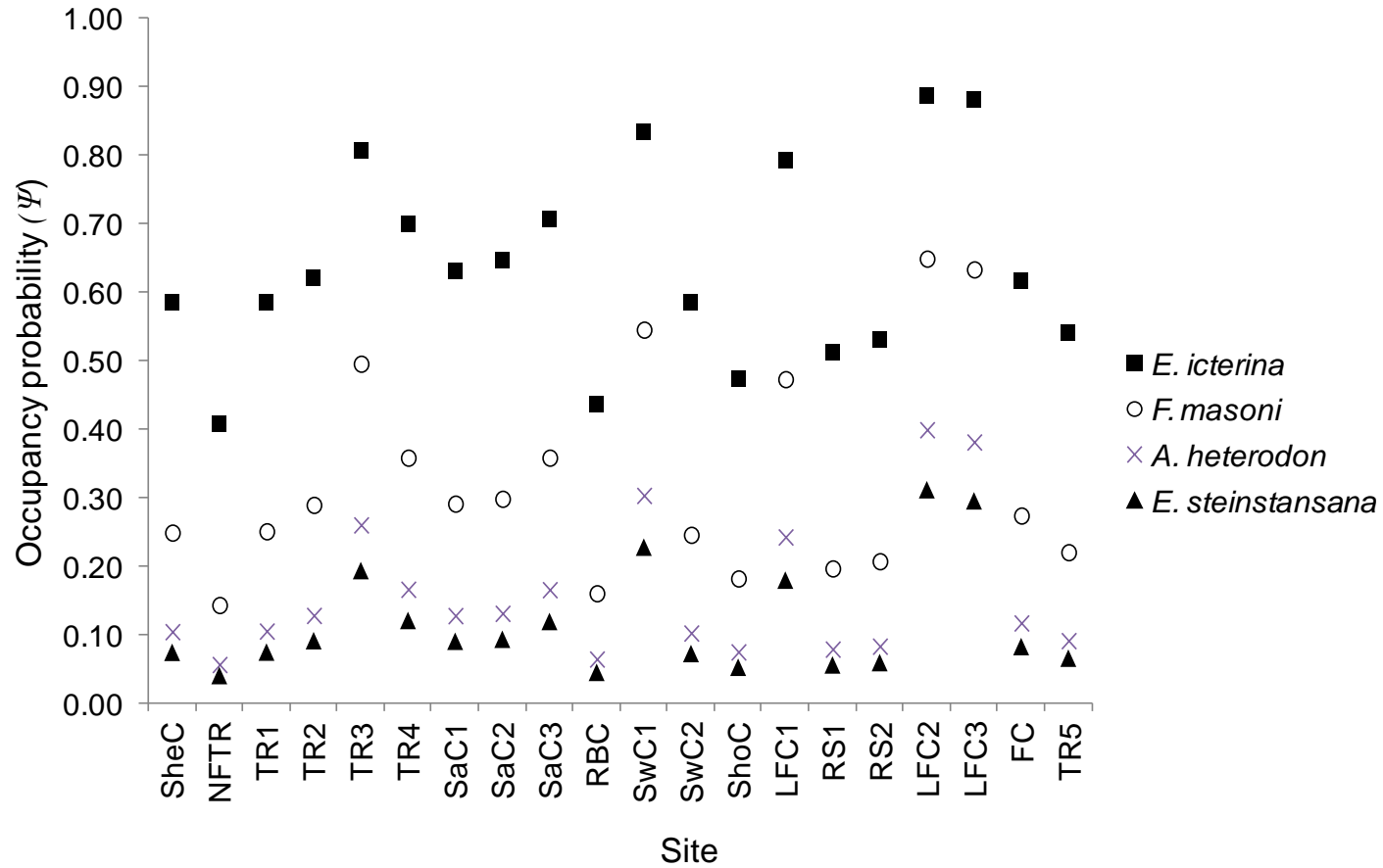


Figure 5. Predicted occupancy probability at 20 sites in the Tar River basin, North Carolina, for four mussel species of varied conservation concern: stable *Elliptio icterina*, NC state endangered *Fusconaia masoni*, and federally endangered *Alasmidonta heterodon* and *E. steinstansana*.

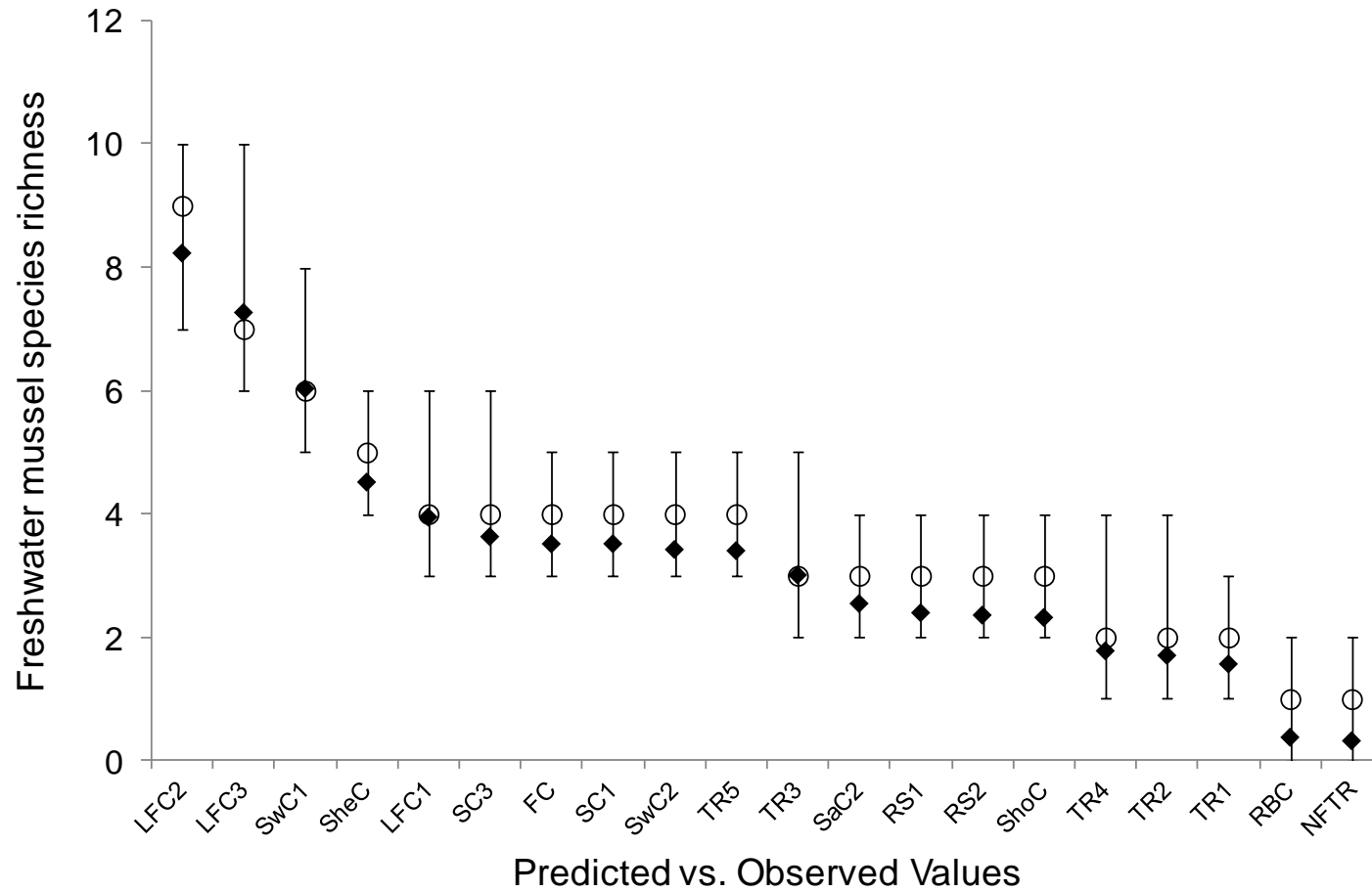


Figure 6. Predicted (♦) and observed (○) mussel species richness at 20 sites in the Tar River basin, North Carolina. Error bars are 95% credible intervals of prediction.

CHAPTER 2

Long-term demographics in a freshwater mussel assemblage: a dynamic occupancy modeling analysis

Abstract

Limited data on long-term changes in freshwater mussel assemblages exist, despite the long life span of mussels. Even fewer data exist on the demographic processes that underlie observed assemblage changes. We used existing survey records from 1986 – 2011 to explore changes in the occurrence of 14 species of freshwater mussels in the Tar River basin of North Carolina. A simple statistical comparison indicated that there were no significant changes in mussel occurrence ($p > 0.05$). However, a dynamic occupancy model that accounted for imperfect mussel detection indicated that occupancy probability for all species decreased over the study period. Occupancy probability in 1986 ranged from 0.19 for *Utterbackia imbecillis* to 0.60 for *Fusconaia masoni*. Occupancy probability at the end of the 26 year study period ranged from 0.10 for *Lampsilis radiata* to 0.40 for *F masoni*. The maximum difference between occupancy probability in 1986 and 2011 was 0.30 for *Alasmidonta undulata*. Mean survival for all species was high (0.97, 95% CI = 0.95 – 0.99). However, the mean colonization probability was very low (< 0.01 , 95% CI = $< 0.01 - 0.01$). These results indicate that mussels persist at sites that they already occupy, but that they are not colonizing sites where they had not been previously, a possible result of poor recruitment success. Our results highlight the importance of long-term monitoring of mussel status and

distribution as well as incorporating detection probability in the determination of demographic rates.

Keywords: freshwater mussel, dynamic occupancy modeling, Bayesian, existing data, trend

Introduction

As one of the most imperiled faunal groups in the world (Williams et al. 1993, Master et al. 2000), freshwater mussels have been the focus of many studies that aim to describe the factors that limit their distributions (e.g., McRae et al. 2004, Poole and Downing 2004). However, a complete interpretation of such investigations is made difficult by the paucity of information on mussel demography (Strayer 2008). Although several studies have focused on the growth, survival, recruitment, and mortality rates of some freshwater mussel populations (Payne and Miller 1989, Miller and Payne 1993, Jones and Neves 2011, Vaughn 2012), many questions remain.

The life span of freshwater mussels is highly variable and can range from 4 to 200 years (Bauer 1992, Bauer and Wachtler 2000), but there is evidence that freshwater mussels may be even more long-lived than previously thought (Anthony et al. 2001). Because freshwater mussels can live for decades or even hundreds of years, a relevant study of population processes must occur over a time span long enough to capture them. Unfortunately, it can be difficult to allocate the resources needed to devote to long-term studies, and the number of existing long term studies on mussels is limited (e.g., Strayer 1980, Metcalfe-Smith et al. 1998, Strayer and Fetterman 1999). The existing studies of long-

term changes in mussel assemblages often span a decade or two (Nalepa et al. 1991, Warren and Haag 2005, Morowski et al. 2009, Galbraith et al. 2010), though some have covered periods of 100 years or longer (Strayer 1980, Metcalfe-Smith et al. 1998). These investigations are necessary to document changes in mussel populations, but few have focused on the underlying processes that drive those changes (but see Vaughn 2012).

Dynamic occupancy models have recently been used to generate information on the processes that underlie population trends (e.g., Betts et al. 2008, Frey et al. 2011, Walls et al. 2011). These models provide estimates of the local colonization and extinction probabilities that are often the focus of long-term monitoring projects (MacKenzie et al. 2003, Royle and Kery 2007). A major benefit to using the dynamic occupancy approach to investigate demographics is that it accounts for imperfect detection (i.e., detection probability < 1 ; MacKenzie et al. 2003, Royle and Kery 2007, Dorazio et al. 2010). Studies that do not account for imperfect detectability assume that non-detection equates to species absence, though this is often not the case (MacKenzie et al. 2002). Estimations of population dynamics that do not account for imperfect detection will be biased, and can lead to faulty conclusions (MacKenzie et al. 2003, Royle and Kery 2007).

We used existing long-term data to construct a dynamic occupancy model that was used to estimate change in mussel occurrence over time, as well as the survival and colonization processes behind those changes. We then compared these results with those from a simple statistical approach that explored coarse scale assemblage changes, but did not account for detection.

Methods

Study sites

Twenty sites from the Tar River basin of North Carolina were selected for this study (Figure 1). The Tar-Pamlico River basin is the fourth largest in North Carolina with a 14,090 km² watershed and about 3,790 km of streams (NCDENR 2008). The freshwater portion of the Tar River covers a distance of about 225 km from its source in Person County to the city of Washington in Beaufort County (NCDENR 2008). The Tar River basin is among the most species-rich river basins of North Carolina; supporting a diverse mussel community of 24 species, 13 of which are imperiled (Bogan 2002). All sites were located in one of three sub-basins: upper Tar River, Swift Creek, or Fishing Creek. These sites were chosen as the focus of a companion study that involved an intensive mussel surveying effort in the summer of 2010. The sites were selected to span a range of environmental conditions and include known occurrences of two federally endangered species, *Alasmidonta heterodon* and *Elliptio steinstansana*.

Mussel survey database

Freshwater mussel survey data from the 26 year period of 1986 – 2011 were compiled from the North Carolina Wildlife Resources Commission (NCWRC) database, which we accessed in late 2012. We used all mussel survey records within 500 m, upstream and downstream, of GPS coordinates from our intensive 2010 mussel survey locations. To minimize mussel misidentification bias, we only included survey data acquired by NCWRC staff or by knowledgeable contractors (R. J. Heise, NCWRC, personal communication). Additionally, because of the potential difficulty in distinguishing *Elliptio complanata*, *E.*

icterina, and *E. congaraea* to species consistently over time with multiple surveyors, they, and any records for *Elliptio* spp., were not included in the analysis. Data base records were condensed from count data to a presence-absence record for each species at all 20 sites. If a site had multiple surveys within a year, they were combined to get an overall presence-absence for the year. There were a total of 127 surveys with suitable mussel data for our 20 sites in the queried time period.

Data analysis

Mussel surveys were not evenly distributed in time, with more surveys conducted later in the time period (i.e., after 1996). Therefore, the data were split into two time periods to assess a general change in mussel occurrence through time. First, we split the data into two time periods with an approximately equal numbers of surveys in each (1986 – 2002, $N = 62$; 2003 – 2011, $N = 65$). With this approach, the length of the time periods was 17 years and nine years, respectively. In the second approach, we divided surveys into two equal time periods, such that the number of surveys in each period varied (1986 – 1998, $N = 43$; 1999 – 2011, $N = 84$). The proportion of surveys in which a species was found was calculated for each time period. The proportions for all 14 species were then compared between time periods using a paired t-test. The purpose of this simple analysis was to look for any coarse scale change in mussel occurrence between the two time periods.

Occupancy modeling

We developed a dynamic occupancy model using the presence-absence data from all 127 surveys to evaluate changes in species occurrence during the 26 years (1986 – 2011) for which records existed. We used a state-space representation of the model wherein we

express the model by its two component processes: a submodel for the observations conditional on the unobserved state process and a submodel for the unobserved or partially observed state process. We followed an approach developed by Dorazio et al. (2010) and Walls et al. (2011) wherein the single-species model of Royle and Kery (2007) was extended to account for variation in model parameters among ecologically similar species. We used the entire community of species (Table 1) wherein each species' individual estimates influence the parameter estimates of every other species in the community and inferences about one particular species are borrowed across all of the species. Essentially the parameter estimates for one species are a compromise between the individual species estimates and the mean estimate of those parameters for the entire community. This is often referred to as “shrinkage” in the statistical literature (Gelman et al. 2003) because each species-specific estimate is shrunk in the direction of the estimated mean parameter value. The amount of shrinkage will depend on the amount of data or information for each species and how closely it resembles the overall mean effect for a particular parameter. One of the major benefits of shrinkage is the ability to estimate parameters for species that are not detected frequently or are rarely detected. These species are an important part of the community dynamics, but if analyzed alone there would be too few data to make worthwhile inference. Instead these species can be included in the analysis and species-specific estimates can be obtained.

Because sites were not surveyed multiple times per year in our aggregation of historical data, we were unable to estimate detection probability. To solve this problem, we used information from a study designed to explicitly estimate detection probability, which found little evidence of species-specific detection rates (see Chapter 1, this Dissertation).

Therefore, in each year of our current study, we randomly-generated a detection probability from a normal distribution with mean and variance estimated from 14 Tar River basin mussel species. We allowed these probabilities to vary each year to account for temporal variability in detection rates. Following Walls et al. (2011), we then specified a model using the randomly-generated detection rates and conditional on the binary occupancy state (present or absent). We defined y_{ikt} for each combination of site (k), year (t), and species (i) where each binary observation indicates whether the species was detected ($y_{ikt} = 1$) or not detected ($y_{ikt} = 0$). We defined the occupancy state as z_{ikt} for species i , site k , and year t , such that $z_{ikt} = 1$ indicates species presence and $z_{ikt} = 0$ indicates absence of the species. It is important to note that if we observe no detections, then there is ambiguity in defining the occupancy state because the site could be occupied and we failed to detect the species or the site could be unoccupied. Therefore, we defined the model for each element of the data as:

$y_{ikt} | z_{ikt}, p_t \sim \text{Bernoulli}(z_{ikt} p_t)$ where p_t denotes the probability of detecting a species in year t given that the species is present. This implies that if the k th site is unoccupied by species i in year t , then $y_{ikt} = 0$ with probability one and otherwise is detected with probability p_t .

We assumed we could model changes in occupancy state for each species using a first-order Markov process (Royle and Kery 2007). We assumed the initial occupancy state for the i th species at site k is modeled as $z_{ik1} | \psi_{i1} \sim \text{Bernoulli}(\psi_{i1})$ where ψ_{i1} denotes the probability of occurrence for species i in year 1. Using a recursive relationship wherein occupancy states in subsequent years ($t + 1, t + 2, \dots, T$) depend on the occupancy states one year earlier can be written as: $z_{ik,t+1} | z_{ik,t}, \phi_{it}, \gamma_{it} \sim \text{Bernoulli}(z_{ik,t} \phi_{it} + \gamma_{it} (1 - z_{ik,t}))$

where $\gamma_{it} = \Pr(z_{ik,t+1} = 1 | z_{ikt} = 0)$ denotes the probability of local colonization (i.e., a site unoccupied at time t will become occupied at time $t + 1$), and $\varphi_{it} = \Pr(z_{ik,t+1} = 1 | z_{ikt} = 1)$ denotes the probability of local survival (i.e., probability of occupied site at time t staying occupied at time $t + 1$). We can define the probability of local extinction, ε_{it} , as the probability of an occupied site at time t becoming unoccupied at time $t + 1$ and can define this as the complement of local survival probability: $\varepsilon_{it} \equiv 1 - \varphi_{it}$.

We used a multivariate normal prior distribution to model species-specific deviations from the mean group-level parameter values (Dorazio et al. 2006, Kery and Royle 2008). We estimated parameters using a Bayesian approach with Markov chain Monte Carlo (MCMC) implemented using R statistical software (with the R2WinBUGS package; Sturtz et al. 2005) and WinBUGS (Lunn et al. 2000) using flat priors for each of the group-level parameters. The MCMC approach allows us to explicitly measure uncertainty in parameter values by examining a posterior distribution for each parameter. We ran three chains of each model for 20000 iterations, thinned by 5, after a burn-in of 10000 iterations (resulting in 12000 posterior samples for each parameter) and assessed model convergence by examining trace plots and Gelman-Rubin statistics using package CODA in R (Gelman et al. 2003). We estimated occupancy, survival (1- extinction), and colonization probabilities for the entire mussel assemblage.

Results

A comparison of the proportion of surveys that detected a species between the periods of 1986 – 2002 and 2003 – 2011 indicated that there was no significant difference between

the time periods ($p = 0.9626$; Figure 2). In the later survey period (2003 – 2011), six species had a slightly lower rate of occurrence than in the early period (1986 – 2002), seven were slightly higher, and one species did not change. The mean absolute difference in occupancy rate for a species was 5% (0.054), but the maximum difference was an almost 17% increase (0.166) for *Alasmidonta heterodon*.

The comparison of surveys between the periods of 1986 – 1998 and 1999 – 2011 also indicated that there was no significant difference in the proportion of surveys where a species was found between the time periods ($p = 0.0850$; Figure 2). In the later time period (1999 – 2011), nine species had a lower rate of occurrence than in the earlier time period (1986 – 1998) and five species had higher rates. The mean absolute difference between time periods for a species was 6% (0.060), with a maximum decrease of 22% (0.219) for *Elliptio lanceolata*.

The predicted overall occupancy probability for all 14 mussel species over 26 years was 0.35 (95% CI = 0.20 – 0.51). Initial occupancy rates (1986) ranged from 0.19 for *Utterbackia imbecillis* to 0.60 for *Fusconaia masoni* (Table 1). Occupancy probability was predicted for each species for all 26 years, and every species exhibited a decline in occupancy probability, regardless of initial occupancy (Figure 3). In the 26th year of the study, 2011, occupancy probability ranged from 0.10 for *Lampsilis radiata* to 0.40 for *F. masoni*. The maximum difference between occupancy rates in 1986 and 2011 was 0.30 for *Alasmidonta undulata*. The mean survival for all species was high (0.97, 95% CI = 0.95 – 0.99), and ranged from 0.93 for *L. radiata* to 0.98 for *A. heterodon*, *E. fisheriana*, *E. lanceolata*, *E. roanokensis*, *F. masoni*, and *V. constricta*. However, the mean colonization

probability was very low (< 0.01 , 95% CI = $< 0.01 - 0.01$). All 14 species had a predicted colonization probability of < 0.01 .

Discussion

The occupancy probability for all 14 freshwater mussel species in our study decreased over our 26 year study period. Mussels had an overall mean occupancy probability of 0.36 in 1986 which decreased to 0.23 in 2011. Survival probability was high, with a mean estimate of 0.97 among species. However, the mean colonization probability among species was very low with an estimate of < 0.01 (i.e., no probability of colonization). These results indicate that poor survival of established mussels is not the driving force behind mussel declines in the Tar River basin, but that mussels are not moving into sites where they did not already occur. We would not have been able to discern this information from our simple statistical comparison of mussel occupancy between two time periods.

With the dynamic modeling approach that we used, we were able to estimate mussel occurrence for each year in the study period, providing evidence that occupancy probability was steadily declining for each mussel species. Our simple statistical analysis of the same data did not produce evidence of a statistically significant change in mussel occurrence. This can probably be partly attributed to the coarse scale of the comparison (i.e., data were divided into two time periods). However, a major advantage of our modeling approach was that it incorporated detection probability for the freshwater mussels in our study, and therefore, we avoided basing demographic inferences on biased data (MacKenzie et al. 2003, Kery and Schmidt 2008). For instance, our statistical comparison suggested that federally endangered

A. heterodon appeared in surveys more frequently in the later time period than in the earlier period. However, our modeling results indicate that occupancy probability of *A. heterodon* has been declining, and regional mussel experts agree that populations of *A. heterodon* are on the decline in the Tar River basin (R. B. Nichols, NCWRC, personal communication). The apparent increase in occurrence is most likely related to detection issues. The species was federally listed as an endangered species in 1990, which is also around the time that research interest in mussels escalated (Strayer et al. 2004). It is likely that survey effort became more targeted for the newly listed species, and thus, surveyors became more adept at finding this small mussel (e.g., Strayer and Smith 2003, Meador et al. 2011). The apparent increase in detection can be misinterpreted as an increase in occupancy probability.

The high survival and low colonization probabilities that we found for mussels in the Tar River basin indicate that sites that already have mussels are staying occupied, but unoccupied sites are not becoming occupied. Although we cannot conclusively state the cause of the extremely low colonization probability in our study, we suspect that it reflects a lack of mussel recruitment. Payne and Miller (1989) documented similar dynamics in their four year study in the Lower Ohio River. Other long-term studies have found that colonization success depends on mussel density and distribution (Warren and Haag 2005, Vaughn 2012) and larval dispersal traits (Vaughn 2012).

Despite the inherent value of a long term demographic study on freshwater mussels, our study had some limitations. We were constrained by the data that were available in an existing database. Most records in the database did not include survey effort information, so we could not investigate abundance, only occupancy (i.e., presence or absence). The simple

act of including survey effort in survey records can greatly enhance their utility. Also, because these data were not collected with the intention of use in an occupancy model, results should be interpreted with caution (Payne and Miller 1989, Metcalfe-Smith et al. 1998). We were able to conduct a dynamic occupancy analysis because we already had complementary detection information that we used to inform the model. Methods of estimating detection are very data-intensive and we would not have been able to model it successfully without our supporting data. The relative paucity of our data also limited us to estimate an overall colonization and survival probability for each species, so we could not examine changes in those probabilities over time. Similarly, we could not include covariates to explore the influences of mussel traits or environmental parameters on the occupancy, survival, or colonization processes.

Regardless of these limitations, we were able to use existing data to get valuable information on freshwater mussel demographics in this ecosystem. Freshwater mussel research has historically been lacking in both long-term community studies and in demographic studies (Strayer 2008). We were able to address both of these topics in our study by estimating occupancy, survival, and colonization probabilities of a mussel assemblage based on 26 years of mussel survey records. Successful mussel conservation depends not only on demographics, but largely on the identification of the causes of decline (Downing et al. 2010). Other long-term mussel studies have suggested the causes to be surface mining and oil extraction (Warren and Haag 2005), land use (Strayer 1980, Morowski et al. 2009), and thermal stress (Galbraith et al. 2010). We were unable to isolate causes in this instance, but as data continue to be added to the database over time, the

potential for using the survey records in further long-term analyses grows (Strayer et al. 2004, Willis et al. 2007). There is an existing wealth of data spread among individuals, agencies, and universities (Reichman et al. 2011), and we should consider the potential uses of the aggregation of these data for improved conservation of freshwater mussels.

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Table 1. Mean and standard deviation of initial occupancy estimates in 1986 (Ψ_{1986}), occupancy probability in 2011 (Ψ_{2011}), survival probability (Φ), and colonization probability (γ) for 14 freshwater mussel species in the Tar River basin, North Carolina.

Species	Occurrence				Survival		Colonization	
	Ψ_{1986}	SD	Ψ_{2011}	SD	Φ	SD	γ	SD
<i>Alasmidonta heterodon</i>	0.30	(0.12)	0.24	(0.08)	0.98	(0.01)	< 0.01	(< 0.01)
<i>Alasmidonta undulata</i>	0.54	(0.15)	0.24	(0.09)	0.96	(0.02)	< 0.01	(< 0.01)
<i>Elliptio fisheriana</i>	0.31	(0.11)	0.23	(0.08)	0.98	(0.01)	< 0.01	(< 0.01)
<i>Elliptio lanceolata</i>	0.34	(0.11)	0.22	(0.08)	0.98	(0.02)	< 0.01	(< 0.01)
<i>Elliptio roanokensis</i>	0.31	(0.11)	0.24	(0.08)	0.98	(0.01)	< 0.01	(< 0.01)
<i>Elliptio steinstansana</i>	0.32	(0.12)	0.20	(0.08)	0.97	(0.02)	< 0.01	(< 0.01)
<i>Fusconaia masoni</i>	0.60	(0.15)	0.40	(0.11)	0.98	(0.01)	< 0.01	(< 0.01)
<i>Lampsilis cariosa</i>	0.49	(0.15)	0.30	(0.09)	0.97	(0.01)	< 0.01	(< 0.01)
<i>Lampsilis radiata</i>	0.23	(0.14)	0.10	(0.06)	0.93	(0.08)	< 0.01	(< 0.01)
<i>Lampsilis</i> sp.	0.22	(0.12)	0.13	(0.06)	0.97	(0.03)	< 0.01	(< 0.01)
<i>Pyganodon cataracta</i>	0.24	(0.12)	0.15	(0.07)	0.97	(0.03)	< 0.01	(< 0.01)
<i>Strophitus undulatus</i>	0.43	(0.14)	0.20	(0.08)	0.96	(0.03)	< 0.01	(0.01)
<i>Utterbackia imbecillis</i>	0.19	(0.11)	0.12	(0.07)	0.96	(0.05)	< 0.01	(< 0.01)
<i>Villosa constricta</i>	0.51	(0.12)	0.39	(0.11)	0.98	(0.01)	< 0.01	(< 0.01)

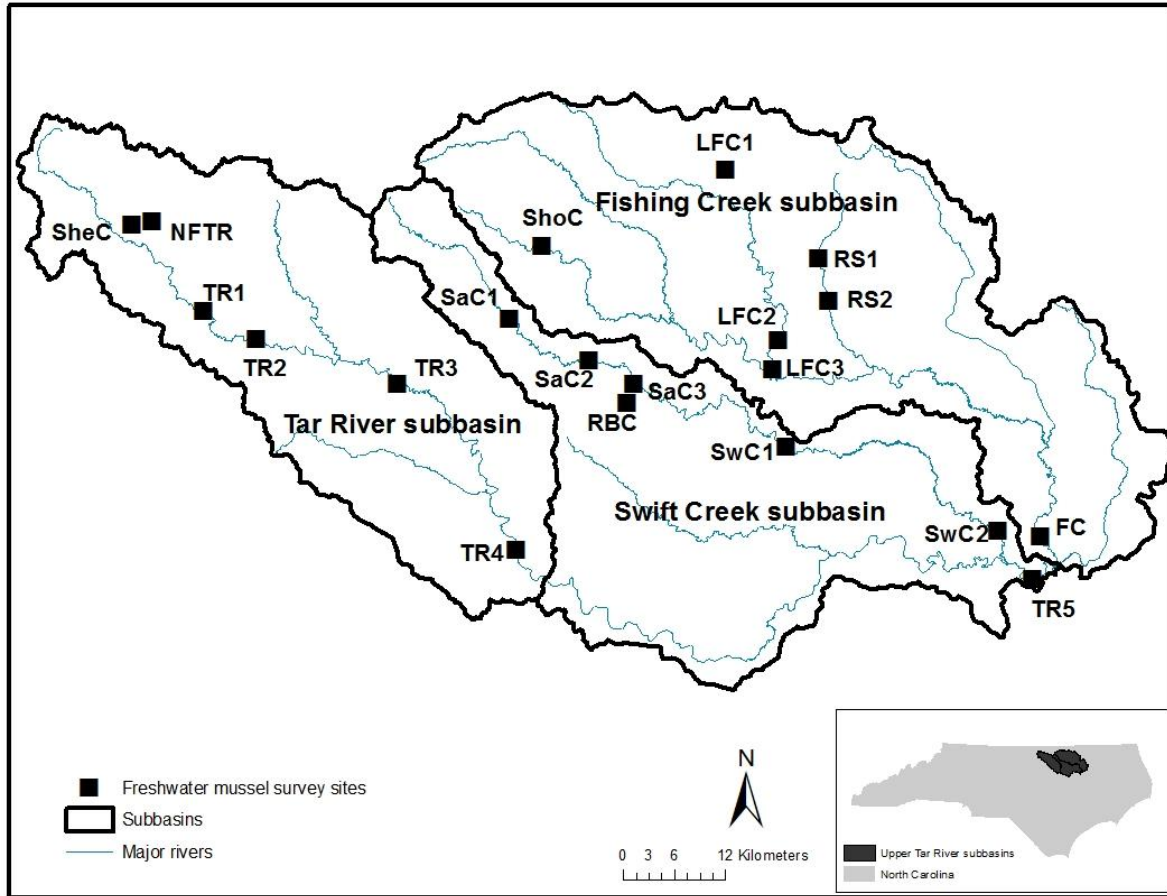


Figure 1. Location of 20 mussel survey record sites in the Tar River basin, North Carolina.

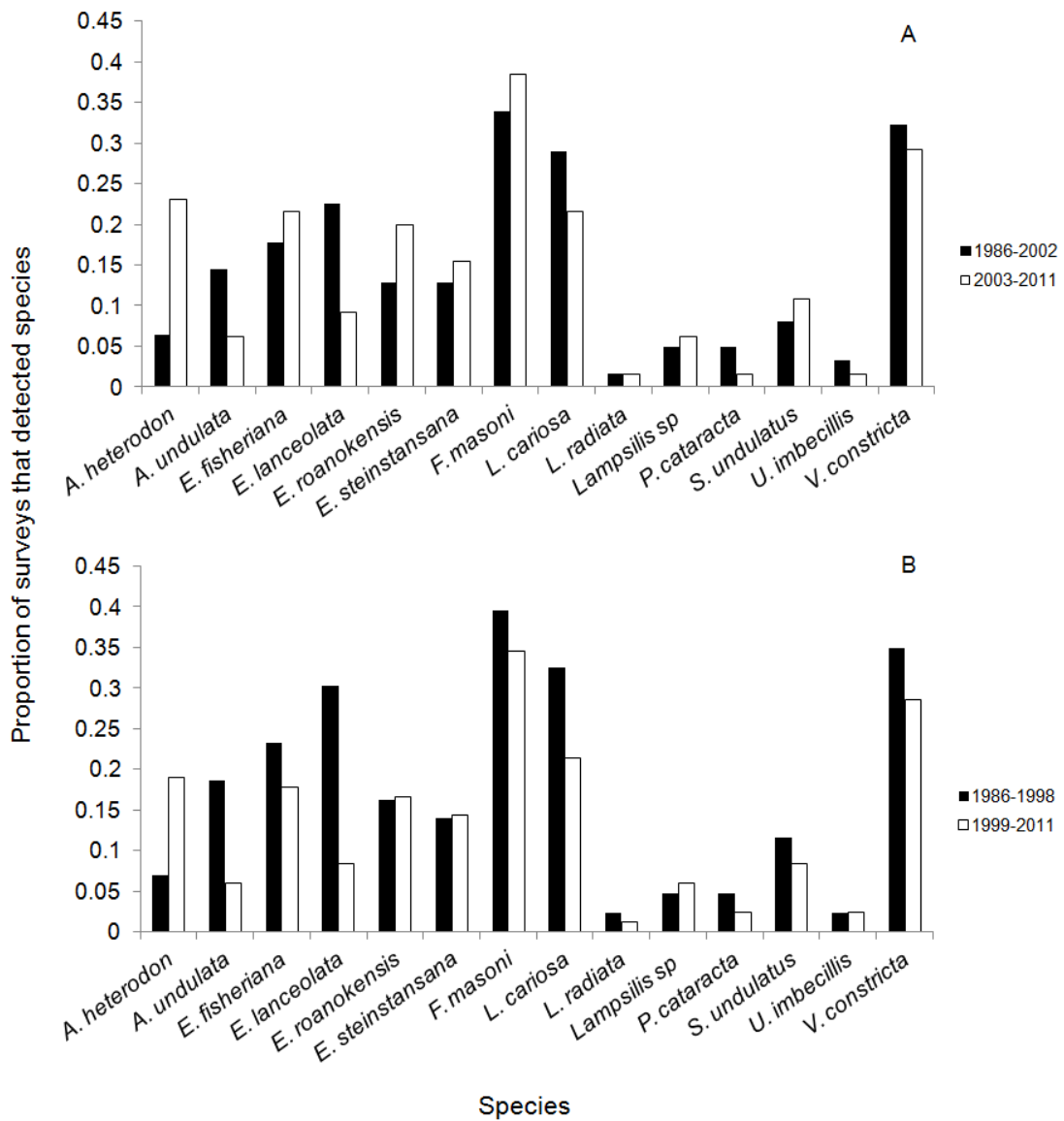


Figure 2. The proportion of surveys where each of 14 species of freshwater mussel species was found between 1986 – 2002 and 2003 – 2011 (A), and 1986 – 1998 and 1999 – 2011 (B).

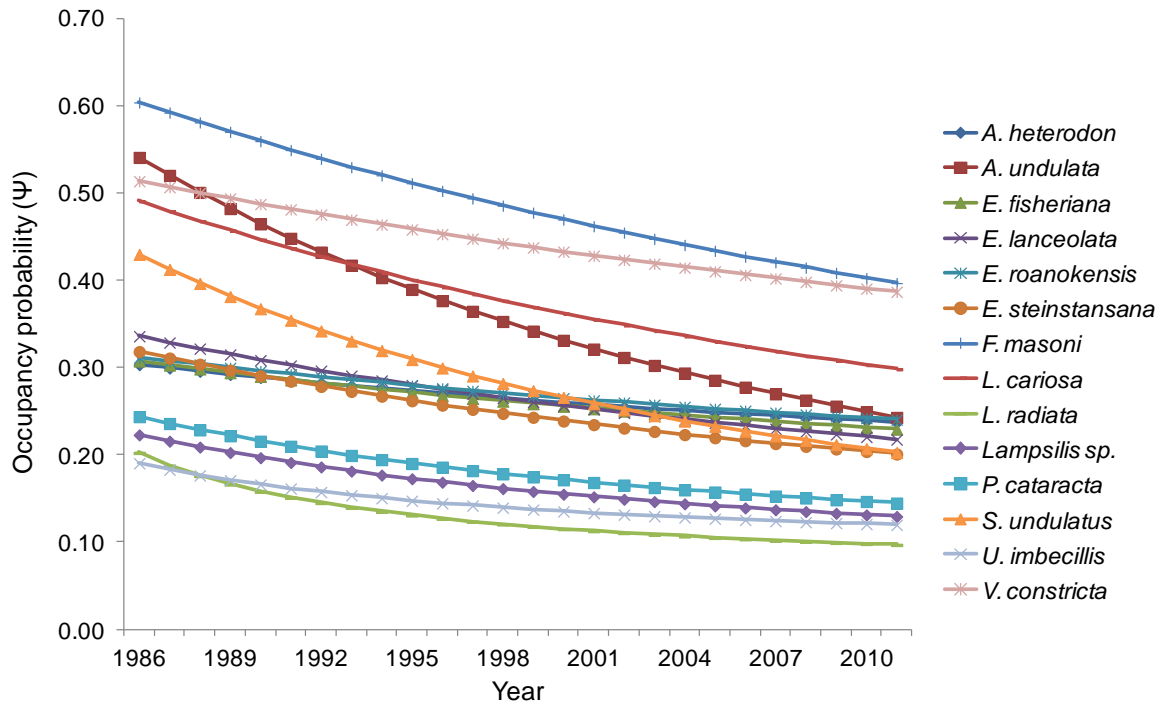


Figure 3. Estimated occupancy probabilities (Ψ) for 14 freshwater mussel species in the Tar River basin, North Carolina from 1986 to 2011.

CHAPTER 3

Thermal threats to freshwater mussels: an empirical stream analysis

Abstract

Freshwater mussels fulfill a considerable ecological role in aquatic environments, but they currently face many threats related to global change, including thermal regime alteration. In this study, we aim to combine extensive field measurements of temperature in the stream water column and substrate at sites where mussels occur, measures of abundance and species richness for mussels and fish, and the current thermal tolerance knowledge for mussels and fish to generate a comprehensive assessment of potential threats mussels face as temperatures continue to rise as a result of global change. Average summer (June – August, 2010 – 2012) temperatures at mussel-occupied sites in the upper Tar River basin of North Carolina ranged 16.2 – 34.7 °C. The mean temperature from the hottest 96 h at each site ranged 23.5 – 31.5 °C. At 80% of sites, a period of moderate drought coincided at least partially with the hottest 96-h period. Temperature threshold exceedance durations indicated that both acute and chronic freshwater mussel thermal tolerance thresholds were met or 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. Water temperatures exceeded 30 °C for at least 24 h at 15% of sites, and six sites never exceeded 30 °C. We found a thermal buffering effect of substrate that may be potentially protective of mussels, and we were able to quantify this relationship with a linear regression. Freshwater mussels currently exist on the edge of their thermal limits, but their abundance and species richness could not be described by temperature patterns alone. However, fish

species richness was related to thermal regime, which indicates that species interactions may drive freshwater mussel responses to global change.

Keywords: thermal buffer, substrate, climate change, unionid, exceedance

Introduction

Global change has already altered species interactions and caused shifts in species distributions (Lake et al. 2000, Parmesan and Yohe 2003, IPCC 2007). Freshwater ecosystems are particularly threatened by present and future change (Allen 2004, Mulholland et al. 1997, Ficke et al. 2007, IPCC 2007), and temperatures and hydrology of rivers and streams are already being affected by human impacts (Allen 2004, Kaushal et al. 2010). Flood frequency and magnitude are expected to increase, while droughts may also become more frequent (Allen 2004, IPCC 2007). These hydrologic changes, along with increasing temperatures, can alter nutrient cycling, decrease habitat availability, decrease water quality, and potentially introduce parasites and pathogens to freshwater ecosystems (Mulholland et al. 1997, Lake et al. 2000, Ficke et al. 2007). These changes will in turn alter species interactions and affect the structure and services of freshwater ecosystems (Lake et al. 2000, Visser and Both 2005, IPCC 2007, Spooner and Vaughn 2008, Walther 2010).

Some potential impacts of changing thermal regime on freshwater ecosystem services have already been demonstrated (Spooner and Vaughn 2008, 2009). 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 (Spooner and Vaughn 2008, 2009). Freshwater mussels fulfill a considerable ecological role in aquatic environments. They filter water, process nutrients, serve as ecosystem engineers, and impact assemblages of other macroinvertebrates (Gutierrez et al. 2003, Howard and Cuffey 2006, Vaughn and Spooner 2006, Vaughn et al. 2008). These key members of freshwater systems are also among the most imperiled taxa globally. Of the 297 species of freshwater mussels in North America, 69% are at risk of extinction, including the 37 species that are already presumed extinct (Williams et al. 1993, Master et al. 2000).

Freshwater mussels are threatened in part due to their unique life history that involves an obligate parasitic larval life stage (glochidia) that requires a suitable, and often a very specific, host fish to transform into a juvenile mussel (e.g., Watters 2007). 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 (Pandolfo et al. 2012). Their sessile nature, unique life history strategies, and imperiled conservation statuses indicate that freshwater mussels are particularly at risk from climate change (Hastie et al. 2003, Cahill et al. 2013).

These considerations have spurred a recent interest in the effects of temperature and flow changes on freshwater mussels (e.g., Pandolfo et al. 2010, Spooner et al. 2011, Archambault et al. 2013, Galbraith et al. 2012, Ganser et al. 2013). Laboratory experiments have generated basic thermal tolerance data for mussels (Pandolfo et al. 2010, Archambault et al. 2014, Ganser et al. 2013), and mussel assemblage changes in relation to temperature and flow have been empirically observed (Haag and Warren 2008, Spooner and Vaughn

2009, Galbraith et al. 2012). However, information relating the current thermal environment of mussels to their thermal tolerances is lacking, and few field temperature studies have been conducted in mussel habitats (e.g., Newton et al. 2013). In this study, we aim 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 are facing as temperatures continue to rise as a result of global change and other human-mediated activities.

Methods

Site selection

Twenty sites were selected within the upper Tar River basin of North Carolina from three 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 six sites in the Upper Tar subbasin. Sites were selected to represent a longitudinal gradient spanning a range of environmental conditions. These sites are grouped by subbasin and ordered by increasing watershed area in all subsequent tables and figures. 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). Drought status for the Tar River basin throughout the study period, determined by area-weighted average of the 7-day average streamflow, was provided by the North Carolina

Department of Environment and Natural Resources, Division of Water Resources
([http://www.ncwater.org/ Drought_Monitoring/](http://www.ncwater.org/Drought_Monitoring/)).

Freshwater mussel and fish surveys

Semi-quantitative snorkel and tactile search freshwater mussel surveys were conducted by the North Carolina Wildlife Resources Commission in the summer of 2010 according to established procedures. A minimum of 6 person-hours of effort was expended surveying for mussels at each site. Fish surveys were conducted in 2010 at each site by two backpack electrofishers and two dipnetters, within a 200-m reach, sampling upstream along the banks followed by a return pass down the center of the stream.

Temperature logger unit design and deployment

Continuous temperature-monitoring iBCod 22L data loggers from Alpha Mach, Inc. (Mont-St-Hilaire, Quebec, CAN) were used in this study. A temperature logger unit (Figure 1B) was assembled with three 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, one data logger (A) recorded temperatures in the water column and the other two recorded substrate temperatures. Each temperature logger unit was fitted with a sharpened bolt at the end for ease of 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 deployment sites were selected based on water depth, substrate type (i.e., no bedrock or large cobble that would prohibit driving the unit in to 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 assist in 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 not consistently uniform among or within sites.

Data analysis

Prior to statistical analysis, data were trimmed to remove the 24-hour 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 one of three temperatures, 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 temperatures (logger A) and temperatures at two substrate depths (loggers B and C). Data were included in regression only if the loggers had temperature data available for the same 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 tested the

relationships between these three parameters in the water column (logger A) and watershed area, percent of non-native fish of the total fish sample, percent of tolerant fish species, and percent of intolerant fish species at each site (NCDENR 2006). Three measures of biological integrity (Kwak and Freeman 2010), freshwater mussel richness, fish 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 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 tested for relationships with the same three biological measures mentioned above. Linear regression was performed to identify one to one relationships in 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 (Jobling 1981). 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).

Results

A total of 16,607 mussels of 16 species were sampled at the 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 8,240 fish belonging to 49 species were sampled (Table 1). The most abundant fish was *Notropis procne* (1,668) and the most ubiquitous was *Lepomis auritus*, the only species to occur at all 20 sites. There were 14 fish species that are documented hosts for Tar River basin mussels (Bogan 2002, Ohio State University Mussel/Host Database, <http://140.254.118.11/MusselHost/>).

The number of data points, i.e., hourly temperature readings, that were collected from the water column and two substrate depths, 5 cm and 15 cm, at 20 sites in the upper Tar River basin at each site ranged from 2,064 to 23,638 with a mean of 16,887 (Table 2). In the Tar River subbasin, mean summer temperature ranged 16.2 – 34.2 °C. Swift Creek subbasin mean summer temperature ranged 18.2 – 34.7 °C, and mean temperature in the Fishing Creek subbasin ranged 17.1 – 31.7 °C. 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. Fluctuation in hourly temperatures (standard deviation) was lower for the loggers in 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 indicates a buffering effect of the substrate

that keeps temperature more constant than in the water column. This effect generally increased as substrate depth increased.

The buffering effect of substrate was also demonstrated by the 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 did not consistently follow this trend. The general relationship among water column temperature and temperature at both substrate depths was quantified via linear regression (Table 3). Water column temperatures accounted for 89% of the variation in temperatures at 5 cm substrate depths and 73% of variation at 15 cm substrate depths. These relationships were significant, and result in a robust predictive equation to estimate substrate temperatures based on water column temperatures (Table 3).

The maximum number of hours for which three temperatures (28, 30, and 33 °C) were exceeded varied greatly among sites (Table 4). 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 in all sites with the exception of the two most upstream sites with the smallest watershed areas. Temperatures exceeded 28 °C for at least 24 h at 11, nine, and seven sites for 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 seven sites, and in 15 cm substrate at six sites. Temperatures exceeded 28 °C only in the water column (logger A) at

five sites. Only one site, Little Fishing Creek 1, never exceeded 28 °C. Temperatures exceeded 30 °C for at least 24 h at three, two, and one sites for the water column, 5 cm substrate, and 15 cm substrate, respectively. There were six sites that never exceeded 30 °C, and five 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 five sites ever exceeded 33 °C, three in the Tar River subbasin and two in the Swift Creek subbasin. The maximum 33 °C exceedance duration was 8 h in the water column at Tar River 5.

The mean temperature from the hottest 96 h at each site ranged 25.3 – 31.5 °C in the water column, 24.5 – 31.0 °C for loggers 5 cm in substrate, and 23.5 – 30.6 °C for loggers 15 cm in 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 seven, 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, six 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 four of those exceeded 30 °C with a maximum of 31.0, again at Tar River 5. Of the eight sites that exceeded a 96-h average temperature of 28°C for loggers 15 cm in substrate, two of those exceeded 30 °C, Sandy Creek 1 and Tar River 5.

The timing of the hottest 96-h period differed among sites, and these dates were compared with the drought index for the Tar River basin for the same time period (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 one 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, nine experienced moderate drought during the hottest 96 h period recorded at all three loggers.

Linear regression 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 existed between the maximum 28°C threshold exceedance of water temperatures and watershed area ($R^2 = 0.766$).

No significant linear relationship was detected among these three temperature measures and the percent of non-native 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 three biological measures 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 biological responses peak, but tests of a nonlinear fit (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 less 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 illustrate the closer relationship between fish richness and thermal condition than that demonstrated by freshwater mussel richness (Figure 6).

Discussion

We found temperatures at mussel-occupied sites in the Tar River basin of North Carolina have already reached thresholds that have been shown to cause harm to mussels in laboratory tests (Dimock and Wright 1993, Pandolfo et al. 2010, Ganser et al. 2013, Archambault et al. 2014). 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 substrate may be protective of mussels when air and

water temperatures are extreme, a concept purported by Archambault et al. (2014) in their laboratory tests of mussel vertical thermal exposures. Although freshwater mussels currently exist on the edges of their thermal limits, their abundance and species richness cannot be explained by temperature patterns alone. However, fish species richness was related to thermal regime, which indicates that species interactions with fish hosts may drive freshwater mussel responses to climate change.

The temperatures selected for use in the exceedance duration analysis in this study are representative of several thermal tolerance benchmarks for freshwater mussels. The lowest temperature, 28 °C, represents a chronic thermal threshold that would be expected to cause long-term mortality or short-term sublethal effects. It is based on average 28-d LT50s for three species of juvenile mussels (Ganser et al. 2013), and the overall average acute LT05 for glochidia and juveniles of eight species (Pandolfo et al. 2010). 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 (Pandolfo et al. 2010), and approximates the 96-h LT50 for juvenile mussels in additional studies (Dimock and Wright 1993, Archambault et al. 2014). The middle temperature, 30 °C, is based on the average of 14-d LT50s for juveniles of three mussel species (Ganser et al. 2013), and the average 96-h LT05 for juveniles of seven mussel species (Pandolfo et al. 2010), and represents a potential for both chronic and acute exceedances.

The choice of 96-h for 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 (ASTM 2006). The majority of thermal tolerance data that exist for freshwater mussels pertain to exposures of 96 h, so the 96-h duration of the hottest period is easily comparable to existing data. Water-only 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 (Hastie et al. 2003), and laboratory tests more closely mimic these extreme conditions.

The maximum average temperature for the hottest 96 h at any site was 31.5 °C at Tar River 5. This did not exceed acute (96-h LT50) thermal tolerance thresholds for most juvenile or adult mussels (Pandolfo et al. 2010, Galbraith et al. 2012, Archambault et al. 2014), but it equaled the 96-h LT50 for juvenile *Utterbackia imbecillis*, a species found in the Tar River study area (Dimock and Wright 1993). 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 five sites had average temperatures > 30 °C during the hottest 96-h period, and seven 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 one or more loggers at nine sites. At these sites, seven loggers in the water column, seven loggers 5 cm in substrate, and six loggers 15 cm in substrate exceeded 28 °C for 96 h or

more. The overall average 96-h LT05 for glochidia and juveniles of eight mussel species was 27.8 °C in laboratory exposures (Pandolfo et al. 2010). 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 three species was 28.8 °C (27.0 – 30.6 °C; Ganser et al. 2013). Tar River basin temperatures narrowly missed exceeding the average 21-d LT50 threshold, but it exceeded that of the most sensitive species, *Lampsilis siliquoidea* (Ganser et al. 2013). Fishing Creek exceeded 28 °C at loggers B and C in the substrate for a period of ~14 d. The 14 d LT05 for three mussel species was 28.0 °C (Ganser et al. 2013). 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 three sites exceeded 30 °C for 24 h or more, the established duration of toxicity tests for larval mussels (glochidia; ASTM 2006). Acute thresholds for glochidia of some species were exceeded at these three sites (Pandolfo et al. 2010), though glochidia are most likely to be abundant in the spring and these temperatures occurred in the summer months. Loggers at five sites exceeded 33 °C for durations lasting 2 – 8 h. This duration of time at 33 °C is probably not enough to harm mussels, but it indicates the potential for mussels to be exposed to extreme temperatures, especially in small streams.

While we make general statements about the exceedance of thermal thresholds for freshwater mussels, species differences are known to exist (Galbraith et al. 2012, Ganser et al. 2013). Specific thermal tolerance data exist only for a few of the mussel species found in our Atlantic Slope study. Temperatures in the Tar River basin did not reach the critical thermal maxima of adult *E. complanata* or *Strophitus undulatus* (Galbraith et al. 2012).

However, the maximum average temperature from the hottest 96-h period at Tar River 5 met the LC50 for juvenile *U. imbecillis* (Dimock and Wright 1993), and *U. imbecillis* was not detected at that site. This may be a spurious relation because *U. imbecillis* only occupied one of the 20 sites in our study, and that site (Tar River 1) was one with warmer 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 one site that never exceeded 28 °C, Little Fishing Creek 1, did not have 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 responses to increasing temperature, including changes in heart rate, lure display, burrowing behavior, and byssus production (Pandolfo et al. 2009, Gascho Landis et al. 2012, Archambault et al. 2013, Block et al. 2013). Temperature and watershed area were closely related, as expected. The maximum duration of 28 °C exceedances was the thermal measure most correlated with 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 longitudinal placement in the river basin, as determined by use of watershed area as a proxy for cumulative catchment temperature.

Laboratory-derived thermal thresholds suggest that freshwater mussels are already at risk from current environmental temperatures. However, laboratory experiments thus far have failed to replicate the potential for substrate to act as a thermal buffer in natural conditions. Archambault et al. (2014) 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 (Archambault et al. 2014). Other studies have shown that mussels most often occupy the top 5 – 10 cm of substrate, with mussels burrowed as deep as 20 cm (Scwalb and Pusch 2007). Thus, additional research with vertical thermal gradients in more natural sediment types and textures (i.e., organic content and particle sizes) are warranted with juvenile mussels.

Results from our study and another study of thermal profiles measured in mussel beds in a larger river system (Newton et al. 2013) both indicate that the thermal buffering capacity of 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 periods, occurred in 67% of cases with 5 cm substrate and 76% of cases with 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 so robust that it enabled the development of highly predictive regression equations for substrate temperatures

using water column temperature with the pooled data from 20 field sites. The thermal buffering effect of substrate was also observed in four 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 (Newton et al. 2013). The substrate was typically within 3 °C of surface water temperatures in their study, but at one site substrate was 7.5 °C cooler than surface water (Newton et al. 2013). 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 equation for water and substrate temperatures will most likely not 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.

It is well-documented that freshwater mussels burrow into substrate as a response to increasing temperature (Amyot and Downing 1997, Watters et al. 2001, Schwalb and Pusch 2007), and some mussels even spend a majority of their lives burrowed (Watters et al. 2001, Eads and Levine 2013). Studies have shown that there is only a difference of a few degrees between temperatures that are lethal to 50% of a mussel population and temperatures that are lethal to only 5% of a population (Pandolfo et al. 2010, Archambault et al. 2014). The few degree difference between water column and substrate temperatures may mean the difference between life and death for individual freshwater mussels and ultimately to survival of a population. This relation will become only increasingly important as global change and other

human-mediated impacts to the landscape (e.g., deforestation) continue to increase surface water temperatures.

Moreover, even if freshwater mussels are able to withstand the physiological stress from increasing temperature, they are susceptible to the prevailing effects of global change on species interactions (Visser and Both 2005, Spooner et al. 2011, Cahill et al. 2013, Warren and Bradford 2014). Because of the obligate nature of their relationship with often very specific host fishes, freshwater mussels may also be indirectly impacted by environmental stressors that affect fish (Spooner et al. 2011, Pandolfo et al. 2012). 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 (Pandolfo et al. 2012). Of the 49 fish species encountered in our surveys, 14 of these are known hosts for freshwater mussels. Thermal data are limited for non-game fishes that often serve as mussel hosts, but lethal thresholds for nine 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) (Wismer and Christie 1987). These thermal tolerance data for nine of the host fish species in the Tar River basin indicate that fishes, too, are living close to their thermal limits.

We found evidence that fish species richness is related to categorical 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 response supports 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 fishes among sites.

Global change is expected to have a vast array of consequences for fisheries and freshwater ecosystems in general (e.g., Eaton and Scheller 1996, Peterson and Kwak 1999, Hastie et al. 2003, Mohseni et al. 2003, Ficke et al. 2007). The expected impacts of global change may be particularly damaging to mussel assemblages in small streams that have less thermal inertia (Hastie et al. 2003, Newton et al. 2013). Hourly temperatures were simulated for the upper Tar River basin under projected climate change scenarios (Daraio et al. in press) 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 (Daraio et al. in press). Currently, no sites in the upper Tar River basin exceed this temperature duration during summer. Increasing temperature in a relatively short time period can interfere with mussel recruitment directly (Roberts and Barnhart 1999, Tæubert et al. 2013) or indirectly, through a variety of mechanisms including mismatched timing with host fish (Phillippart et al. 2003, Pandolfo et al. 2012).

Predicted changes in fish habitat resulting from global change in the United States could affect the availability of host fish for freshwater mussels (Eaton and Scheller 1996, Mohseni et al. 2003). 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 with climate and land use change (Allen 2004, IPCC 2007). Many studies have examined the

effects of both high and low flows on freshwater mussels (e.g., Haag and Warren 2008, Peterson et al. 2011, Archambault et al. 2013). Burrowing behavior and byssus production of juvenile mussels declined in laboratory-simulated drought conditions (Archambault et al. 2013), and assemblage level effects have been modeled and observed empirically (Haag and Warren 2008, Peterson et al. 2011, Spooner et al. 2011, Galbraith et al. 2010). Peterson et al. (2011) found a simulated mussel extinction that was at least eight times greater with current increased water use than under historical conditions, but mussel survival was also negatively related to high flows. Host-generalist mussel assemblages were more likely to be extirpated than specialist assemblages in a study that modeled the potential for fish and mussel coextirpation in relation to changing flows (Spooner et al. 2011). Empirically, mussel density in small streams in Alabama and Mississippi was 65 – 83% lower after a severe drought in 2000 (Haag and Warren 2008). Analysis of 15 years of mussel surveys in the Kiamichi River in Oklahoma indicated that mussel assemblages shifted from thermally sensitive to thermally tolerant species, and that these changes corresponded with a period of drought in the river (Galbraith et al. 2010). In the Tar River basin, at least one temperature logger at 16 sites (80%) coincided with moderate drought during the hottest 96-h period over three summers. This indicates that low flows will likely exacerbate the effects of rising temperatures for freshwater mussels and other aquatic organisms.

Unfortunately, climate change is not the only threat facing freshwater mussels. Other agents of global change are being encountered simultaneously. Simulated future land use increased daily mean stream temperatures in headwater streams in the Tar River basin (Daraio et al. in press), and a series of predicted future land use scenarios all resulted in net

increases in urban cover, with freshwaters in the southeastern United States particularly at risk (Martinuzzi et al. 2014). Invasive species are also expected to establish populations in currently unaffected freshwater systems due to shifting distributions of native species, increased reservoir construction, and the introduction of pathogens resulting from climate change (Rahel and Olden 2008).

We have conducted a thorough analysis of the current thermal environment of freshwater mussels over a range of sites in a southeastern U.S. river basin. Our results clearly demonstrate that freshwater mussels and their host fishes are currently experiencing temperatures that sometimes exceed their upper thermal tolerances. Shifts in mussel assemblages have already been documented as a result of temperature and flow impacts (Haag and Warren 2008, Galbraith et al. 2010), and the wide-ranging impact of these changes are experienced at an ecosystem level (Spooner and Vaughn 2008, 2009). The buffering capacity of 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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Table 1. Species and numbers of fish sampled at 20 sites in the Tar River basin, North Carolina.

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 auritus</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	-	-	-	-

Table 1 Continued.

Species	Site																			
	SheC	NFTR	TR1	TR2	TR3	TR4	SaC1	SaC2	SaC3	RBC	SwC1	SwC2	ShoC	LFC1	RS1	RS2	LFC2	LFC3	FC	TR5
<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	-

Table 2. Overall and logger-specific averages, minimum, maximum, and standard deviation from temperature loggers that recorded hourly measurements at 20 sites in the Tar River basin, NC, during the summers (June, July, August) 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 substrate, and Logger C was 15 cm below the interface in substrate; *N* = number of temperature logger measurements taken at each site.

Site	Watershed Area (km ²)	Overall					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	<i>N</i>	Mean	Min	Max	SD	
<i>Tar River Subbasin</i>																						
NFTR	North Fork Tar River	40.1	23,638	22.9	16.2	28.7	1.6	9,095	23.3	16.2	28.7	1.8	7,512	22.6	17.2	25.7	1.6	7,031	22.6	19.6	25.2	1.2
SheC	Shelton Creek	58.5	19,834	23.6	17.7	33.7	1.4	7,913	23.9	17.7	33.7	1.5	6,312	23.4	20.2	33.7	1.3	5,609	23.2	18.6	26.2	1.2
TR1	Tar River 1	359.0	12,048	27.6	23.7	34.2	1.5	4,752	28.0	23.7	34.2	1.8	4,752	27.4	23.7	31.2	1.3	2,544	27.2	24.7	29.6	1.1
TR2	Tar River 2	557.1	22,392	26.3	19.6	33.2	2.0	9,000	26.8	19.6	33.2	2.1	9,000	26.2	20.7	31.2	1.7	4,392	25.4	20.6	28.6	1.6
TR3	Tar River 3	948.0	21,648	26.3	19.1	32.6	1.9	7,416	26.6	19.1	32.6	2.1	6,816	26.7	22.2	32.2	1.7	7,416	25.7	19.7	29.2	1.7
TR4	Tar River 4	1428.7	21,648	27.4	21.7	32.6	1.6	7,704	27.6	21.7	32.6	1.8	6,240	27.5	22.2	31.7	1.6	7,704	27.0	22.2	30.2	1.4
<i>Swift Creek Subbasin</i>																						
RBC	Red Bud Creek	36.2	16,488	24.9	18.2	31.7	1.9	5,496	24.8	18.2	31.7	2.1	5,496	24.9	19.2	29.7	1.9	5,496	24.9	19.7	28.7	1.8
SaC1	Sandy Creek 1	138.9	7,200	27.2	22.6	33.1	1.6	2,400	27.3	22.6	32.6	1.8	2,400	27.3	23.2	32.7	1.6	2,400	27.1	23.7	33.1	1.5
SaC2	Sandy Creek 2	252.1	20,736	24.5	18.1	30.2	1.8	6,912	24.8	18.1	30.2	2.0	6,912	24.6	19.1	29.2	1.8	6,912	24.2	19.2	28.2	1.7
SaC3	Sandy Creek 3	287.4	18,072	25.0	19.7	30.7	1.6	6,024	25.7	19.7	30.7	1.7	6,024	24.9	20.2	28.7	1.4	6,024	24.3	19.7	27.2	1.3
SwC1	Swift Creek 1	471.6	15,456	24.5	19.2	30.7	1.9	5,136	25.8	19.6	30.7	1.8	5,160	24.3	20.0	28.1	1.5	5,160	23.3	19.2	26.7	1.5
SwC2	Swift Creek 2	693.5	14,688	26.3	20.6	31.1	1.7	3,552	26.3	20.7	31.1	2.0	5,736	26.2	20.6	30.7	1.7	5,400	26.4	21.1	29.6	1.5
TR5	Tar River 5	3364.1	11,112	27.0	18.6	34.7	2.7	4,320	28.1	21.7	34.7	2.4	2,472	27.3	21.2	32.1	2.4	4,320	25.7	18.6	30.6	2.6
<i>Fishing Creek Subbasin</i>																						
RS1	Rocky Swamp 1	27.1	11,376	23.3	20.2	29.7	1.5	3,792	24.0	20.2	29.7	1.6	3,792	23.1	20.2	27.6	1.4	3,792	23.0	20.6	26.7	1.3
RS2	Rocky Swamp 2	51.1	22,176	23.4	17.1	29.1	1.6	7,392	23.8	17.1	29.1	1.8	7,392	23.3	18.6	27.6	1.5	7,392	23.1	19.2	26.7	1.4
ShoC	Shocco Creek	62.9	20,664	23.1	17.1	29.1	1.8	6,888	23.8	17.1	29.1	2.0	6,888	23.1	18.1	27.2	1.7	6,888	22.5	18.2	25.7	1.5
LFC1	Little Fishing Creek 1	73.6	17,616	22.1	19.0	26.6	1.3	6,864	22.5	19.1	26.6	1.3	4,656	22.4	19.0	25.6	1.2	6,096	21.4	19.2	23.7	0.9
LFC2	Little Fishing Creek 2	460.7	18,984	24.6	19.7	28.7	1.4	6,888	24.7	19.7	28.7	1.6	6,888	24.7	20.2	28.2	1.4	5,208	24.5	21.7	27.2	1.2
LFC3	Little Fishing Creek 3	485.7	19,896	24.5	18.6	30.7	1.8	7,368	25.2	18.6	30.7	2.0	5,160	24.4	19.1	27.7	1.7	7,368	23.9	19.6	27.1	1.5
FC	Fishing Creek	2035.5	2,064	28.5	25.7	31.7	1.3	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

Table 3. Predictive relationships 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	N	R^2	F - stat	p - value
<i>Water column and upper substrate (5 cm)</i>				
Logger B = 2.231 + 0.894*Logger A	108,096	0.8879	856,000	< 0.001
<i>Water column and lower substrate (15 cm)</i>				
Logger C = 4.624 + 0.780*Logger A	105,729	0.7312	287,600	< 0.001

Table 4. Maximum duration (h) that temperatures exceeded 28, 30, and 33 °C at 20 sites in the Tar River basin, NC, during the summers (June, July, August) 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 substrate, and Logger C was 15 cm below the interface in substrate.

Site		Maximum Exceedance Duration (h)								
		28 °C			30 °C			33 °C		
		A	B	C	A	B	C	A	B	C
<i>Tar River Subbasin</i>										
NFTR	North Fork Tar River	5	0	0	0	0	0	0	0	0
SheC	Shelton Creek	9	9	0	6	6	0	2	2	0
TR1	Tar River 1	179	156	156	68	16	0	6	0	0
TR2	Tar River 2	140	104	16	17	13	1	4	0	0
TR3	Tar River 3	71	127	64	14	12	0	0	0	0
TR4	Tar River 4	164	308	266	66	67	15	0	0	0
<i>Swift Creek Subbasin</i>										
RBC	Red Bud Creek	17	18	17	7	0	0	0	0	0
SaC1	Sandy Creek 1	108	111	112	15	16	17	0	0	2
SaC2	Sandy Creek 2	66	65	19	2	0	0	0	0	0
SaC3	Sandy Creek 3	116	15	0	12	0	0	0	0	0
SwC1	Swift Creek 1	70	7	1	11	0	1	0	0	0
SwC2	Swift Creek 2	89	91	111	13	10	0	0	0	0
TR5	Tar River 5	511	513	469	88	88	43	8	0	0
<i>Fishing Creek Subbasin</i>										
RS1	Rocky Swamp 1	10	0	0	0	0	0	0	0	0
RS2	Rocky Swamp 2	8	0	0	0	0	0	0	0	0
ShoC	Shocco Creek	7	0	0	0	0	0	0	0	0
LFC1	Little Fishing Creek 1	0	0	0	0	0	0	0	0	0
LFC2	Little Fishing Creek 2	12	7	0	0	0	0	0	0	0
LFC3	Little Fishing Creek 3	19	0	0	6	0	0	0	0	0
FC	Fishing Creek	165	380	328	16	19	10	0	0	0

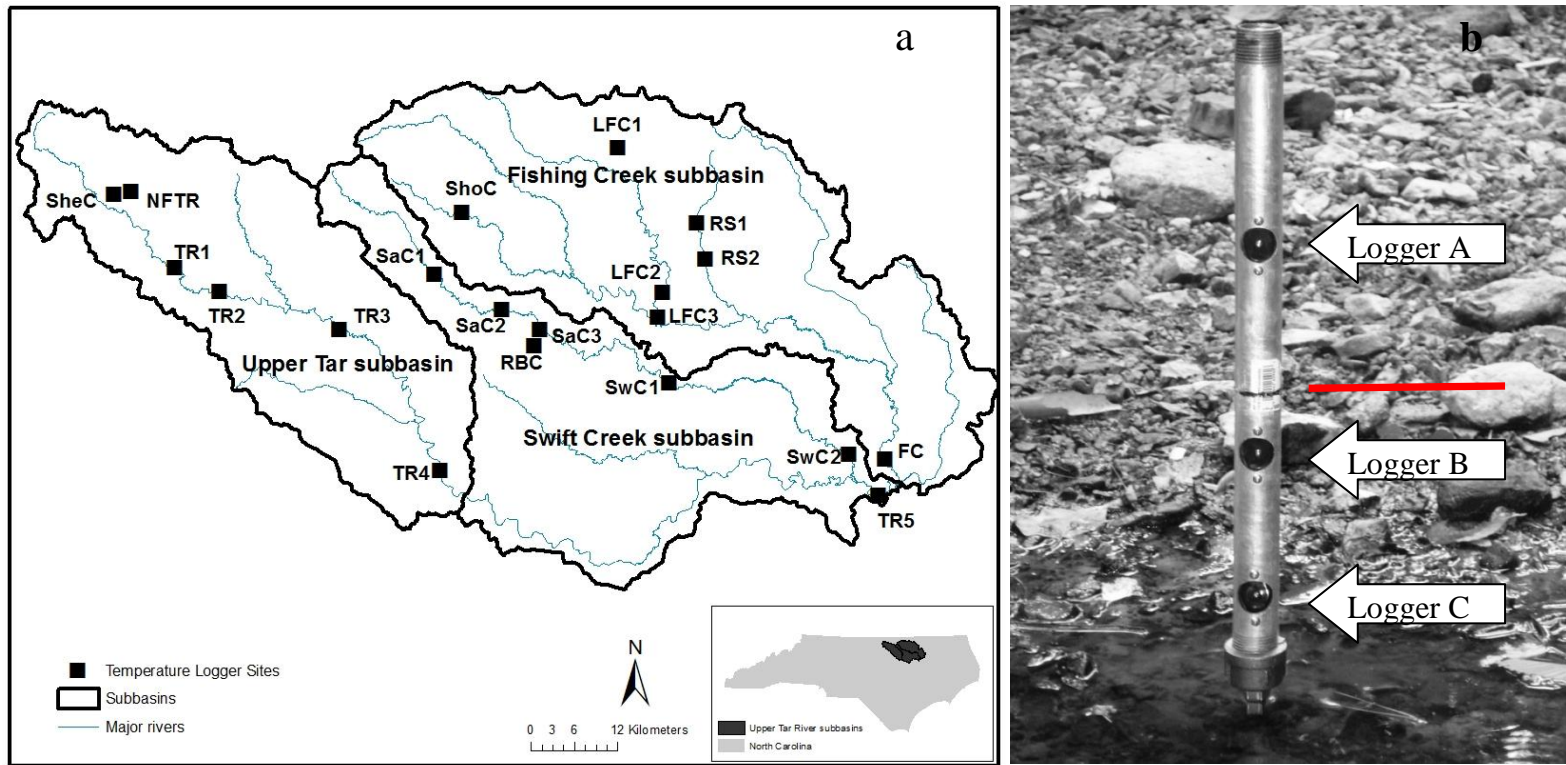


Figure 1. Location of temperature loggers at 20 sites in the upper Tar River basin, NC (a), and photograph showing temperature logger deployment design (b). Logger A is 10 cm above the water/substrate interface in the water column, red line denotes location of water/substrate interface when deployed, Logger B is 5 cm below the interface in substrate, and Logger C is 15 cm below the interface in substrate. See Table 2 for site abbreviations.

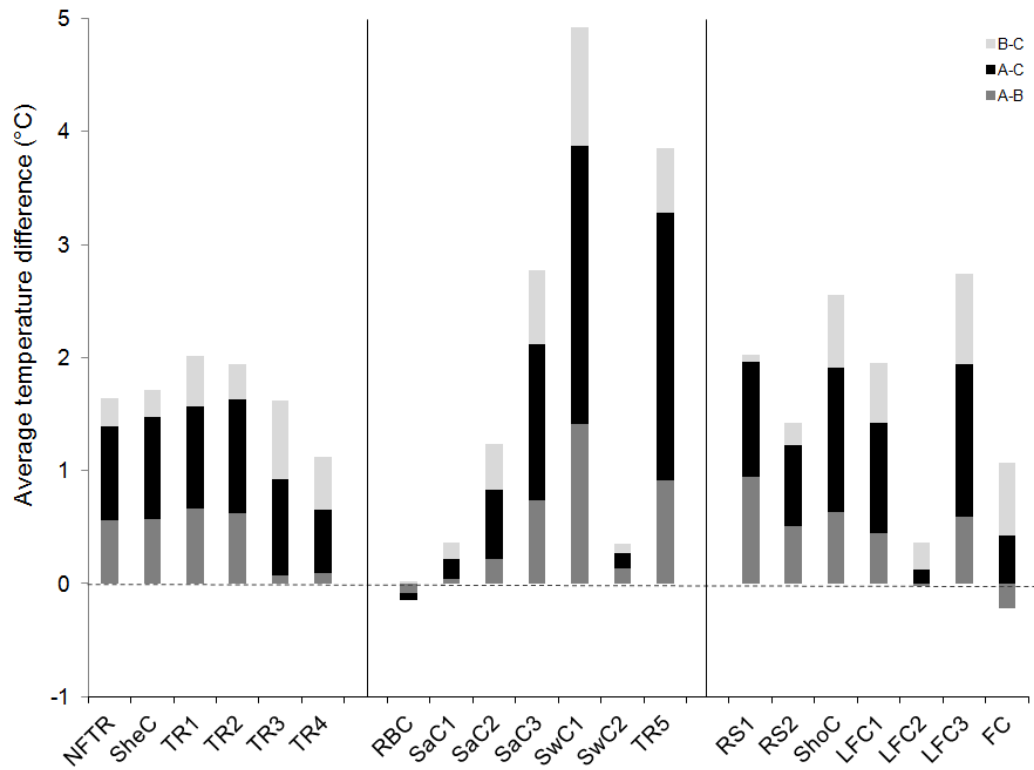
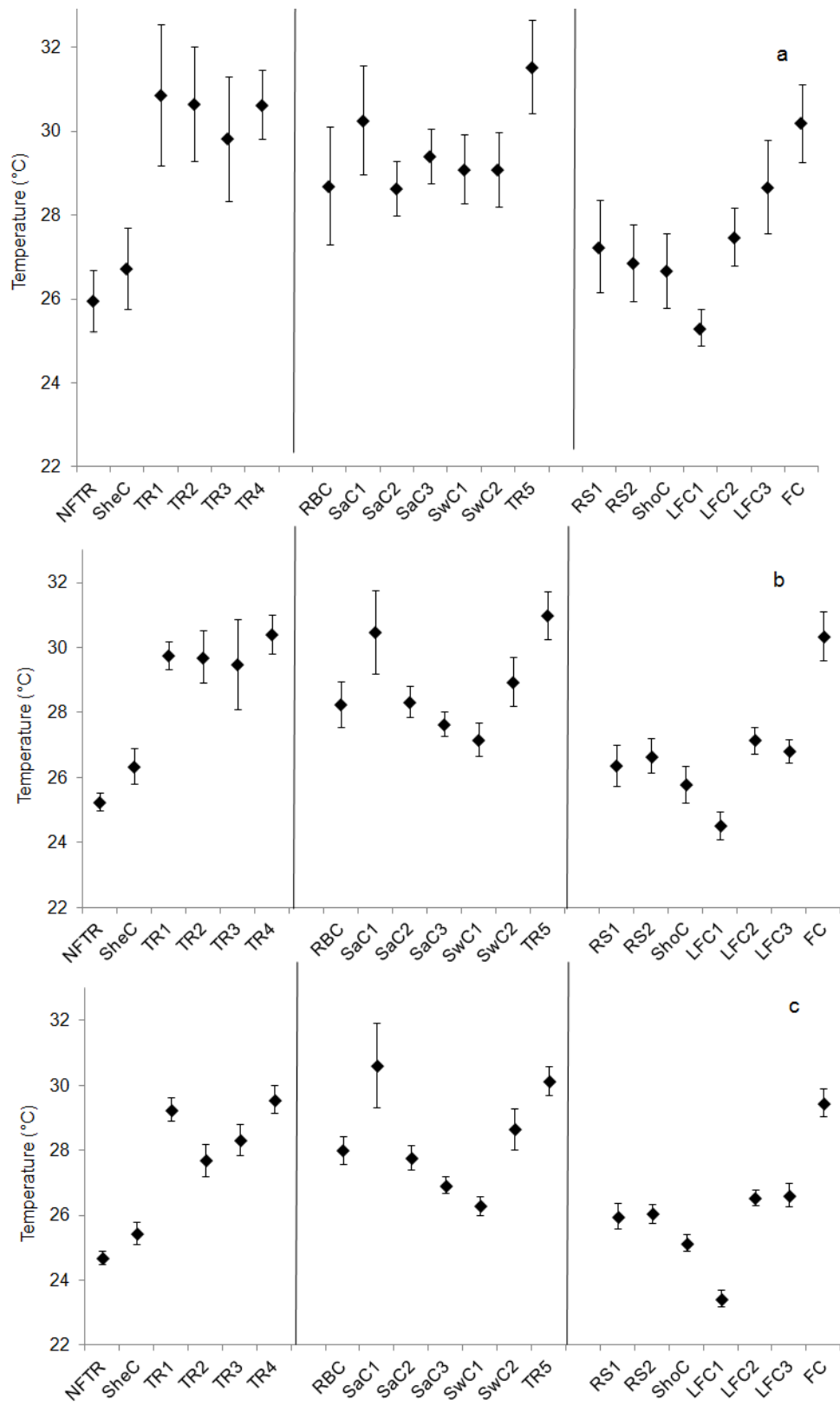


Figure 2. Average differences between temperatures in the water column and 5 cm in the substrate (A-B), temperatures in the water column and 15 cm in the substrate (A-C), and temperatures 5 cm and 15 cm in the substrate (B-C) at 20 sites in the Tar River basin, NC. Vertical lines separate subbasins. See Table 2 for site abbreviations.

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, NC. Temperatures 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.



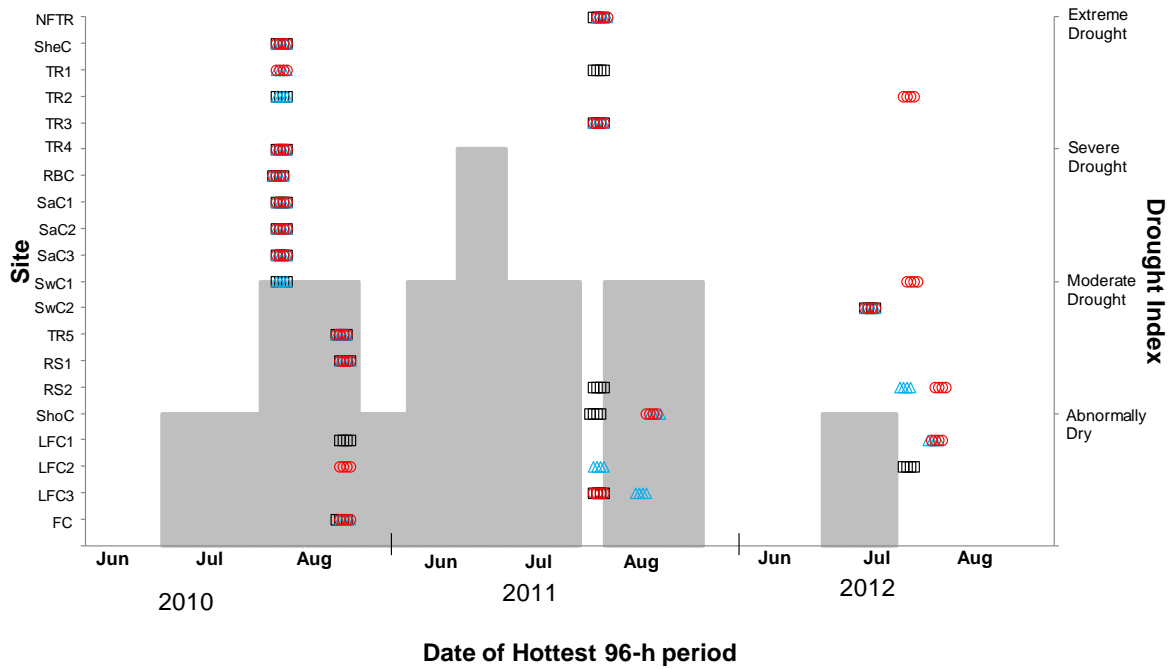


Figure 4. Timing of the hottest 96-h period (symbols) during the summers of 2010 – 2012 at 20 sites in the Tar River basin, NC, and drought status (shaded area) for the Tar River basin during the same time periods. Timing of hottest period measured for the water column (□), 5 cm in the substrate (Δ),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

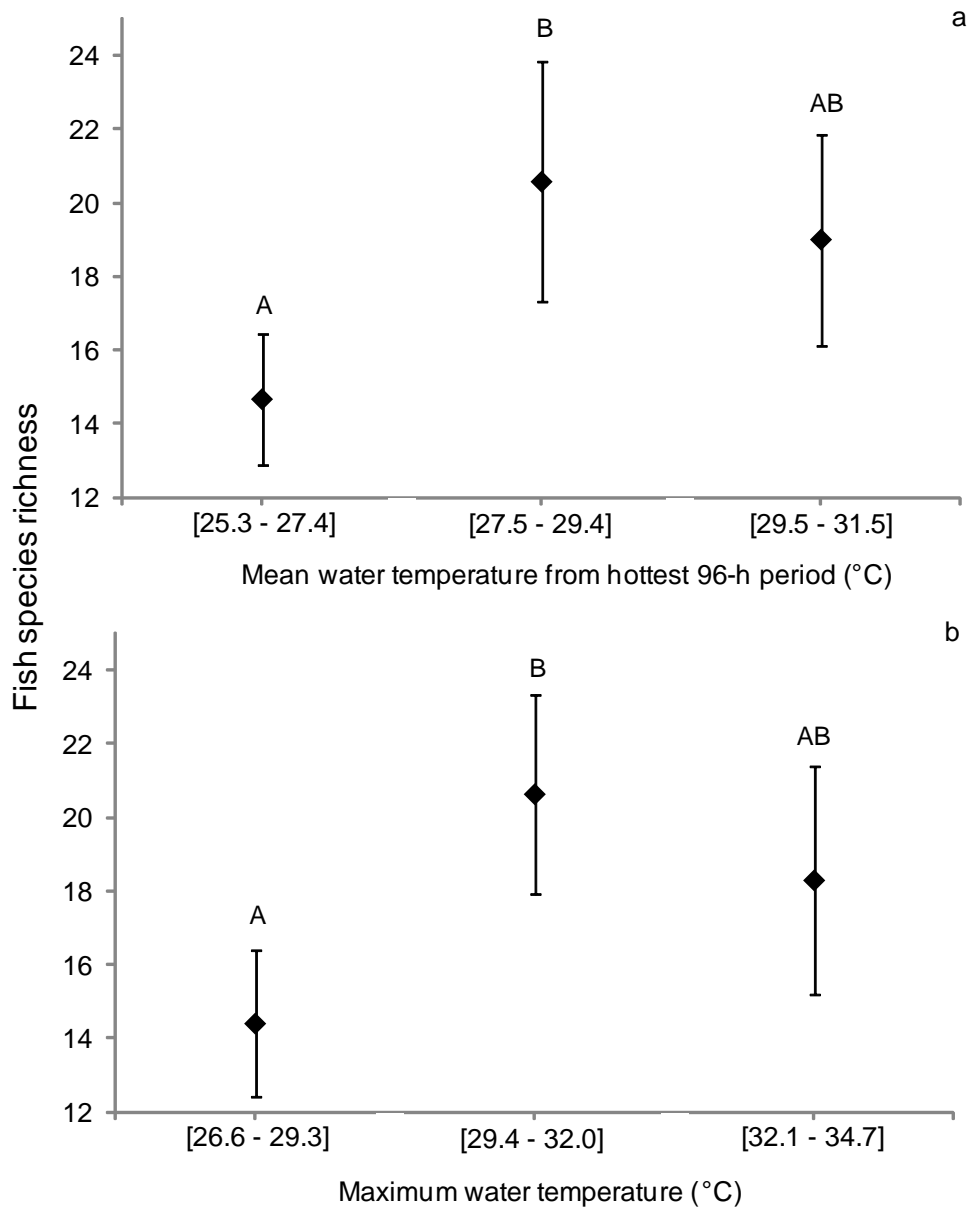
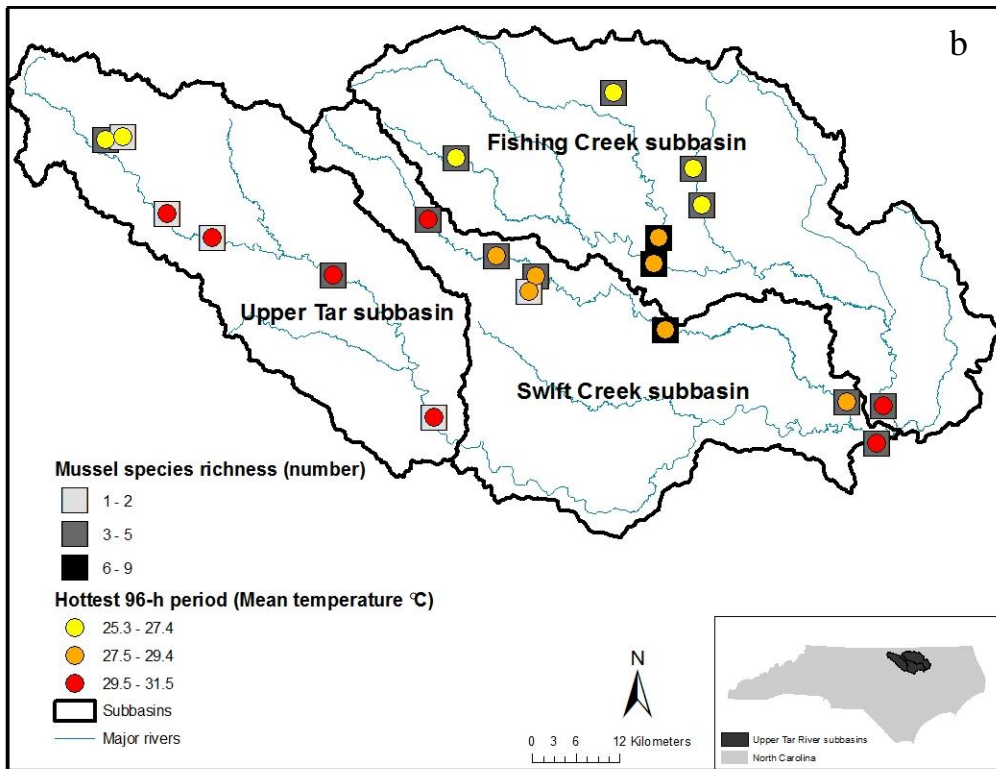
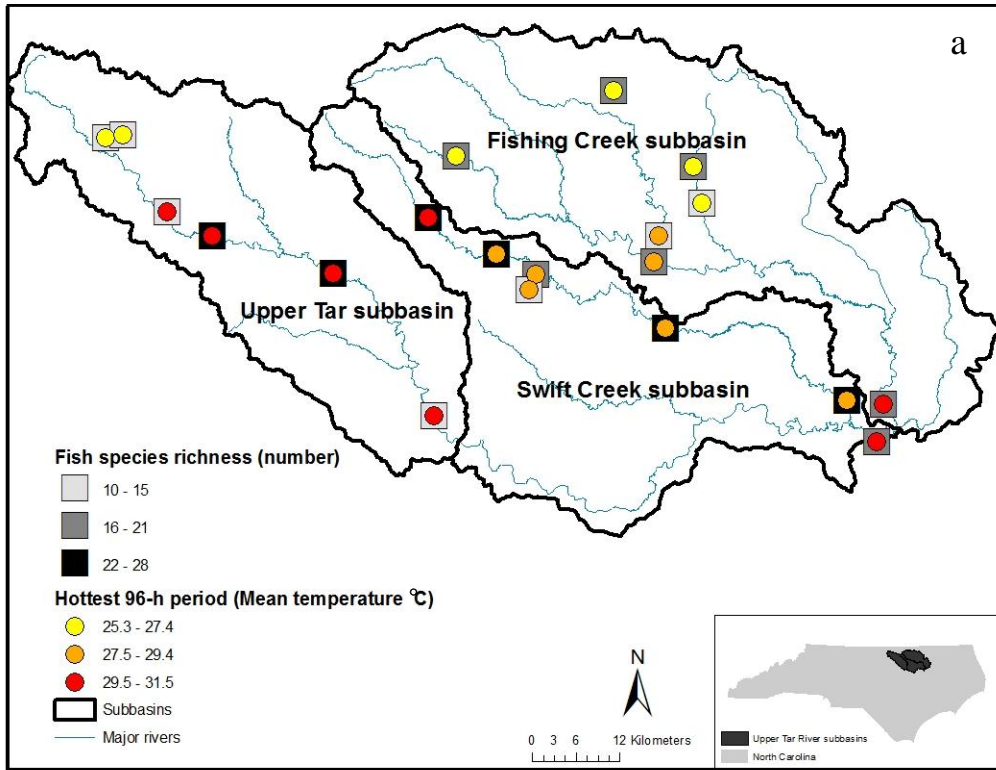


Figure 5. Fish species richness as a function of mean water temperature from the hottest 96-h period (a) and maximum water temperature (b) from 20 sites in the Tar River basin, NC. Different letters indicate statistically significant differences in means among temperature ranges at $\alpha = 0.10$.

Figure 6. Map of fish (a) and freshwater mussel (b) species richness and the average temperature from the hottest 96-h period during the summers of 2010-2012 at 20 sites in the Tar River basin, NC.



CHAPTER 4

Microhabitat suitability and niche breadth of common and imperiled

Atlantic Slope freshwater mussels

Abstract

Freshwater ecosystems are experiencing a rapid loss of biodiversity that surpasses the loss in any other environment, and a primary cause of this widespread species loss is habitat alteration and degradation. Freshwater mussels present a critical case for the need to identify habitat requirements, because many imperiled mussels may require human intervention to persist. We empirically measured microhabitat use for 10 freshwater mussel species, including the federally endangered *Alasmidonta heterodon*, at 20 sites in the Tar River basin, North Carolina. Habitat availability at each site was also quantified, and habitat suitability was calculated for each mussel species. The majority of available habitat across all sites consisted of shallow, slow moving water with penetrable silt or sand substrate. Among species, mean water depth of occupied habitats ranged 0.23 – 0.54 m, mean bottom velocity ranged 0.001 – 0.055 m/s, average mean-column velocity ranged 0 – 0.055 m/s, and mean substrate penetrability ranged 0.11 – 11.67 on an index scale. The most commonly measured dominant substrate materials were silt, sand, very coarse sand, pea gravel, and coarse gravel. The most commonly documented cover types were coarse woody debris and fine woody debris. We found a relationship between the niche breadth and conservation status of a species. Federally endangered *A. heterodon* consistently showed a narrower suite of suitable microhabitats than the common mussel *E. complanata*. The range of suitable habitat

characteristics for *F. masoni* and *V. constricta*, listed as North Carolina state endangered and special concern, respectively, was typically narrower than those of *E. complanata* and wider than those of *A. heterodon*. These habitat suitability criteria will be useful to guide identification of suitable sites for habitat protection, mussel relocation, or site restoration.

Keywords: Habitat Suitability Index, HSI, use, availability, unionid, instream flow, IFIM

Introduction

Freshwater ecosystems are losing biodiversity at a higher rate than terrestrial or marine systems (Ricciardi and Rasmussen 1999, Dudgeon et al. 2006). Among North American freshwater species, 20 – 30% of fishes, amphibians, crayfishes, and gastropods are considered to be imperiled, and at least 123 freshwater species have gone extinct since 1900 (Neves et al. 1997, Ricciardi and Rasmussen 1999, Johnson et al. 2013). The situation is not improving; a revision of the conservation status of North America's fishes found that 89% of imperiled fishes were as or more imperiled than they were in 1989 (Jelks et al. 2008). Freshwater invertebrates are in an even more dire condition than vertebrates, with freshwater mussels (order Unionida) being the most imperiled taxonomic group in North America (Master et al. 2000, Lydeard et al. 2004). Of the 297 species of freshwater mussel in North America, 69% are at risk, including the 37 species that are already presumed extinct (Williams et al. 1993, Master et al. 2000).

These widespread declines in freshwater fauna have been broadly attributed to habitat degradation, toxic contaminants, stream fragmentation and flow alteration, and the presence

of nonindigenous species (Neves et al. 1997, Richter et al. 1997, Strayer et al. 2004, Dudgeon et al. 2006, Jelks et al. 2008). Among these and many other possible causes, habitat degradation or destruction is ranked the most detrimental threat to about 50% of the imperiled species in the United States (Richter et al. 1997). For freshwater mussels in the eastern United States, habitat degradation due to increased sediment load from agricultural land use is the greatest threat (Richter et al. 1997). The role of habitat preservation in the conservation of animals is clear, and a lack of information regarding the habitat requirements of freshwater species impedes conservation (Abell 2002). Freshwater mussels may present an extreme case for the importance of the identification of habitat requirements because many imperiled mussels may require human intervention to persist. It is important to identify optimal and suitable habitat characteristics to assist in habitat protection, management, and restoration, as well as mussel relocation site selection.

The majority of habitat studies conducted with freshwater mussels to date aim to produce a predictive model for mussel distribution or abundance (e.g., Brim Box et al. 2002, McRae et al. 2004, Gangloff and Feminella 2007, Allen and Vaughn 2010). These models have met with mixed success (Layzer and Madison 1995, Johnson and Brown 2000), but there is currently a general agreement that microhabitat characteristics alone are not effective predictors of mussel distribution (e.g., Strayer and Ralley 1993, Haag and Warren 1998, Brim Box et al. 2002). Freshwater mussel habitat preferences have also been examined in controlled laboratory studies (Michaelson and Neves 1995, Downing et al. 2000). However, studies on habitat suitability indices for freshwater mussels are lacking [but see Layzer and Madison (1995)].

Habitat suitability indices have been widely developed for fishes and other aquatic organisms (e.g., Hamilton and Nelson 1984, Raleigh et al. 1986), particularly for use in stream flow modeling via the Instream Flow Incremental Method (IFIM) (Bovee 1986). The IFIM was developed by the U.S. Fish and Wildlife Service as a water resource management tool, and it was widely applied in the 1980s, with variations developed and applied more recently. IFIM uses site-specific stream flow and habitat suitability data for a species to determine how the availability of suitable habitat may change with fluctuations in stream flow (Bovee 1986). Habitat suitability index empirical data that are used to provide the biological input for these models describe the relative importance, or suitability, of different microhabitats based on measures of habitat use in proportion to availability of that habitat (Bovee 1986). The habitat suitability data that are generated for use in these models may also be valuable for use in different applications, such as targeted field surveys (Midway et al. 2010), animal relocations or reintroductions (Fisk et al. 2014), site restoration (Quinn and Kwak 2000, Hewitt et al. 2009), or conservation planning (Spooner et al. 2011). To strengthen the potential for freshwater mussels to benefit from these applications, we investigated the habitat suitability of common and imperiled mussel species in lotic ecosystems. Microhabitat use characteristics were measured at 20 stream sites for 10 species of freshwater mussels. Habitat availability surveys were conducted at the same sites, and habitat suitability for a suite of microhabitat parameters was determined for 10 species. These suitability results can be used to infer relative selectivity of freshwater mussels for a variety of microhabitats and target suitable ranges of habitat parameters for conservation and management (Johnson 1980).

Methods

Field methods

Twenty sites were selected within the upper Tar River basin, North Carolina, from three subbasins with similar drainage areas: the Upper Tar subbasin, Swift Creek subbasin, and Fishing Creek subbasin (Figure 1). Seven sites were chosen within the Swift Creek and Fishing Creek subbasins, and six sites were chosen in the Upper Tar subbasin. Surveys of mussel microhabitat use were conducted at these sites concurrent with freshwater mussel snorkel surveys in the summer of 2010. Precise mussel locations were flagged, and microhabitat characteristics at these precise locations were measured. For the common species, *Elliptio complanata*, up to 20 individuals were flagged per site and recorded during a 6 person-hour survey. For all other species, microhabitat characteristics were measured for all mussels detected during a survey.

Measurements of six microhabitat parameters were recorded for each mussel, including water depth (m), bottom water velocity (m/s), mean-column water velocity (m/s), substrate penetrability (index), dominant substrate type, and closest cover type. Depth and water velocity were measured using a top-set wading rod and a Marsh-McBirney Model 2000 digital flow meter, following established methods for stream habitat assessment (Bain and Stevenson 1999). Dominant substrate type (Table 1) was determined using a modified Wentworth particle size scale (Bovee and Milhous 1978). Substrate penetrability (Table 1) was measured using a Lang Penetrometer (Johnson and Brown 2000). The closest cover type was the nearest material that could slow water velocity or potentially provide shelter or habitat for a mussel (Table 1). Mussel survey reach lengths were measured using a digital

rangefinder, and GPS coordinates were taken to ensure habitat availability surveys would be conducted at locations corresponding to mussel microhabitat use surveys.

Microhabitat availability was measured in instream habitat surveys at each site during base flow conditions. At each site, a mean stream width was determined and then, starting with a randomly placed cross-sectional transect, 10 transects were spaced every two mean-stream-widths apart (Simonson et al. 1994). At about 10 equally-spaced points within each transect, the six microhabitat parameters that were measured in the mussel microhabitat use assessment were measured to characterize microhabitat availability.

Data analysis

Mussel species with at least five individuals sampled at one site were considered for further analysis. Nine species met this criterion, including *E. complanata*, *E. icterina*, *E. congaraea*, *E. roanokensis*, *E. fisheriana*, *Alasmidonta heterodon*, *Villosa constricta*, *Fusconaia masoni* and an undescribed *Lampsilis* species. There were also limited data for an endemic federally endangered species, *E. steinstansana*. Though this species did not meet the analysis criteria, because information on the species is so limited, we have included an anecdotal analysis of the habitat suitability for the three individuals sampled.

Microhabitat suitability values were calculated and graphed as distributions for 10 species using the microhabitat use and availability data collected from the field for six habitat parameters. For each habitat parameter, suitability was calculated by dividing microhabitat use at a site by availability at that site over a range of values for each parameter (Bovee 1986). The results for a parameter's entire range of values were normalized to a maximum of 1.0 to provide a scale where 1.0 indicates the most optimal, or suitable, habitat and 0

indicates the least suitable. When a mussel species was encountered at more than one site, data from multiple sites were combined by weighting suitability for each site by the number of individuals at that site, and then summing the weighted suitability values and again normalizing to a maximum of 1.0. In cases where proportional use for a particular interval or category of a parameter was greater than its availability, suitability was set to 1.0.

Data for four species, *A. heterodon*, *F. masoni*, *V. constricta*, and *E. complanata*, were further analyzed, because sufficient sample sizes were attained and they represent a range of conservation statuses. The habitat suitabilities of the six parameters for these four species were graphed together to compare the range of suitabilities according to species and conservation status. Data for these species were also analyzed using a bootstrap two-sided Kolmogorov-Smirnov test (R statistical software, Sekhon 2011) to test for significant differences between use and availability distributions for each habitat parameter, i.e., non-random use of habitat by a mussel species.

Results

Microhabitat use was measured for 10 freshwater mussel species (Table 2), and sample size varied greatly among species. The most ubiquitous species, *E. complanata*, was represented by 357 individuals from 20 sites, whereas the rarest species, *E. steinstansana*, was represented by three individuals from two sites. Among species, mean depth of occupied habitats ranged 0.23 – 0.54 m, mean bottom velocity ranged 0.001 – 0.055 m/s, average mean-column velocity ranged 0 – 0.055 m/s, and mean substrate penetrability ranged 0.11 – 11.67 on an index scale. The most commonly measured dominant substrate materials

were silt, sand, very coarse sand, pea gravel, and coarse gravel. The most commonly documented cover types were coarse woody debris and fine woody debris.

Habitat availability was surveyed at a mean of 120 (range 80 – 161) points within each of 20 sites containing a variety of mussel species (Figure 2). The majority of available habitat across all sites consisted of shallow, slow moving water with penetrable silt or sand substrate. The most abundant cover type was coarse woody debris. These characteristics are consistent with current stream channel conditions in the coastal plain physiographic region.

Habitat suitability distributions for depth, bottom velocity, mean velocity, substrate penetrability, dominant substrate, and closest cover type were developed for each of 10 mussel species (Figures 3 – 12). Suitability distribution forms varied among species, reflecting differences among habitat niches occupied, but influenced by the range in sample sizes, i.e., suitability distributions of species with the greatest numbers of habitat use measurements, *E. complanata* and *E. icterina*, more closely resemble a continuous distribution.

Differences among species microhabitat selectivity were evident. *A. heterodon* tended to occupy shallow, slow-flowing sites with penetrable silt, coarse sand, and gravel. Tree roots and vegetation provided suitable cover, in addition to woody debris. *V. constricta* also utilized shallow slow-flowing locations, though moderately penetrable gravels and cobble were the most suitable substrates. Boulders and woody debris provided the most suitable cover. Suitable habitat for *F. masoni* was similar to that of *V. constricta*, though slightly deeper and faster flowing water was more suitable. The undescribed *Lampsilis* species was most suited to habitats like those preferentially occupied by *V. constricta*.

The most common species, *E. complanata* was at least marginally suited to almost all available habitat. The most suitable habitats for this species were shallow, slow-flowing sites with penetrable substrates. *E. icterina* had similar suitabilities, but moderately penetrable coarse sand was the most suitable substrate. *E. congaraea* occurred in slightly deeper water with slow velocity, though they tolerated even the swiftest flows (> 0.50 m/s). Many substrates were suitable for *E. congaraea*, but silt was not. *E. fisheriana* was suited to shallow, slow-flowing habitats with moderately penetrable substrates. *E. roanokensis* was suited to coarse gravel habitats with deeper and swifter water than the other species. The federally endangered *E. steinstansana* was anecdotally associated with moderately penetrable coarse sand and slow velocity with woody debris and boulders as cover.

Habitat suitabilities for four species with different conservation statuses, *E. complanata*, *A. heterodon*, *F. masoni*, and *V. constricta*, were plotted together for relative species comparisons (Figure 13). Most suitable depths for *A. heterodon*, *F. masoni*, and *E. complanata* ranged 0.3 – 0.5 m, whereas slightly deeper waters of 0.6 – 0.7 m were most suitable for *V. constricta*. All four species were suited to velocities up to 0.025 m/s, which were also the most widely available. A range of substrates could be considered at least moderately suitable for all species, but the species differed in penetrability suitability. *A. heterodon* was suited to the most penetrable substrates, although those were the only substrates available at the sites where it occurred. *E. complanata* was also most suited to highly penetrable substrates, whereas *V. constricta* and *F. masoni* found mid- to high-range compaction most suitable. Woody debris was suitable cover for all four species. *V. constricta* and *F. masoni* also utilized boulders, and *A. heterodon* was associated with

vegetation, roots, and undercut banks. Federally endangered *A. heterodon* consistently showed a narrower suite of suitable microhabitats than the common mussel *E. complanata*. The range of suitable habitat characteristics for *F. masoni* and *V. constricta*, listed as North Carolina state endangered and special concern, respectively, was typically narrower than those of *E. complanata* and wider than those of *A. heterodon*.

Habitat use of *E. complanata*, *A. heterodon*, *F. masoni*, and *V. constricta* was tested against habitat availability to detect randomness in habitat selectivity among species (Table 3). Depth, bottom velocity, and substrate penetrability were non-randomly selected among all four species. *E. complanata* exhibited non-random habitat use for all six measured microhabitat parameters. Mean-column velocity use was also non-random for *A. heterodon*, and *F. masoni* showed non-random dominant substrate and closest cover selectivity.

Discussion

Our results indicate that freshwater mussels generally occupy microhabitat non-randomly and that mussel conservation status may correspond to niche breadth. Although freshwater mussels are broadly described as habitat generalists (Tevesz and McCall 1979), our results demonstrate that some characteristics are more suitable than others when habitat use is adjusted for availability. Habitat requirements are thought to be one of the primary controls on animal distribution and abundance (Haag and Warren 1998). However, defining this relationship for freshwater mussels has been complicated. Microhabitat is greatly surpassed by complex reach-scale hydraulic variables, such as shear stress, in the ability to predict the distribution and abundance of freshwater mussels (Layzer and Madison 1995,

Zigler et al. 2008, Allen and Vaughn 2010). Despite the general lack of broad predictive value, multiple investigators have found correlative relationships between some microhabitat parameters and freshwater mussel occurrence and abundance (Salmon and Green 1983, Strayer and Ralley 1993, Johnson and Brown 2000). These mixed conclusions suggest that microhabitat may not directly control mussel occurrence per se, but it may be one of many factors influencing the distribution of freshwater mussels (Strayer and Ralley 1993, Layzer and Madison 1995, Haag and Warren 1998, Downing et al. 2000, Strayer 2008). Habitat is almost certainly a limiting factor in mussel distributions, but the relationship is complex and involves dynamics at multiple interacting spatial and temporal scales (e.g., McRae et al. 2004).

The microhabitat characteristics that have been found to correlate with freshwater mussel occurrence most often are related to substrate composition and flow (Salmon and Green 1983, Holland-Bartels 1990, Strayer and Ralley 1993, Johnson and Brown 2000), though there is not always a strong relationship (Neves and Widlak 1987, Strayer et al. 1994, Layzer and Madison 1995, Haag and Warren 1998). The microhabitat parameters that we examined in our study were aligned with these two characteristics. We measured water depth, velocities, dominant substrate, substrate penetrability, and cover type. We found that depth, velocity, and substrate penetrability were selected non-randomly by all four species tested (*E. complanata*, *A. heterodon*, *V. constricta*, and *F. masoni*). This further supports the findings of previous studies that demonstrated the importance of flow and substrate stability for freshwater mussel habitat.

In those studies that observed a correlation among mussels and microhabitat, mussel abundance, recruitment, and density were most often positively associated with slow to moderate flows and moderately coarse substrates with few fines (e.g., Salmon and Green 1983, Holland-Bartels 1990, McRae et al. 2004, Geist and Auerswald 2007). Measures of substrate compaction via penetrometer have been applied in a limited number of studies (Johnson and Brown 2000, Geist and Auerswald 2007), and those studies have shown that this microhabitat measure is relevant to mussel ecology. Sediment compaction was positively related to mussel abundance, but negatively affected recruitment (Johnson and Brown 2000, Geist and Auerswald 2007).

We found that the common mussel, *E. complanata*, exhibited non-random selectivity of all habitat parameters tested. However, suitability values for dominant substrate indicated that broad substrate suitability ranging in size from silt to large cobble. Other studies with *E. complanata* have found a similar broad tolerance of substrate types. In the coastal plain of the Apalachicola, Chattahoochee, and Flint River basins, the presence of *E. complanata* and *E. ictarina* were not correlated with substrate composition (Brim Box et al. 2002). A study of *E. complanata* in Virginia found no habitat characteristics that explained the mussels' clumped distribution (Balfour and Smock 1995). In a laboratory study, *E. complanata* most commonly occurred in muddy substrates, which differed from the sand and gravel that were most commonly occupied in their lake environment (Downing et al. 2000). These cumulative results concur to describe the wide niche breadth of *E. complanata* that is reflected in its ubiquitous distribution throughout eastern North America (Johnson 1970).

In our study, the federally endangered *A. heterodon* was most suited to slow flowing, shallow locations with fine to medium fine substrate. These results generally agree with habitat suitability criteria from the Delaware River suggesting moderately deep, slow-flowing water, and laboratory studies that confirm a preference for slow to moderate velocity (Michaelson and Neves 1995, Parasiewicz et al. 2012). Field and laboratory studies also suggest fine substrates are most suitable for *A. heterodon* (Strayer and Ralley 1993, Michaelson and Neves 1995). Empirically, the other federally endangered species in the Tar River basin, *E. steinstansana*, often occurs in fast-flowing, well-oxygenated water and relatively silt-free substrate composed of gravel or coarse sand (USFWS 1992). Our very limited data on *E. steinstansana* suggest a slow velocity with moderately compacted sand or coarse sand substrate.

The importance of microhabitat influence on mussel distribution may depend on the species (Huehner 1987, Brim Box et al. 2002). Minor microhabitat differentiation among species has been shown in some species (Salmon and Green 1983, Holland-Bartels 1990). In the Mississippi River, mussels occurred in a broad range of sediment types that indicated a general lack of species differences; the endangered *L. higginsii* was present in habitats similar to those as the most common species, *A. plicata* (Holland-Bartels 1990). Holland-Bartels (1990) found subtle differences in habitat dynamics among mussel species, however, and they could be broadly grouped into those preferring fine to medium fine sands and those that prefer coarser sands. Salmon and Green (1983) suggest that these slight microhabitat differences among species may explain niche partitioning that allows the coexistence of numerous mussel species within a single bed.

We found subtle and distinct among-species differences in habitat suitability distributions for the 10 species examined. There was evidence of some species occupying habitat non-randomly for specific parameters, whereas other species occupied habitat randomly for the same parameter. For instance, *A. heterodon* and *E. complanata* appeared to select mean velocity non-randomly whereas this was not true for *F. masoni* and *V. constricta*. We also found evidence of differences among species related to their conservation status. It is probable that conservation status serves as a proxy for niche breadth and degree of habitat specialization. Conservation status was positively related to the range of suitable habitats for a species, which suggests, as would be expected, that the rarest mussels have smaller microhabitat niches than ubiquitous species. However, our results also show that the most ubiquitous species, *E. complanata*, was the only one that demonstrated non-random habitat use for all habitat parameters.

Habitat suitability index models are a useful method for identifying environmental factors that may limit species occurrence, but these relationships are not necessarily causal and should be considered only as a premise for further investigation and management planning (Morrison et al. 1998). Absolute statements regarding the suitability of habitats are not recommended, but relative comparisons of suitabilities can be informative (Johnson 1980). Any habitat suitability study is constrained by the researcher's options and choice of available habitat (Johnson 1980), and suitable conditions that were not measured or present in the defined study area may exist. However, given a region with similar habitat characteristics, i.e., in coastal plain systems, our results represent a valid relative comparison of the suitability of a variety of habitat components (Johnson 1980).

Another consideration in the applicability of habitat suitability studies in general is that the use of habitat by an animal does not necessarily imply active selection, rather than an unmotivated presence (Johnson 1980, Beyer et al. 2010). In addition, substrate use by freshwater mussels is probably more complex than can be measured via simple microhabitat use (Layzer and Madison 1995). Mussels may require combinations of fine substrate materials for burrowing, and also coarser substrates to function as cover and velocity breaks (Layzer and Madison 1995). It is also possible that the apparently random habitat use measured by some parameters (mean velocity, substrate, and cover) for three species in our study was influenced by sample size. Brim Box and Mossa (1999) suggested that in some cases the lack of correlation between substrate and freshwater mussel distribution or abundance may be due to an inadequate sampling effort. In our study, *E. complanata* had the largest sample size, and non-random habitat use was exhibited for all six measured habitat variables. The species with fewer microhabitat use measurements exhibited both random and non-random use of habitat according to parameter. This may be due to the lack of statistical power in these samples, or it may be due to an actual ecological difference among species. We would not expect a ubiquitous species to occupy habitat non-randomly, whereas the rarer species appear to be generalists that use some habitat randomly, but it is possible. The complications arising from the limited number of rare mussels encountered during habitat use surveys is a common problem when working with rare species (Brim Box et al. 2002). Our ability to detect and measure microhabitat use for representative numbers of rare species was limited, even with intensive sampling effort. The typical level of effort applied in timed search mussel assemblage surveys in streams is 1.5 person-hours per site (Metcalf-Smith et

al. 2000), yet we expended 6.0 person-hours at each sampling site, suggesting that our low sample sizes for some species reflect actual low site densities, rather than low detection probability. This was particularly the case with the federally endangered *E. steinstansana*, of which we found only three individuals. This highlights the difficulty of studying the rarest species; it is often very difficult to collect information on the species in greatest need of conservation and in which we are most interested. Future studies that aim to characterize the microhabitat of rare species would be enhanced by developing sampling designs and methods that will allow these species to be sampled more frequently (Brim Box et al. 2002).

Studies of microhabitat requirements for freshwater mussels tend to overlook the requirements of juvenile mussels (Holland-Bartels 1990, Layzer and Madison 1995). The measurements made for adults are probably not transferable to juveniles and therefore do not reflect recruitment and early life stage microhabitat needs (Layzer and Madison 1995). It has been observed that juvenile and adult mussels occupy different microhabitats despite occurring in the same bed (Geist and Auerswald 2007). Geist and Auerswald (2007) suggested that substrate measures are an important, and perhaps the only, measurable habitat factor in juvenile mussel distribution.

The ability to quantify the characteristics of suitable habitat distinguishes habitat suitability indices from anecdotal descriptions of microhabitats (Bovee 1986). The habitat suitability method adopted in our study empirically measured habitat use and availability independently for each site, thus allowing the relative selectivity for habitats to be quantified (Bovee 1986). Microhabitat characteristics are simple to measure and can be quickly assessed in the field, making habitat suitability a useful tool in practical applications, if not in

predictive modeling exercises. This knowledge can be useful in targeting field surveys for rare species (Midway et al. 2010), identification of relocation sites for imperiled species (Fisk et al. 2014), or for the planning of conservation measures, including site restoration (Quinn and Kwak 2000). Microhabitat is one in a suite of variables to be considered, it does not limit distribution on its own, but neither is it inconsequential. Particularly in small systems where larger-scale attributes are more static, microhabitat characteristics can be the best option for evaluating habitat suitability (Johnson and Brown 2000). It appears that no one scale and approach of habitat assessment may adequately describe the ecological relationships between freshwater mussel populations and their environment.

Habitat degradation is among the most prominent threats facing freshwater mussels, and the habitat requirements of mussels must be understood to develop the best spatial scale and specific conservation practices to protect them from future degradation. It is particularly crucial to understand the habitat requirements of juvenile mussels and the dynamics of habitat conditions that influence recruitment. The assessment of microhabitat can be useful in quantifying suitable and optimal habitat to guide conservation strategies and management plans for endangered mussel species (Johnson and Brown 2000). Microhabitat preferences are already being used to relocate the endangered *Margaritifera hembeli* to suitable sites when their beds are threatened by channel alterations (Johnson and Brown 2000). Habitat suitability criteria such as those we developed for 10 species can similarly be used to target habitat protection, mussel relocations, reintroductions, or site restorations within acceptable macrohabitats.

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Table 1. Classification of substrate, cover type, and substrate penetrability for habitat use and availability analyses.

Covariate	Value	Abbreviation/ Index
<i>Substrate</i>		
	<i>mm</i>	
Silt-clay	<0.062	Silt
Sand	0.062-1	Sand
Very coarse sand	1-2	VCS
Pea gravel	2-4	PG
Fine gravel	4-8	FG
Medium gravel	8-16	MG
Coarse gravel	16-32	CG
Very coarse gravel	32-64	VCG
Small cobble	64-130	SC
Large cobble	130-250	LC
Small boulder	250-500	SB
Medium boulder	500-1,000	MB
Large boulder	1,000-2,000	LB
Very large boulder	2,000-4,000	VLB
Mammoth boulder/bedrock	>4,000	Bedrk
<i>Cover Type</i>		
Coarse woody debris		CWD
Fine woody debris		FWD
Vegetation		Veg
Roots		Roots
Undercut bank		Bank
Small boulder		SB
Medium boulder		MB
Very large boulder		VLB
Mammoth boulder/bedrock		Bedrk
Tire, trash, misc.		Other
<i>Substrate Penetrability</i>		
	<i>Force pounds</i>	
(highest penetrability,	3.57	1
lowest compaction)	4.64	2
	5.72	3
	6.79	4
	7.86	5
	8.94	6
	10.01	7
	11.09	8
	12.16	9
	13.24	10
	14.31	11
	15.39	12
	16.46	13
	17.54	14
	18.61	15
	19.68	16
	20.76	17
	21.83	18
(lowest penetrability,	22.91	19
most compaction)	23.98	20

Table 2. Count (*N*), mean, range, and standard deviation of microhabitat use measurements of depth (m), bottom and mean-column velocity (m/s), substrate penetrability, dominant substrate, and cover type for 10 species of freshwater mussel.

Species	<i>N</i>		Depth (m)		Bottom Velocity (m/s)		Mean Velocity (m/s)		Substrate Penetrability (Index)		Dominant Substrate		Closest Cover	
	indivs	sites	Mean (Range)	SD	Mean (Range)	SD	Mean (Range)	SD	Mean (Range)	SD	Mode	Types	Mode	Types
<i>Alasmidonta heterodon</i>	19	2	0.26 (0.08 - 0.55)	0.11575	0.003 (0.00 - 0.02)	0.00671	0.004 (0.00 - 0.02)	0.00607	0.11 (0 - 1.5)	0.35664	VCS, PG	6	CWD	4
<i>Elliptio complanata</i>	357	20	0.38 (0.02 - 1.36)	0.23787	0.019 (0.00 - 0.31)	0.04621	0.047 (0.00 - 0.64)	0.08435	3.42 (0 - 20)	4.92669	Silt	14	CWD	9
<i>Elliptio congaraea</i>	22	3	0.53 (0.24 - 0.80)	0.17154	0.055 (0.00 - 0.43)	0.10098	0.074 (0.00 - 0.56)	0.12963	6.98 (0 - 18.5)	5.93512	Sand	9	CWD	5
<i>Elliptio fisheriana</i>	11	2	0.30 (0.10 - 0.53)	0.14887	0.017 (0.00 - 0.04)	0.01421	0.033 (0.00 - 0.06)	0.01902	8.36 (0 - 20)	7.50364	Sand	6	CWD	5
<i>Elliptio icterina</i>	62	7	0.43 (0.06 - 1.02)	0.24025	0.016 (0.00 - 0.31)	0.04639	0.051 (0.00 - 0.52)	0.07926	5.60 (0 - 20)	5.84088	Sand	11	CWD	7
<i>Elliptio roanokensis</i>	13	2	0.52 (0.14 - 1.19)	0.33321	0.050 (0.00 - 0.18)	0.05477	0.151 (0.00 - 0.32)	0.10332	3.42 (0 - 16)	4.6451	Sand, CG	5	CWD	3
<i>Elliptio steinstansana</i>	3	2	0.47 (0.13 - 0.65)	0.29195	0.013 (0.00 - 0.04)	0.02309	0.023 (0.00 - 0.07)	0.04041	11.67 (7 - 14.5)	4.07226	Sand	2	CWD	2
<i>Fusconaia masoni</i>	14	2	0.54 (0.41 - 0.69)	0.08389	0.036 (0.00 - 0.14)	0.05761	0.080 (0.00 - 0.30)	0.10997	9.29 (0 - 14.5)	4.07957	Sand	5	CWD	4
<i>Lampsilis</i> sp.	5	1	0.23 (0.07 - 0.40)	0.1253	0.000 (0.00 - 0.00)	0	0.004 (0.00 - 0.01)	0.00548	NA	NA	NA	5	CWD	3
<i>Villosa constricta</i>	24	2	0.48 (0.05 - 0.86)	0.24871	0.001 (0.00 - 0.02)	0.00408	0.010 (0.00 - 0.05)	0.01601	6.98 (0 - 13)	4.92438	Sand	8	FWD	4

Table 3. Results from a two-sample Kolmogorov-Smirnov test of the difference between microhabitat use and microhabitat availability distributions. Statistically significant results ($p < 0.05$, in bold font) indicate non-random use of habitat.

Species	Depth (m/s)		Bottom Velocity (m/s)		Mean Velocity (m/s)		Substrate Penetrability (Index)		Dominant Substrate		Closest Cover	
	<i>D</i> -statistic	<i>p</i> -value	<i>D</i> -statistic	<i>p</i> -value	<i>D</i> -statistic	<i>p</i> -value	<i>D</i> -statistic	<i>p</i> -value	<i>D</i> -statistic	<i>p</i> -value	<i>D</i> -statistic	<i>p</i> -value
<i>Alasmidonta heterodon</i>	0.579	0.001	0.833	<0.001	0.667	0.018	0.850	<0.001	0.364	0.328	0.500	0.214
<i>Elliptio complanata</i>	0.478	<0.001	0.541	<0.001	0.480	<0.001	0.498	<0.001	0.230	<0.001	0.202	0.010
<i>Fusconaia masoni</i>	0.750	<0.001	0.571	0.010	0.353	0.124	0.703	<0.001	0.708	<0.001	0.688	0.001
<i>Villosa constricta</i>	0.524	0.004	0.800	0.002	0.444	0.256	0.588	<0.001	0.300	0.229	0.364	0.365

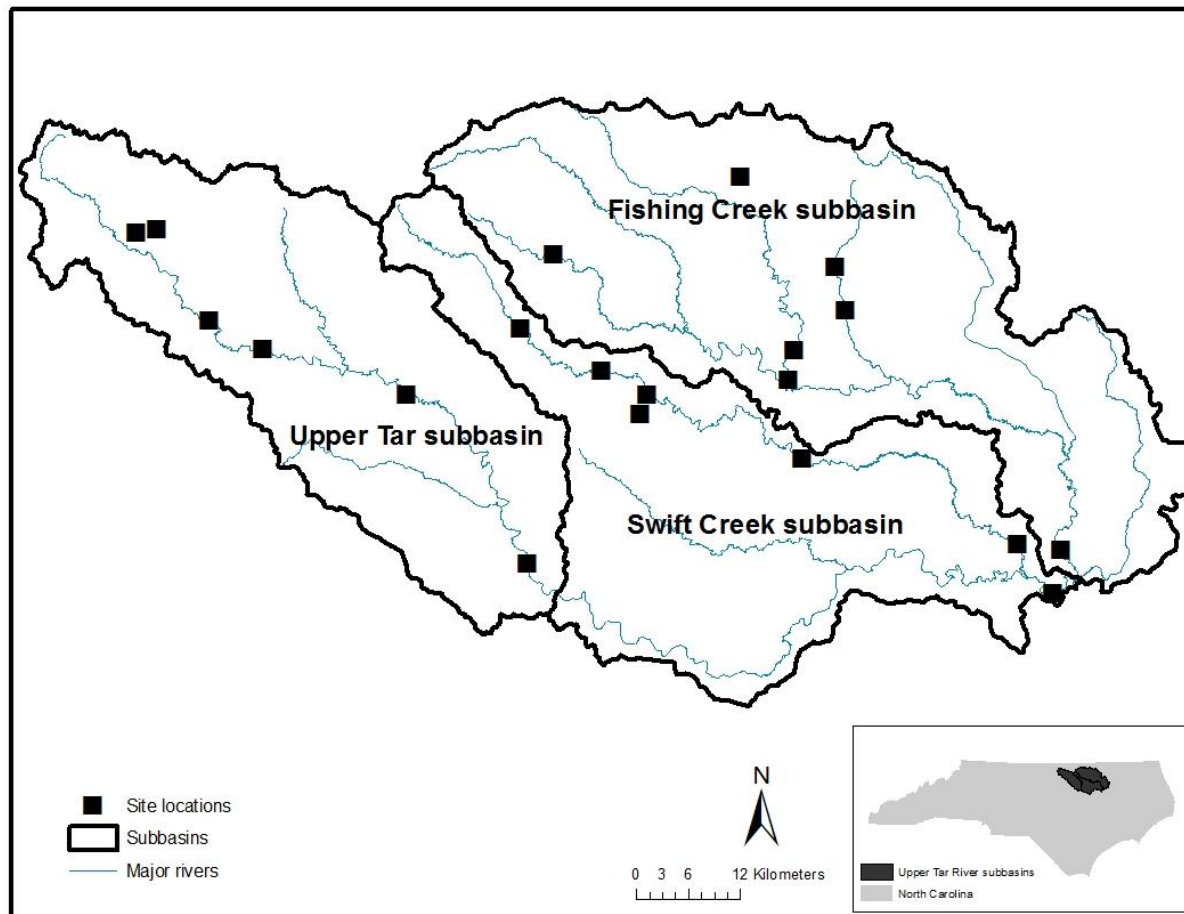


Figure 1. Locations of 20 mussel and habitat survey sites in the Tar River basin, North Carolina.

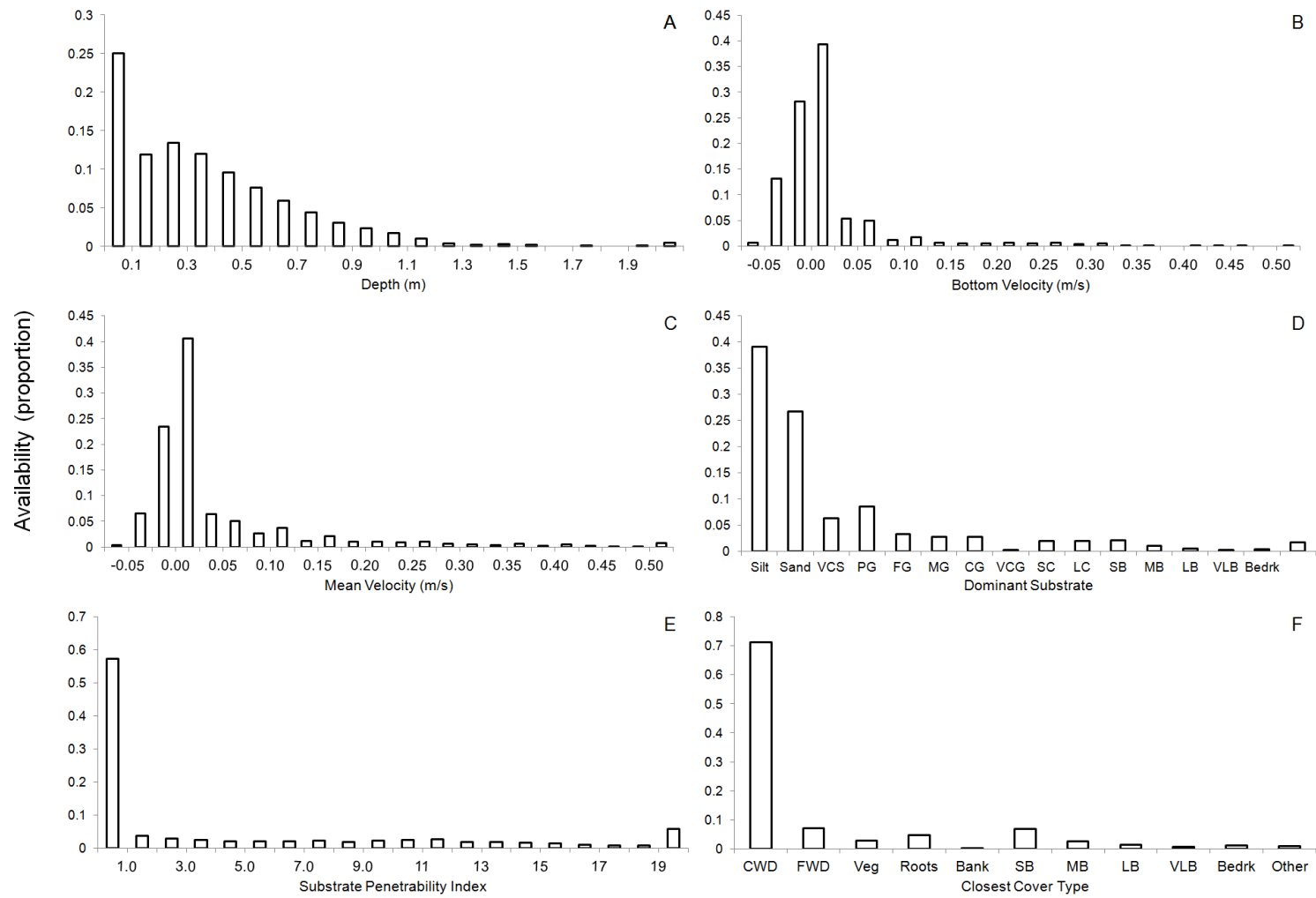


Figure 2. Availability of six microhabitat parameters from 20 sites in the Tar River basin, North Carolina.

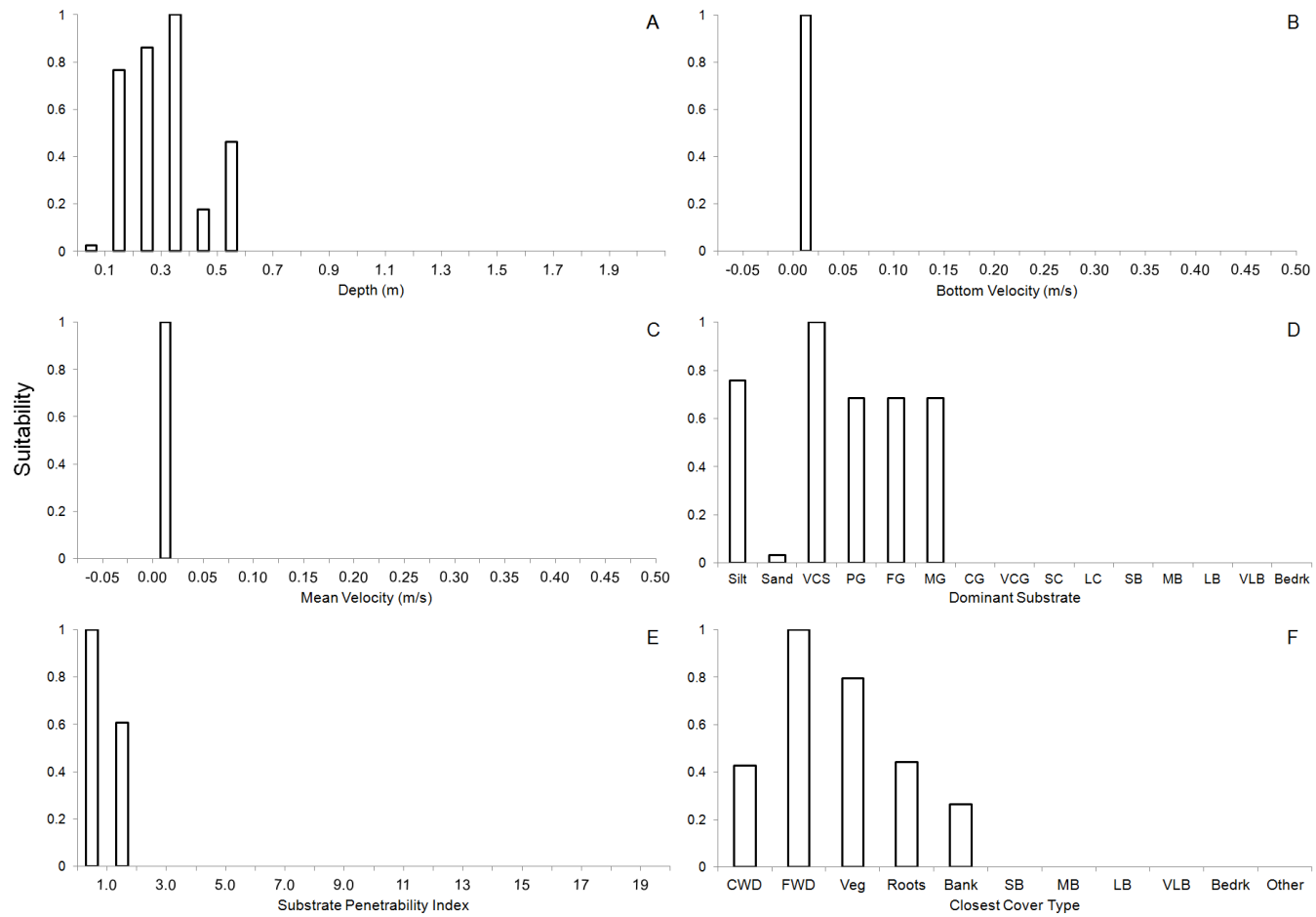


Figure 3. Microhabitat suitability for *Alasmidonta heterodon*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

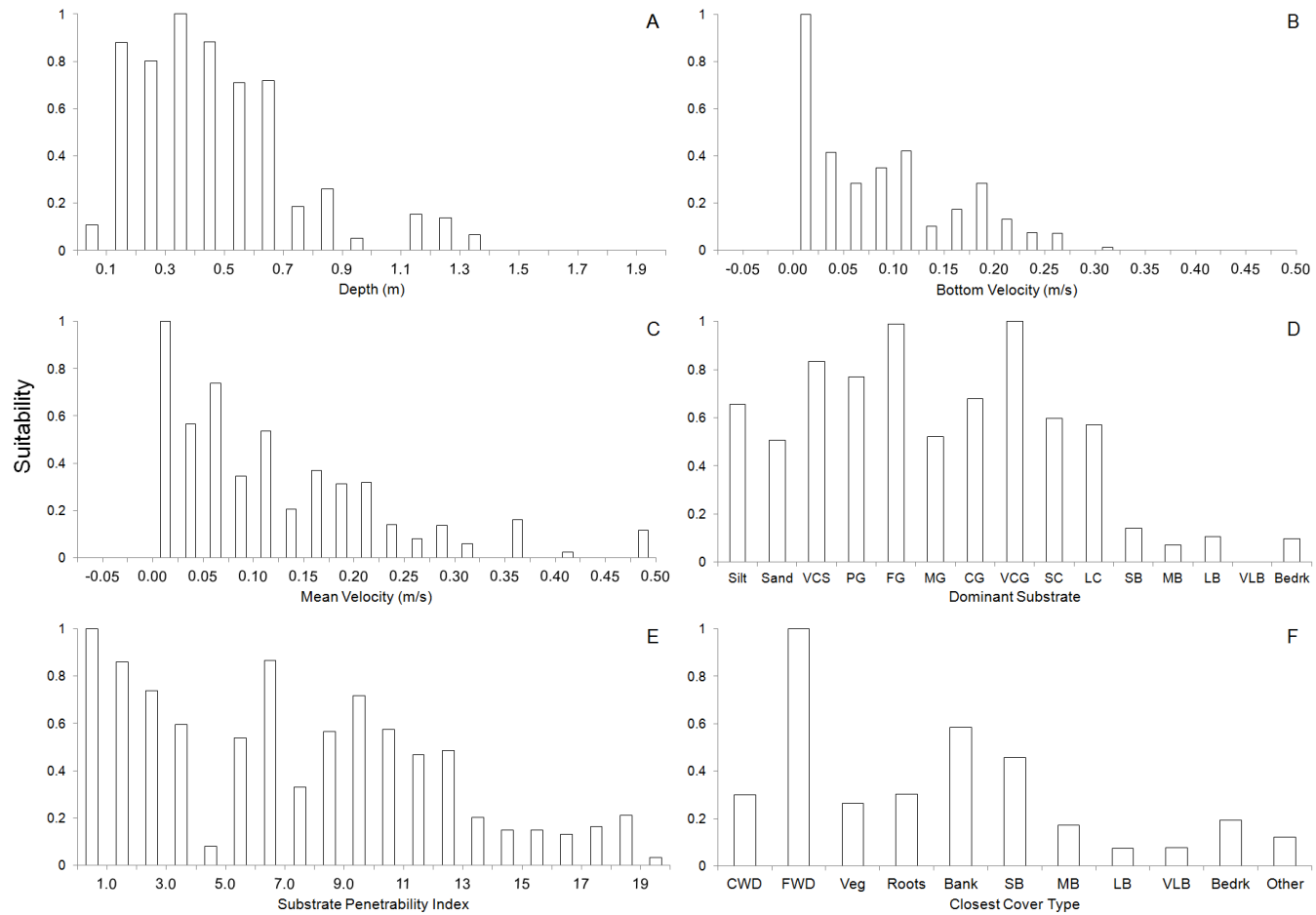


Figure 4. Microhabitat suitability for *Elliptio complanata*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

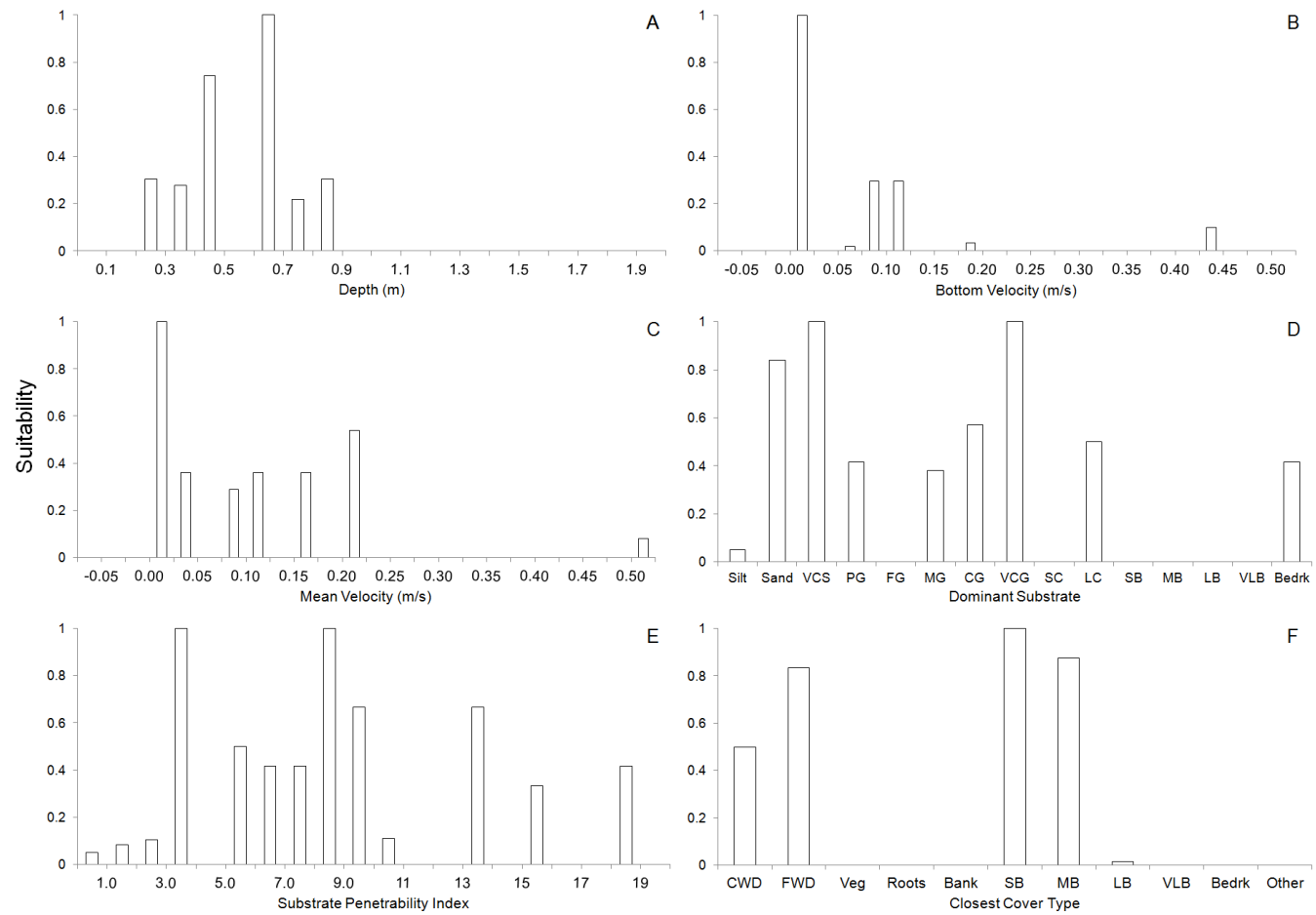


Figure 5. Microhabitat suitability for *Elliptio congaraea*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

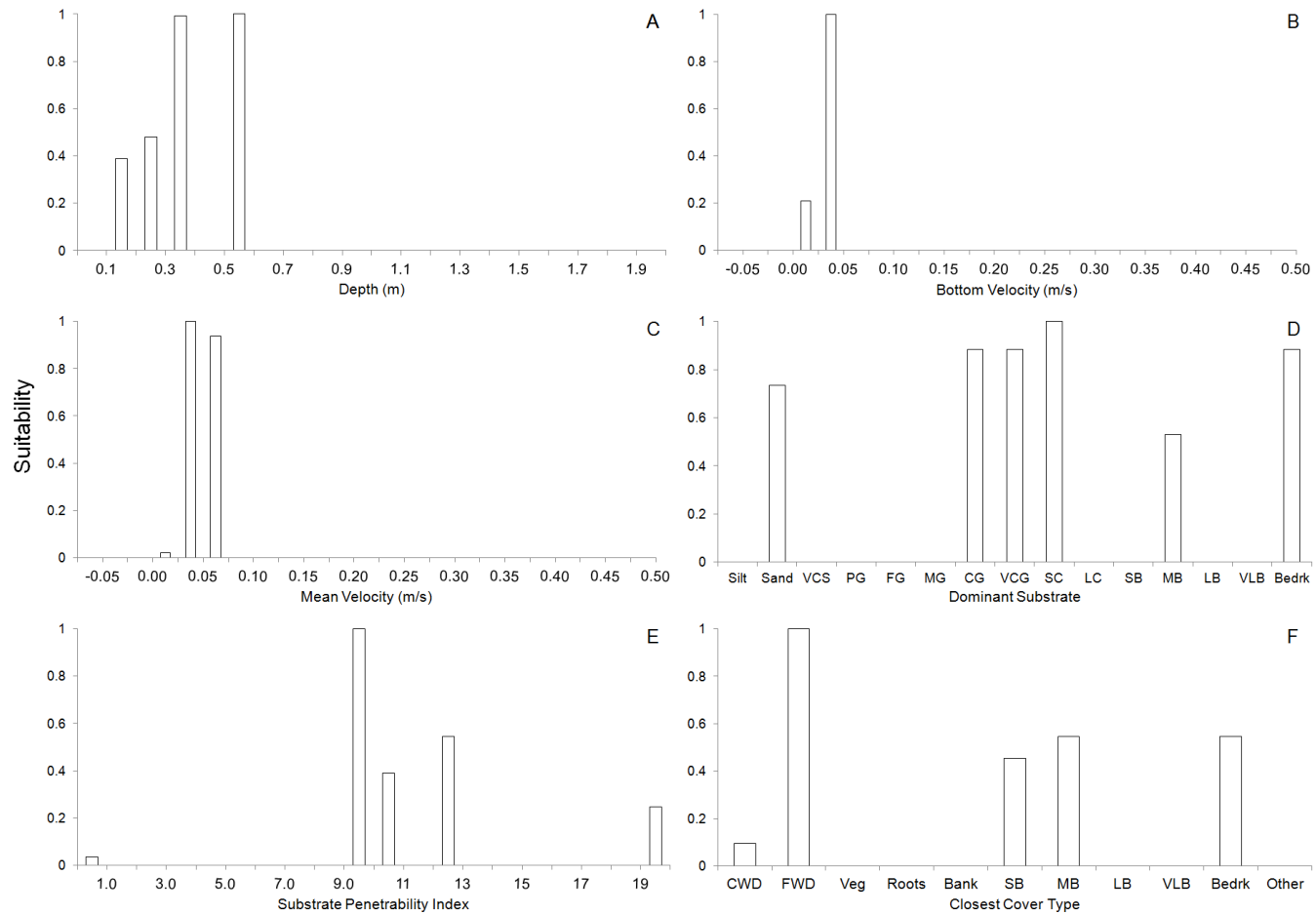


Figure 6. Microhabitat suitability for *Elliptio fisheriana*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

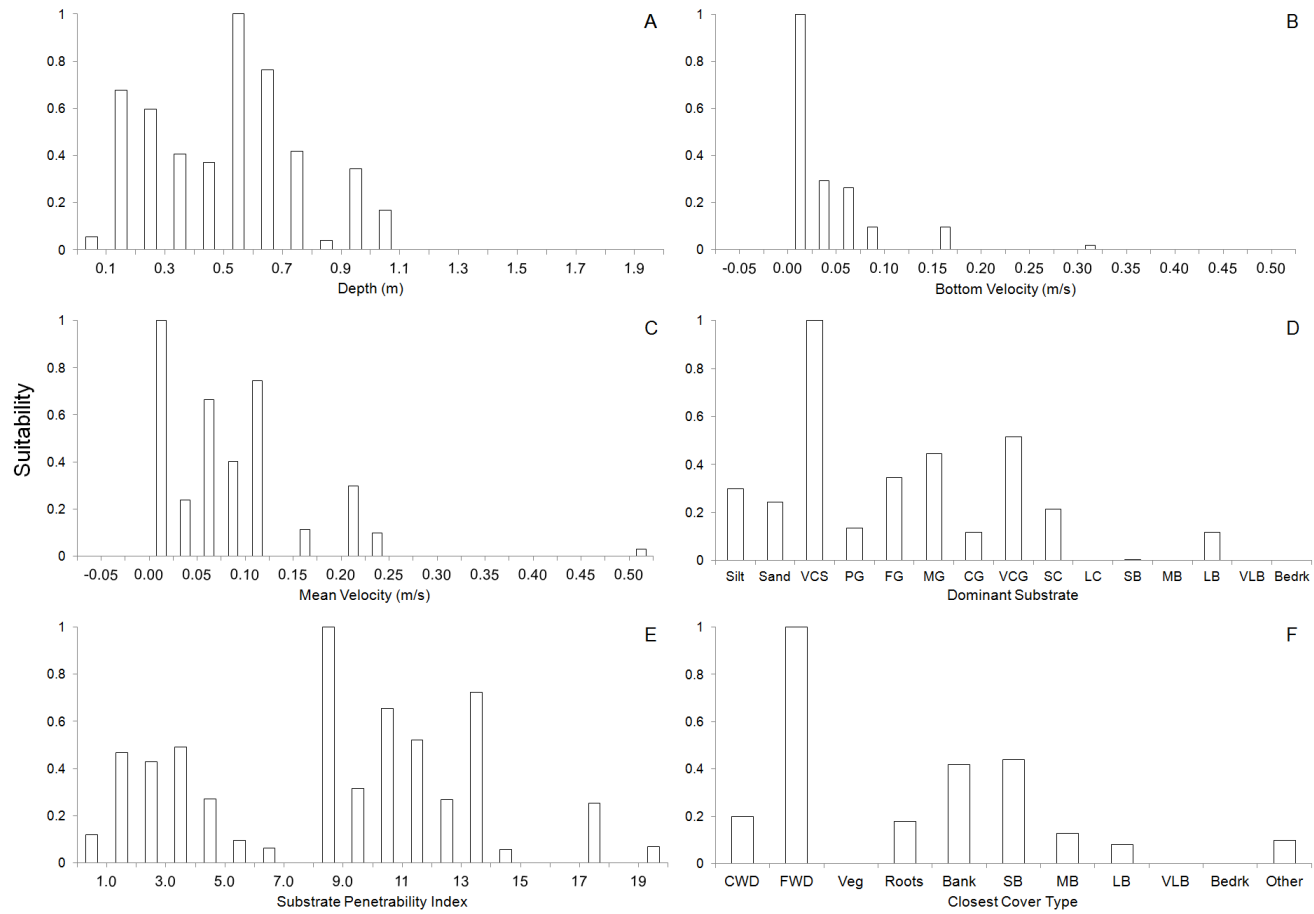


Figure 7. Microhabitat suitability for *Elliptio icterina*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

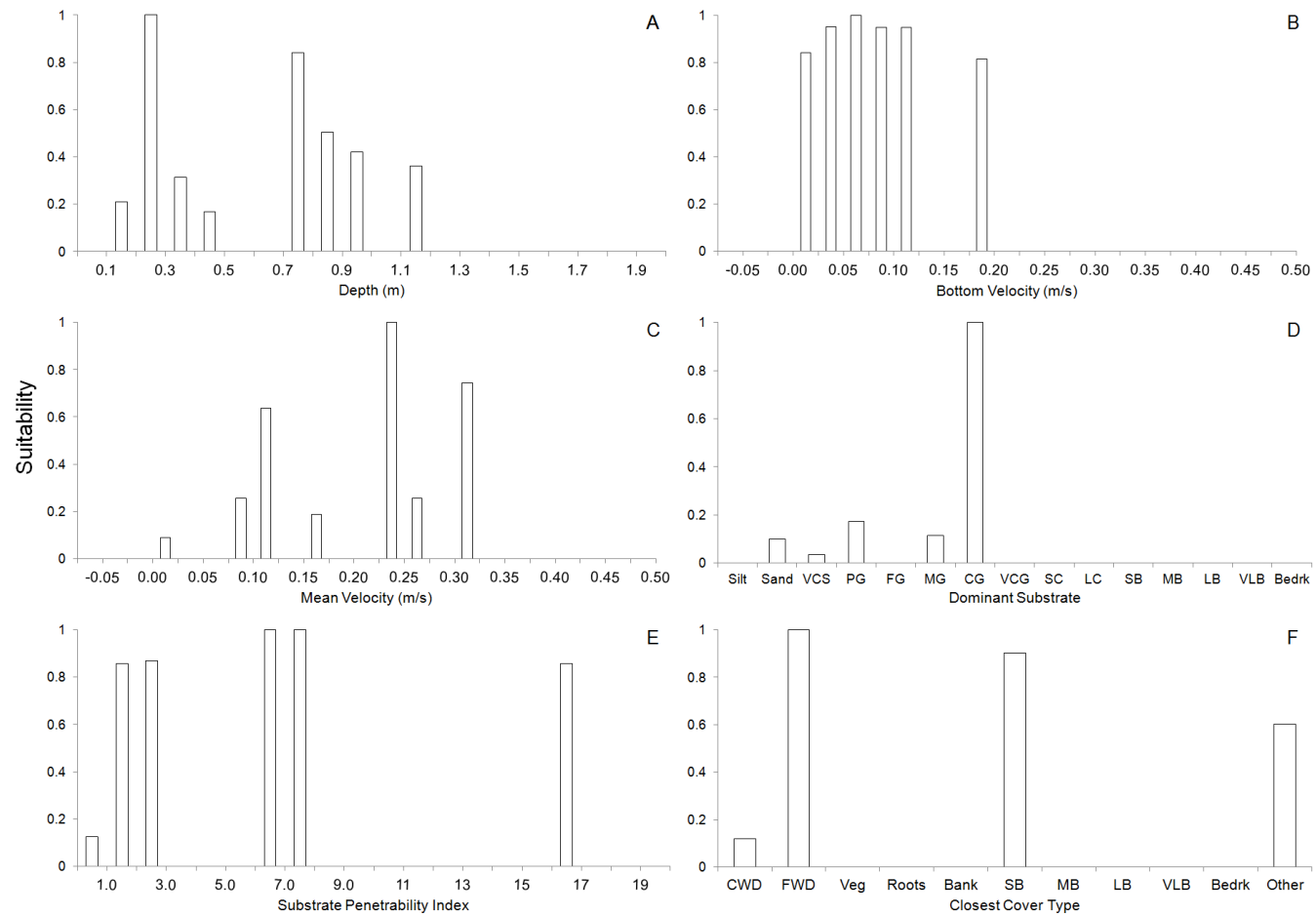


Figure 8. Microhabitat suitability for *Elliptio roanokensis*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

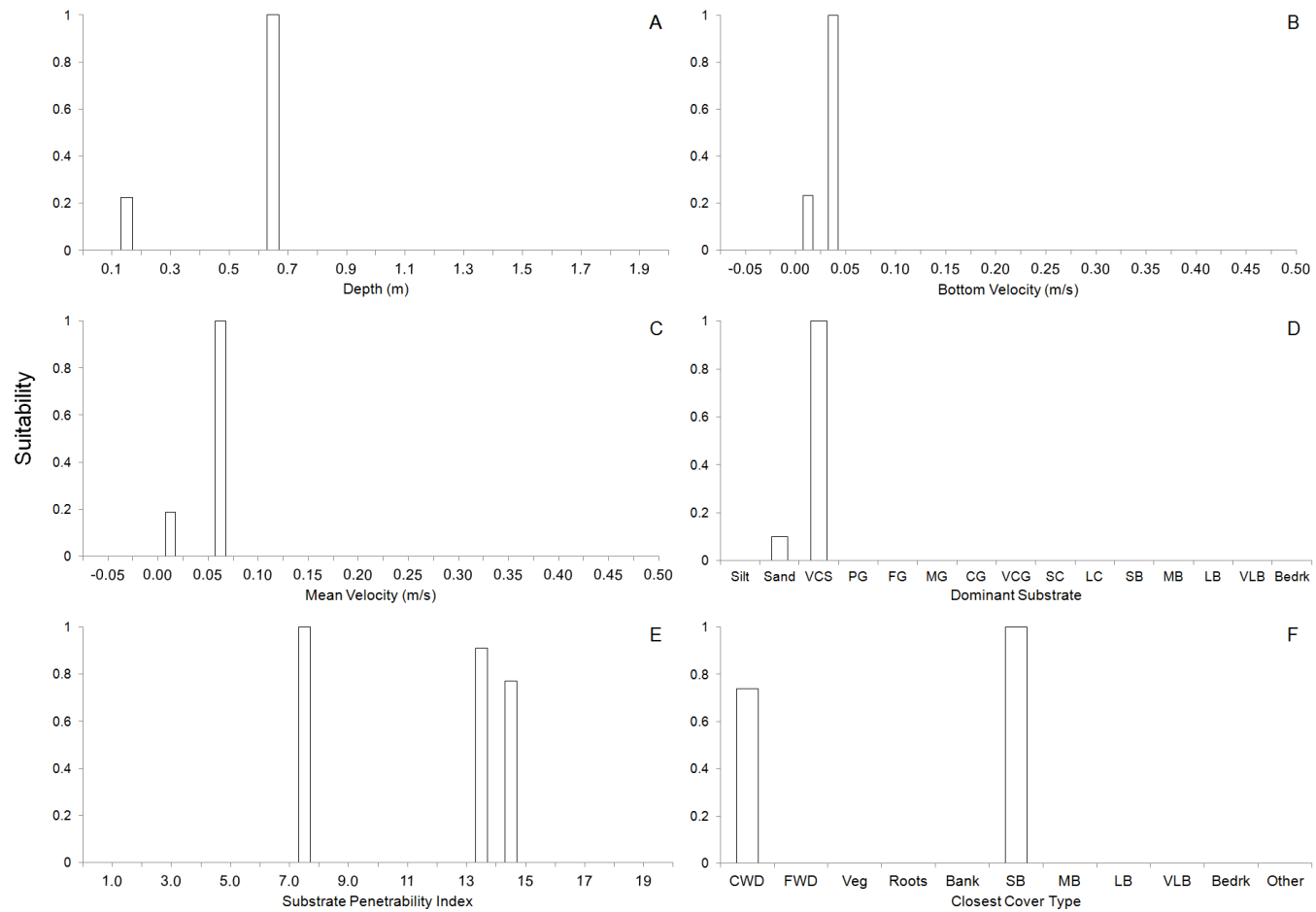


Figure 9. Microhabitat suitability for *Elliptio steinstansana*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

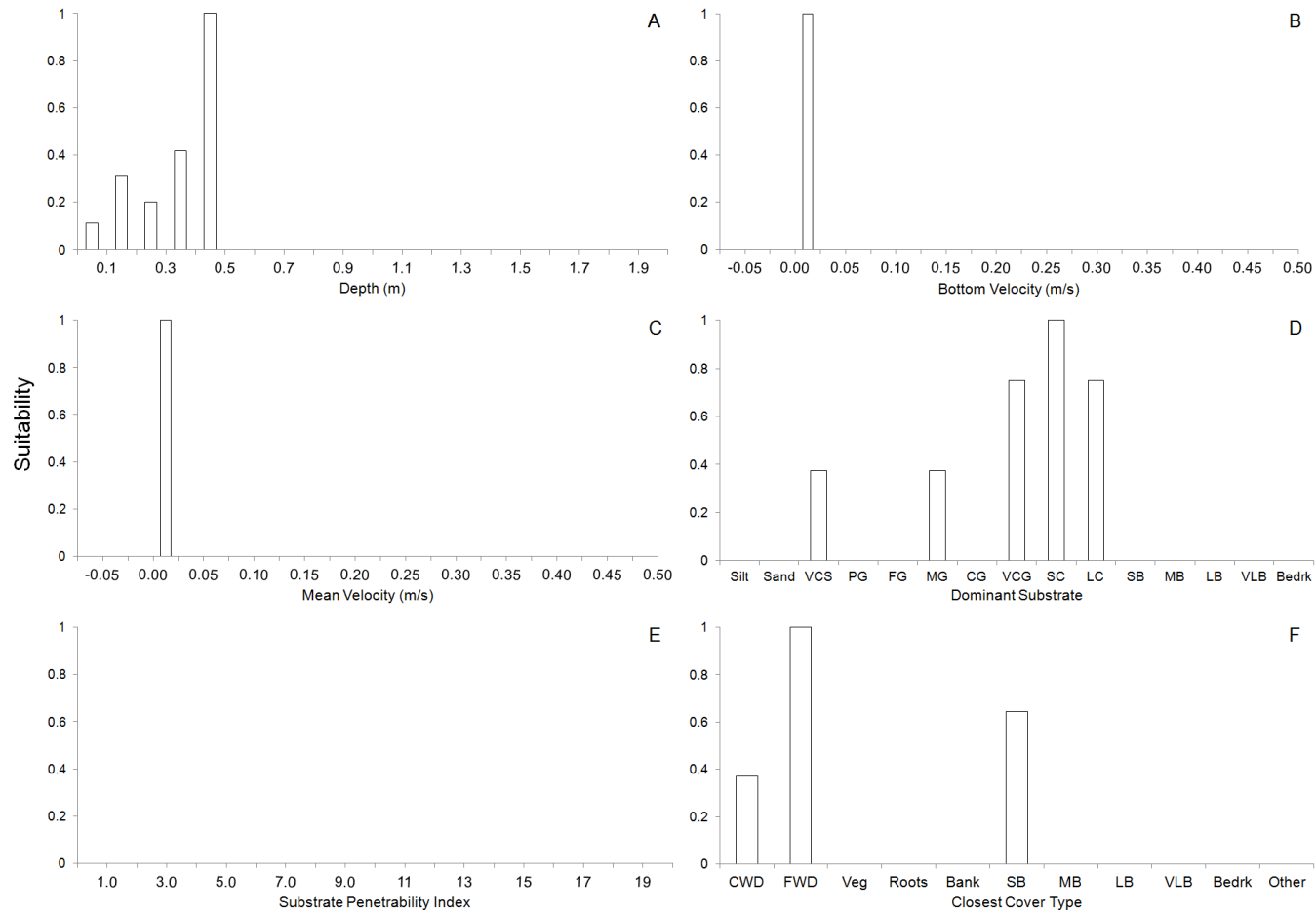


Figure 10. Microhabitat suitability for an undescribed *Lampsilis*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

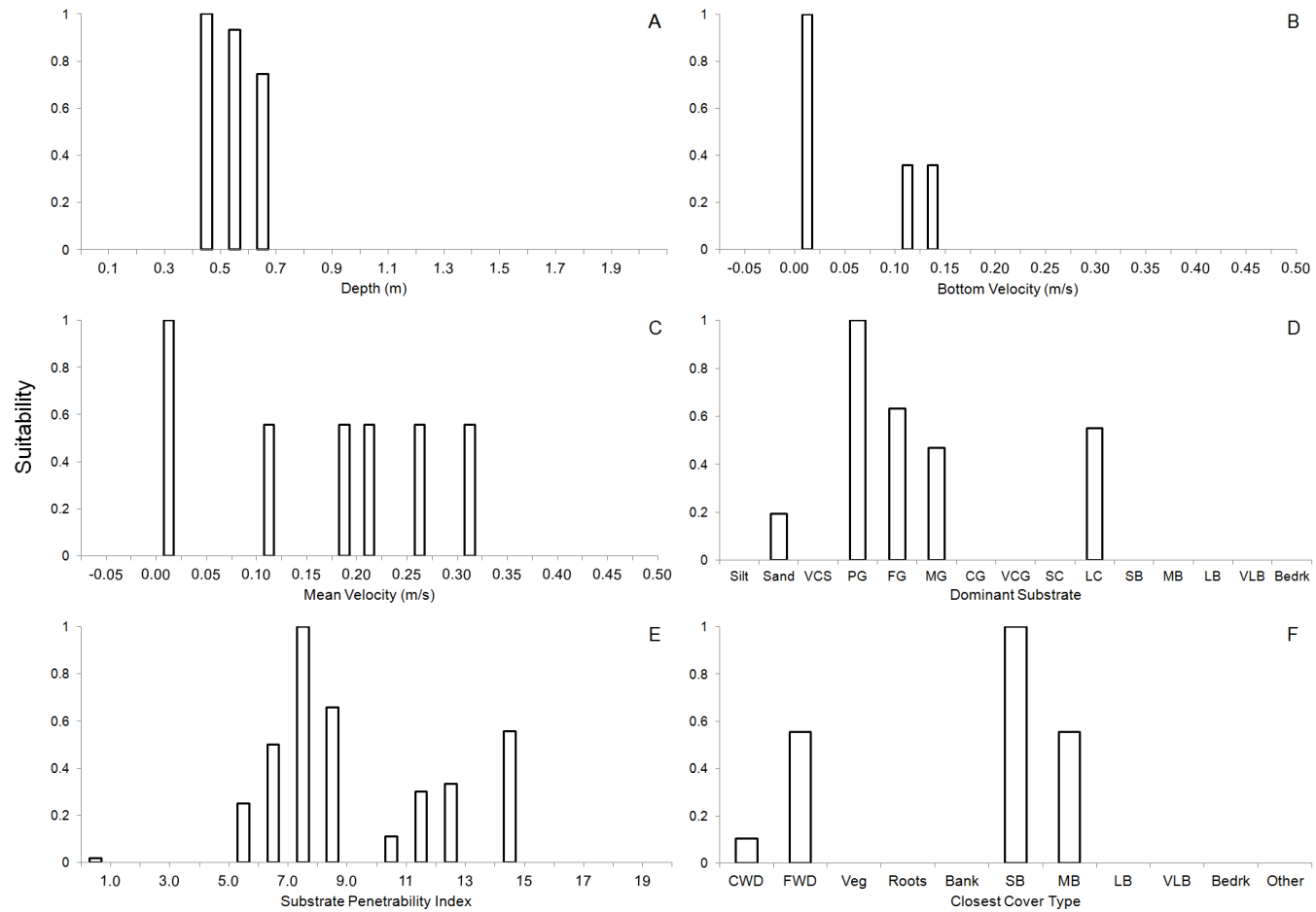


Figure 11. Microhabitat suitability for *Fusconaia masoni*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

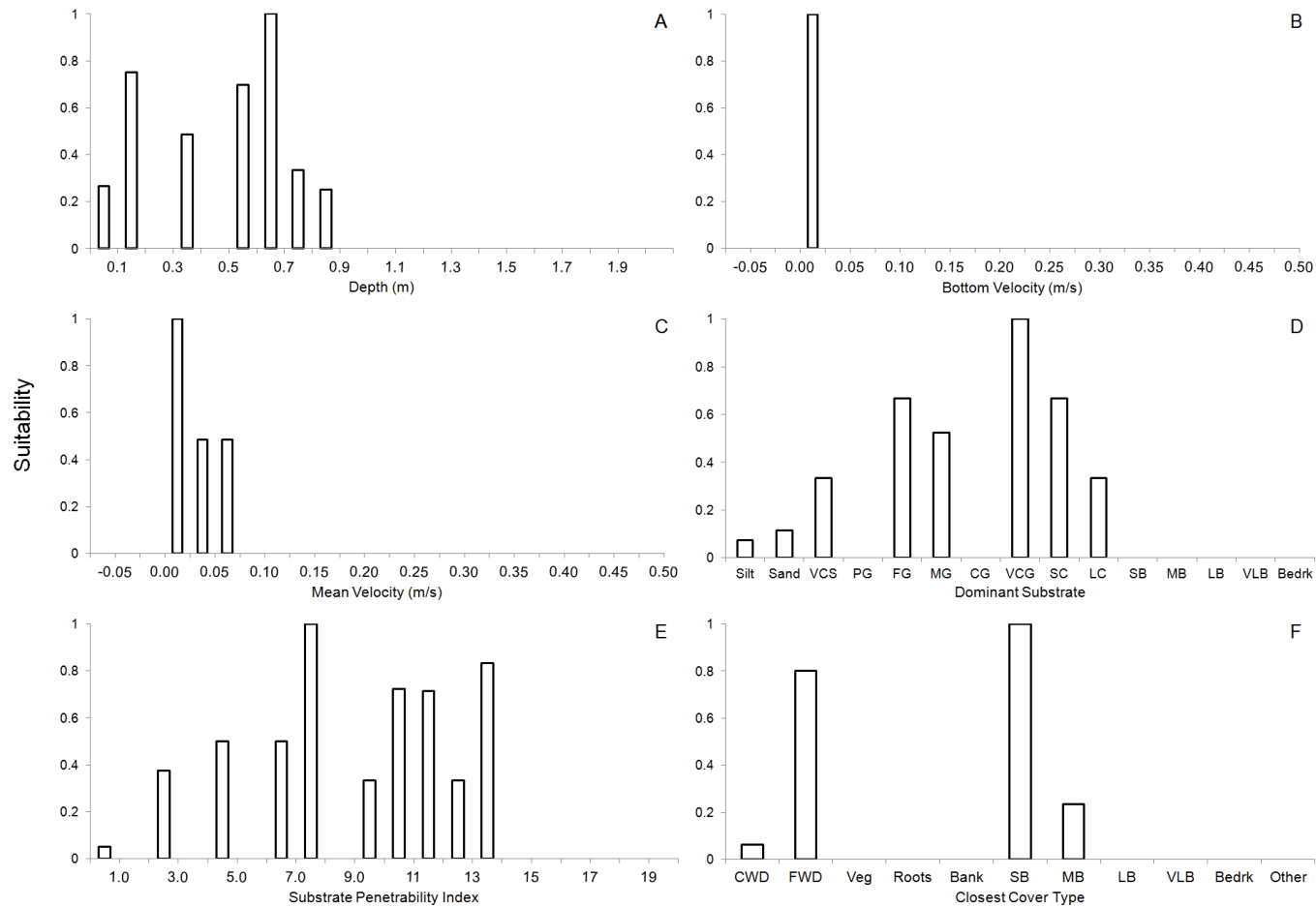


Figure 12. Microhabitat suitability for *Villosa constricta*. Suitabilities for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

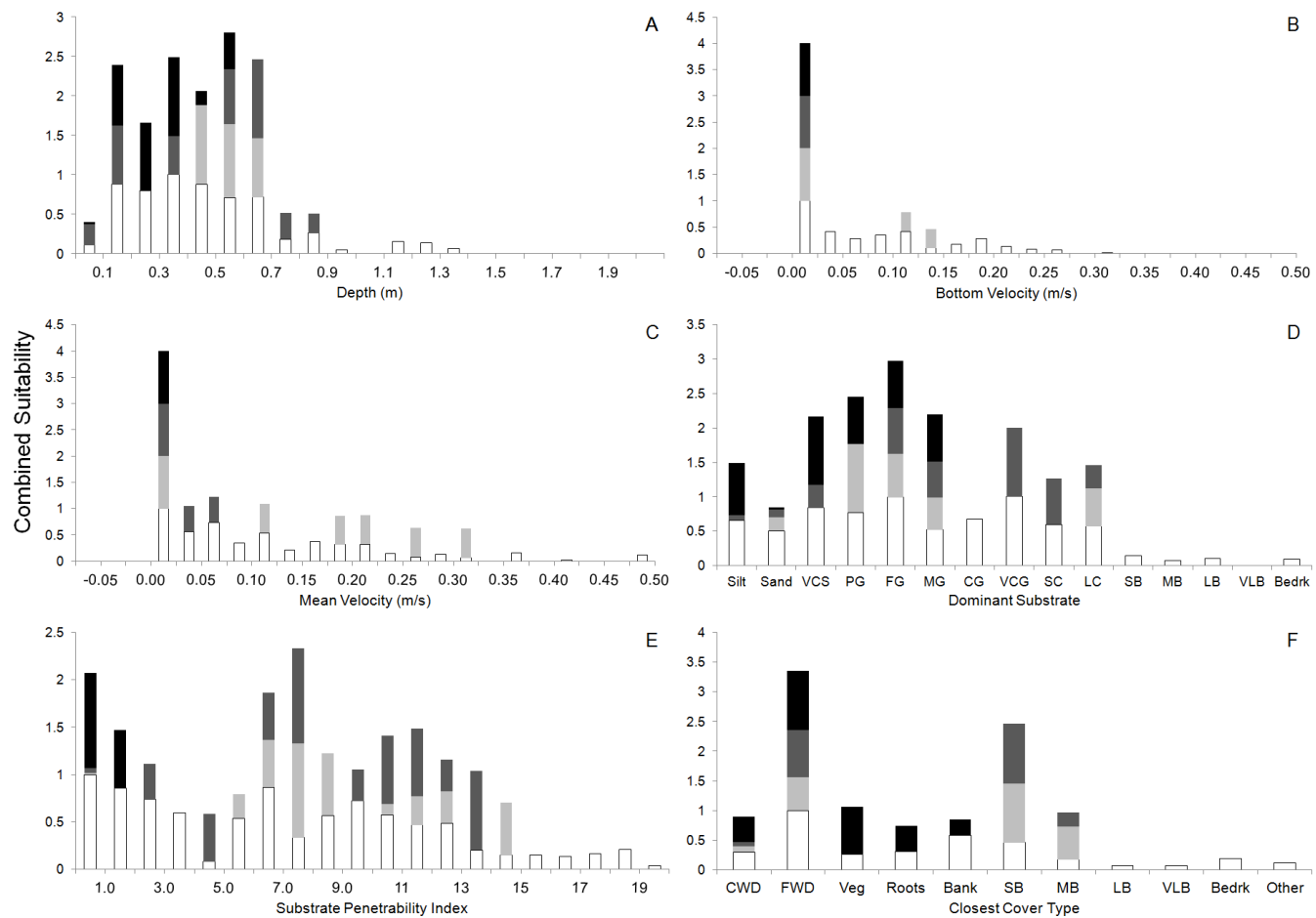


Figure 13. Habitat suitabilities for four freshwater mussel species with different conservation status: federally endangered *Alasmidonta heterodon* (black), North Carolina state endangered *Fusconaia masoni* (light gray), North Carolina state special concern *Villosa constricta* (dark gray), and stable *Elliptio complanata* (white). Combined suitability is for relative comparison only.