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Variation in microhabitat use of the threatened spotfin chub (*Erimonax monachus*) among stream sites and seasons

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Abstract – The spotfin chub *Erimonax monachus* is a rare cyprinid fish endemic to the Tennessee River drainage, and it is federally listed as threatened in the USA. Microhabitat use of spotfin chubs was studied via stream snorkelling in the Emory River watershed, Tennessee, one of the last remaining populations of this species. We used a Bayesian generalised linear mixed model to evaluate microhabitat covariates related to the presence/absence of spotfin chubs among eight stream sites across three seasons (early summer, late summer and fall). In general, spotfin chubs were more likely to be present in microhabitats characterised by boulder/bedrock substrates, medium to high velocity, and medium depth (typical of the run habitat). However, the patterns were not necessarily consistent among seasons or stream sites, due partly to interactions between microhabitat and macrohabitat covariates. Specifically, spotfin chubs were more selective of bedrock and boulder substrate at smaller stream sites where they were less abundant, and they were more selective of higher velocity at warmer stream temperatures (early and late summer). Our data indicate that spotfin chubs may exhibit flexible microhabitat use to some extent, and their microhabitat use may differ by macrohabitat characteristics such as stream size and water temperature. This study provided a refined understanding of microhabitat use of spotfin chubs and suggests that effective conservation of this declining species should identify and protect available suitable habitat across space and time.

Key words: microhabitat use; spotfin chub; Tennessee; hierarchical; streams; conservation

Introduction

Stream fish select microhabitat locations based on various features such as depth, velocity and substrate (e.g., Labonne et al. 2003; Hazelton & Grossman 2009; Midway et al. 2010). Identifying the multidimensional microhabitat niche of stream fish is required for assessing their stream habitat and flow requirements (Ahmadi-Nedushan et al. 2006; Petts 2009). Such research is valuable as many stream fishes continue to decline in their distribution and abundance (Jelks et al. 2008).

Microhabitat use of stream fish often changes seasonally and spatially. Temperate streams are characterised by distinct seasonality (e.g., stream discharge and temperature). Such seasonality affects the availability of habitat and food, energetic requirements and predation risk for stream fish, which respond actively or passively by shifting their microhabitat locations (Grossman & Ratajczak 1998; David & Closs 2003; Labonne et al. 2003; Gillette et al. 2006). Lotic habitat also changes spatially in key broad-scale habitat features (e.g., stream size), but many stream fishes occupy a range of habitat conditions that exist longitudinally. Spatial heterogeneity in broad-scale habitat templates may interact with microhabitat selection of stream fish, resulting in frequently observed spatial variation in microhabitat use (Heggenes 2002;

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Labonne et al. 2003; Copp & Vilizzi 2004). Accordingly, studies of microhabitat use of stream fish should aim to quantify ecological patterns over space and time.

Hierarchical or mixed models provide a useful framework to quantify variation in microhabitat use among stream sites and seasons. They accommodate and quantify inherent variability among groups (i.e., stream sites and seasons), while accounting for the overall pattern across groups (Wagner et al. 2006; Gelman & Hill 2007; Zuur et al. 2009). Plus, group-level covariates (i.e., factors that change seasonally and spatially such as stream discharge and temperature) can be included in the model to assess their influence on the effect size of microhabitat-level covariates (e.g., stream depth and velocity). Thus, these models are suitable for quantifying the hierarchical organisation of stream habitats (Fausch et al. 2002; Lowe et al. 2006) and their interactions across scales (Deschênes & Rodríguez 2007; Ebersole et al. 2009).

The spotfin chub (*Erimonax monachus*) is a small, rare cyprinid endemic to the Tennessee River drainage, USA (Jenkins & Burkhead 1984). It is typically found in clear, warmwater streams or rivers with moderate gradients. The species is historically known from five states: Alabama, Georgia, North Carolina, Tennessee and Virginia. Today, spotfin chub populations are considered extirpated in two states, and they are restricted to only few tributary systems in North Carolina (Little Tennessee River), Tennessee (Emory, Buffalo and Holston rivers) and Virginia (North and Middle Fork Holston rivers) (Etnier & Starnes 1993; Jenkins & Burkhead 1994; Boschung & Mayden 2004). Spotfin chubs were federally listed as threatened in 1977 under the U.S. Endangered Species Act, and it is considered endangered in all three states that harbour remaining populations.

The declining population status of spotfin chub has led to conservation and reintroduction efforts in several rivers within the species' historical range, but ecological information that can guide species recovery is limited for spotfin chubs. Adults measure 55-90 mm in standard body length (Jenkins & Burkhead 1994), and sexual maturity is attained in 1–2 years (Boschung & Mayden 2004). Spawning occurs between May and August, and this crevice-spawning minnow utilises narrow slots and fissures on boulder and bedrock substrate (Jenkins & Burkhead 1984, 1994). Spotfin chubs are sight-feeding insectivores, preying predominantly on immature benthic aquatic insects (Jenkins & Burkhead 1984). General habitat descriptions are available in regional ichthyological references (Etnier & Starnes 1993; Jenkins & Burkhead 1994), and macro-habitat use was quantified by Russ (2006): spotfin chub selected run habitats over boulder and bedrock substrates from spring to fall, but few winter observations indicated that they shifted to pool habitats over sand substrate. Microhabitat use of spotfin chubs has not been previously quantified to our knowledge.

The goal of this study was to quantify microhabitat use of spotfin chub among eight stream sites and three seasons (early summer, late summer and fall) in the Emory River watershed, Tennessee, USA, one of the last remaining populations of this species. Present and absent microhabitats of the species were identified via snorkelling observations, and an array of microhabitat characteristics were measured (e.g., stream depth, velocity and substrate). A Bayesian generalised linear mixed model was used to quantify variation in microhabitat use among stream sites and seasons, and assess the effect of macrohabitat features (stream size and temperature) on the expression of microhabitat use. In addition to gaining ecological knowledge of this rare stream fish, this study was motivated by the need to inform conservation and management of aquatic resources in the study area. In particular, a multispecies Habitat Conservation Plan (HCP), a provision under the authority of the U.S. Endangered Species Act, is in the process of development to balance environmental conservation and land development in this rapidly growing region. The spotfin chub is one of the target species that should benefit from the implementation of the multispecies HCP, but effective conservation actions need to be based on the best available scientific knowledge.

Materials and methods

Study areas

This study was conducted in 2006 at eight 200-m sites in the Emory River watershed located in the Cumberland Plateau and Cumberland Mountain regions of central Tennessee, USA (Fig. 1). The Emory River watershed is generally characterised by clear and confined streams that run through the rugged topography of the plateau. Regional geologic formations are typified by Pennsylvanian-age conglomerate, sandstone, siltstone and shale covered chiefly by well-drained, acidic soils of low fertility. The watershed is predominantly forested along with agriculture, coal mining and urban development, and it is home to a handful of endemic and rare aquatic species, including four mussel species that are federally listed as endangered and one fish species that is federally listed as threatened (spotfin chub). The study area contains characteristic natural areas represented by the Obed Wild and Scenic River and Catoosa Wildlife Management Area (Fig. 1). A recent watershed-wide survey indicates that it retains fish community characteristics comparable to historical records (Russ

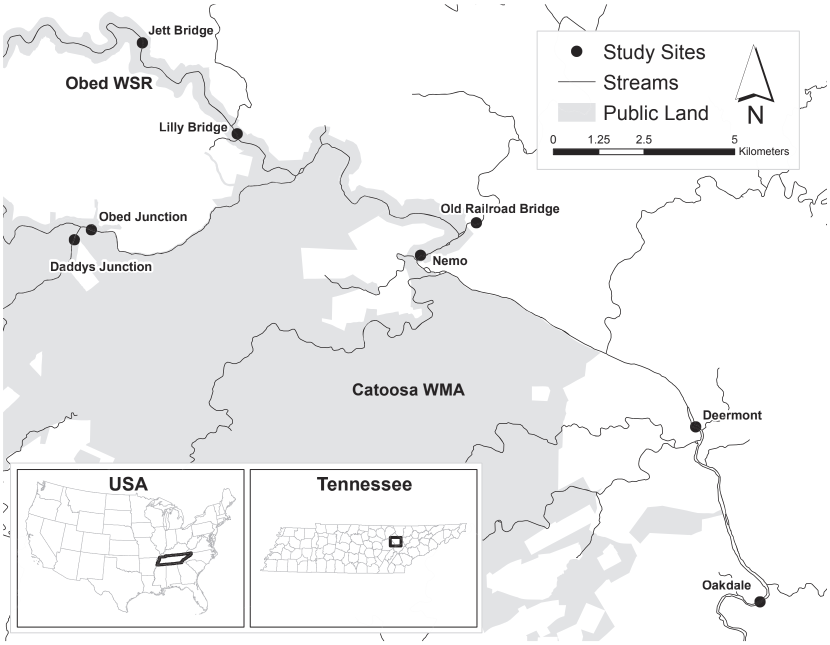

Fig. 1. Map of study sites in the Emory River watershed located in Tennessee, USA.

2006), but it is faced with an increasing pressure from population growth and land development.

Eight study sites were selected based on known occurrences of spotfin chubs and accessibility, and each site was sampled once in early summer (June 6–21), late summer (July 24–August 9) and fall (September 17–October 10). The study sites were characteristic of spotfin chub macrohabitats in the region (Jenkins & Burkhead 1984; Etnier & Starnes 1993). Notably, substrate was primarily characterised by bedrock (solid rock) and boulder (256–512 mm) (>50% in each site) with riffle-run-pool sequences. Stream size differed among study sites and seasons, ranging 8.8–38.9 m in wetted stream width (Table 1). Stream temperature at study sites tended to be cooler during fall (mean, 19.3 °C; range, 16.0–25.1 °C) compared to early summer (mean, 26.3 °C; range, 21.0–30.0°C) and late summer (mean, 28.9 °C; range, 26.7–31.0 °C) (Table 1).

Data collection

Present and absent microhabitats of spotfin chubs were identified via snorkelling at eight 200-m sites across three seasons (early summer, later summer and fall) in 2006. The winter season was not included because Russ (2006) observed a total of only 24 individuals during the winter survey despite significant efforts to locate fish in his macrohabitat study. Stream snorkelling was a reliable and efficient technique for detecting this minnow species, and a noninvasive sampling method was required for the federally threatened species. Deep (>1.0 m) and shallow (<0.1 m) areas prevented thorough examinations by snorkelling, and where possible, deep areas were examined by diving and shallow areas were observed from above the water's surface. This limitation was not considered a significant hindrance because spotfin chubs are typically found at depths of 0.3–1.0 m (Jenkins & Burkhead 1984), and pools are not typically occupied except during winter (Russ 2006).

Snorkelling was conducted once per season at each stream site between 0800 and 1700 h and during periods of low turbidity. Prior to snorkelling, underwater lateral visibility measurements were recorded using a secchi disc in a nonshaded area. One individual held the secchi disc stationary and completely submerged, while another individual wearing a snorkelling mask moved laterally away from the disc until black and white sections were no longer distinguishable underwater.

Snorkelling observations followed a two-step approach, in which present microhabitats were identified in the first snorkelling attempt and then absent microhabitats were determined in the subsequent attempt. During the initial attempt, snorkelling proceeded upstream (larger streams or high gradients) or downstream (smaller streams or low gradients) to locate spotfin chub individuals. Snorkelling was conducted in a zig-zag fashion by one (smaller sites) or two individuals (larger sites), and snorkelers moved at an average rate of about 100 m per 40 min and communicated to avoid duplicate counts. Upon encountering spotfin chubs, the snorkeler placed a lead-weighted (142 g) fluorescent flag on the substrate, marking the microhabitat location where a fish or a group of fish was first observed. When multiple individuals were observed within <24 cm of each

Table 1. Macrohabitat characteristics of stream sites by season. Mean wetted width, mean depth and stream temperature were recorded in the field following microhabitat use observations.

Season	Stream site	Sampling date	Mean wetted width (m)[†]	Mean depth (cm)[‡]	Temperature (°C)[§]
Early summer	Nemo	6/20/2006	31.5	29.9	30.0
	Deermont	6/21/2006	27.3	31.4	28.8
	Oakdale	6/16/2006	26.5	47.2	27.6
	Old Railroad Bridge	6/19/2006	12.2	22.6	24.9
	Daddys Junction	6/15/2006	37.9	27.4	25.8
	Obed Junction	6/13/2006	33.7	30.8	26.0
	Jett Bridge	6/20/2006	17.9	23.4	26.4
	Lilly Bridge	6/6/2006	31.9	43.7	21.0
Late summer	Nemo	8/8/2006	29.0	30.2	31.0
	Deermont	8/1/2006	34.1	46.0	30.8
	Oakdale	7/31/2006	30.3	40.5	30.4
	Old Railroad Bridge	7/26/2006	8.8	22.1	27.4
	Daddys Junction	8/3/2006	38.9	23.5	28.8
	Obed Junction	8/9/2006	30.7	37.1	28.9
	Jett Bridge	7/24/2006	18.5	32.3	26.7
	Lilly Bridge	7/25/2006	28.5	37.5	27.3
Fall	Nemo	10/3/2006	34.4	33.5	18.2
	Deermont	9/19/2006	26.9	36.6	25.1
	Oakdale	9/17/2006	26.1	41.8	24.5
	Old Railroad Bridge	10/9/2006	13.8	33.5	16.0
	Daddys Junction	9/29/2006	34.1	31.7	17.3
	Obed Junction	10/2/2006	31.5	29.0	18.2
	Jett Bridge	10/3/2006	23.1	43.6	16.9
	Lilly Bridge	10/10/2006	31.1	25.9	18.4

[†] Mean wetted width was based on measurements at seven transects at each 200-m site.

[‡] Mean depth was based on measurements at five points on each of the seven transects.

[§] Stream temperature was the mean value across absent microhabitat locations (≤21) for each site.

other, as visually estimated by the snorkeler, a single flag was placed at the approximate centre location. Distances > 24 cm between individuals necessitated the placement of an additional flag. This distance threshold was chosen because direct behavioural interaction was not common when individuals was >24 cm apart (C.U. Schmidt, personal observation). A maximum number of 40 markers were placed for present microhabitat locations at each site. Number of adults (>50 mm TL) and subadults (<50 mm TL) was recorded at each flag location using a PVC sleeve with a #2 pencil, but the two size classes were lumped together for analysis (see Statistical Analysis below). Two size classes were reliably identified during snorkelling, and it follows descriptions by Jenkins & Burkhead (1994).

Absent microhabitats were identified following the initial snorkelling attempt for locating present microhabitats. At each 200-m site, eleven permanent transects (20-m intervals) were established, placed perpendicular to stream flow. Seven of eleven transects were selected by discarding four transects in the closest proximity to the greatest number of present microhabitat locations. The wetted width at the seven transects was measured, and five equidistant points were established along the transects. Three of the five points were randomly chosen as absent microhabitat locations (i.e., maximum of 21 absent locations per site). Each absent microhabitat was observed by snorkelling for a minimum of one minute to ensure absence of spotfin chubs within a 50-cm radius (Turgeon & Rodríguez 2005). When a spotfin chub was observed, the location was discarded and replaced by another randomly selected location along the transect. Absence was not recorded on rare occasions when no new suitable absent locations could be established. Few sampling occasions had <21 absent microhabitat locations.

An identical set of microhabitat variables was recorded at present and absent microhabitats. They were stream depth, velocity, substrate, habitat unit and distance to nearest bank. Depth was measured to the nearest cm with a PVC pipe marked in 1-cm increments. Velocity was measured at the substrate and at 60% of stream depth (Marsh-McBirney Flowmate Model 2000, Hatch Inc., Loveland, CO, USA). A single predominant substrate category was visually estimated at the location of each lead weight according to the modified Wentworth scale (McMahon et al. 1996): bedrock (solid rock), boulder (256–512 mm), cobble (64–256 mm), gravel (2–64 mm) and sand (<2 mm). Habitat unit was visually identified as a riffle, run or pool. Distance to nearest bank was calculated perpendicular to flow by measuring from the surface location above the flagged microhabitat to the nearest bank using a Bushnell Yardage Pro rangefinder (Bushnell Inc., Overland Park, KS, USA) or a tape measure.

Statistical analysis

The objective of our analysis was to identify microhabitat covariates that are correlated with presence⁄absence of spotfin chubs among eight study sites across three seasons. Adults (>50 mm TL) and subadults (<50 mm TL) were combined for statistical analysis because of the predominance of adults in our field observations. Of the total of 507 present microhabitat observations across stream sites and seasons (see Results), <10% (48 observations) were based on microhabitat use of subadults only, while 358 observations were based on adult fish only and 101 observations included both adults and subadults observed together. In addition, subadult spotfin chubs were absent from a stream site (Jett Bridge) or some seasons at several sites (Daddys Junction, Obed Junction and Lilly Bridge). As a result, adults and subadults data were combined and analysed together in all subsequent analyses.

Categorical microhabitat covariates were assessed by chi-squared tests to determine whether their proportional composition differed between present and absent locations. Data were pooled across stream sites and seasons, and this analysis was intended as an approximate measure of habitat unit and substrate type preferences.

A Bayesian generalised linear mixed model (GLMM) was used to quantify site- and season-specific effect size of microhabitat covariates on the probability of spotfin chub presence and to assess the influence of macrohabitat covariates (stream size and temperature) on the effect size of microhabitat covariates. Mixed models can appropriately handle the variance structure when individual observations are nested within broader groups (e.g., microhabitat use observations among stream sites and seasons). It should be stressed that our model does not predict probability of spotfin chub presence in an absolute term, but rather it modelled the probability of presence relative to observed absent microhabitats. This is because maximum numbers of observations were bound for present (≤40 observations) and absent (≤21 observations) microhabitats at each site (see above); therefore, an unknown fraction of available microhabitats was occupied by spotfin chubs at each site.

A subset of measured microhabitat covariates was used in the GLMM to avoid colinearity. The microhabitat covariates used in the GLMM were stream depth (continuous), current velocity at 60% depth (continuous), distance to stream bank (continuous) and substrate type (binary). Substrate type was a binary covariate, where it was 1 if bedrock or boulder, and 0 otherwise. The categorisation was because of the preference of spotfin chubs for bedrock and boulder substrate (see Results, and also Russ (2006)). To address the issue of potential outliers in continuous microhabitat-scale covariates, the range of values that included 95% of observations was identified for each continuous covariate. The observations smaller than the 2.5 percentiles were fixed at the 2.5 percentile value, and those larger than 97.5 percentiles were fixed at the 97.5 percentile value. All microhabitat covariates were then standardised by subtracting their mean and dividing by two times their standard deviation, so that a one-unit change in the newly created covariates correspond to a change in twice the standard deviation on the original scale. This standardisation is an effective approach when continuous and binary covariates are included (Gelman & Hill 2007). No single pair of standardised microhabitat covariates was strongly correlated with one another (Spearman's rank correlation: $r_s < 0.2$), and the lack of pair-wise correlation was visually confirmed with a scatter plot.

The GLMM model consisted of two parts. First, the multiple logistic regression model at the microhabitat scale was a 'varying-intercept, varying-slope' model (Gelman & Hill 2007). Specifically, the intercept ($\alpha_{(\text{site}[i],\text{season}[i])}$) and slopes ($\beta_{(\text{site}[i],\text{season}[i])}$) were allowed to vary among site × season combinations and specified as random effects:

$$\Pr(y_i = 1) = p_i \tag{1}$$

$$\text{logit}(p_i) = \alpha_{(\text{site}[i],\text{season}[i])} + X_i \beta_{x(\text{site}[i],\text{season}[i])} \tag{2}$$

where $y_i = 1$ denotes the presence of spotfin chubs and p_i denotes the probability of presence for observation i ($i = 1, 2, 3, \ldots, 1003$: the total number of present and absent observations among sites and seasons), and X_i represents a vector of microhabitat covariates including stream depth (linear and quadratic terms), current velocity at 60% depth (linear and quadratic), distance to bank (linear and quadratic) and substrate (linear term only) (i.e., vector length of $X_i = 7$). Naturally, the vector length of β_x was similarly seven (i.e., $\beta_1, \beta_2, \ldots, \beta_7$).

The second component of the GLMM was a multiple linear model that quantified the effect of macrohabitat covariates on the effect size of linear terms of microhabitat covariates:

$$\begin{aligned}\beta_{x(\text{site},\text{season})} = {} & \text{g0.x} \\ & + \text{g1.x} * \text{stream}\cdot\text{size}\cdot\text{index}_{(\text{site},\text{season})} \\ & + \text{g2.x} * \text{temperature}_{(\text{site},\text{season})}\end{aligned} \tag{3}$$

where β_x corresponds to site- and season-specific regression coefficients of linear terms (depth, velocity, distance to bank, and substrate; i.e., $x = 1$–4) in Eq. (2); g0.x indicates intercept terms; g1.x represents coefficients for the ‘stream size index’; and g2.*x* represents coefficients for stream temperature at each site and each season. The stream size index was calculated as a function of mean wetted width and mean depth for each site × season combination. Specifically, values of each covariate were divided by the maximum observed value for the covariate, and they were then multiplied to assign a measure of stream size for each site × season combination. This was the best available approximation to stream discharge at each site and season in the absence of gauging stations at every stream site. Macrohabitat covariates were similarly standardised by subtracting their mean and dividing by two times their standard deviation.

The GLMM was fitted using Markov chain Monte Carlo (MCMC) methods in WinBUGS 1.4 (Spiegelhalter et al. 2003) called from R (R Development Core Team 2010) with the R2WinBUGS package (Sturtz et al. 2005). ‘Vague’ priors were used throughout the Bayesian model; most notably, normal distributions with a mean of zero and a wide variance were used for regression coefficients. Marginal posterior distributions of model parameters were estimated by 20,000 iterations of three chains after discarding 5000 burn-in iterations. Model convergence was checked by visually examining plots of the MCMC chains for good mixture and using the Brooks and Gelman diagnostic (Brooks & Gelman 1998). This statistic compares variance within and between chains, and model was considered to have converged when the value was <1.1 for all model parameters (Gelman & Hill 2007). Effect of microhabitat and macrohabitat covariates was considered statistically significant when the 95% credible interval did not overlap with zero. We constructed microhabitat response curves for stream depth and velocity by setting the values of other continuous microhabitat variables at their mean (i.e., 0 because of the standardisation above). The curves were constructed for each stream site and season by specifying the substrate type (bedrock/boulder or not).

Results

Underwater lateral visibility was excellent for snorkelling observations, averaging 5.2 m across all stream sites and seasons (range: 1.8–8.6 m). Spotfin chubs were observed at all eight study sites during all seasons; however, they were more common at downstream sites (Nemo, Deermont and Oakdale) and rare at some upstream sites (Jett Bridge and Lilly Bridge) (Table 2 and Fig. 1). Across the eight study sites, we recorded a total of 155 present microhabitats (660 individuals) during early summer, 195 present microhabitats (753 individuals) during late summer and 157 present microhabitats (552 individuals) during fall (Table 2).

Table 2. Count of present and absent spotfin microhabitats at eight stream sites during three seasons.

	Early summer		Late summer		Fall	
Stream site	Present	Absent	Present	Absent	Present	Absent
Nemo	16	21	40	18	40	16
Deermont	39	21	40	9	40	18
Oakdale	40	21	40	21	40	21
Old Railroad Bridge	15	21	24	21	11	21
Daddys Junction	15	21	26	21	14	21
Obed Junction	20	21	8	21	5	21
Jett Bridge	6	21	7	21	5	21
Lilly Bridge	4	21	10	21	2	21
Total	155	168	195	153	157	160

Spotfin chubs occupied a wide range of stream depth, current velocity and distance to stream bank. Across the eight study sites, spotfin chubs were present at stream depths ranging 9–85 cm (mean 33 cm), current velocity at 60% depth ranging 0–0.88 $\mathrm{m \cdot s^{-1}}$ (mean 0.24 $\mathrm{m \cdot s^{-1}}$) and distance to bank ranging 0.9–25.0 m (mean 8.3 m) during early summer. In late summer, they were observed at depths 5–73 cm (mean 31 cm), current velocity at 60% depth 0–1.05 $\mathrm{m \cdot s^{-1}}$ (mean 0.20 $\mathrm{m \cdot s^{-1}}$) and distance to bank 0.9–19.0 m (mean 7.1 m). In fall, they were observed at depths 9–82 cm (mean 37 cm), current velocity at 60% depth 0–0.61 $\mathrm{m \cdot s^{-1}}$ (mean 0.19 $\mathrm{m \cdot s^{-1}}$) and distance to bank 0.3–22.1 m (mean 7.0 m). During the course of this study, spotfin chubs were observed at all types of habitat units (runs, riffles and pools) and all types of substrate (bedrock, boulder, cobble and gravel) except sand, which was inherently limited in the study stream sites (Fig. 2). However, spotfin chubs were more likely to be present at runs ($\chi^2 = 193.2827$, d.f. = 2, *P*-value < 0.0001; data pooled across sites and seasons), and bedrock and boulder substrates ($\chi^2 = 173.2029$, d.f. = 4, *P*-value < 0.0001; data pooled across sites and seasons) (Fig. 2).

The Bayesian GLMM identified some general microhabitat use patterns exhibited by spotfin chubs, but patterns also varied among stream sites and seasons. In general, spotfin chubs were more likely to be present in microhabitats characterised by boulder/bedrock substrates, high to medium velocity, and medium depth (Table 3; Figs 3 and 4). Bedrock and boulder substrate was the most consistently used microhabitat across stream sites and seasons, and its regression coefficient was significantly positive in 18 of 24 site × season combinations (Table 3). This pattern is graphically observable when the predicted probability of spotfin chub presence is compared between bedrock/boulder versus cobble/gravel/sand substrate (compare left versus right columns on Figs 3 and 4).

The middle range of stream depth was generally favoured by spotfin chubs. The quadratic term of stream depth was significantly negative in half of 24

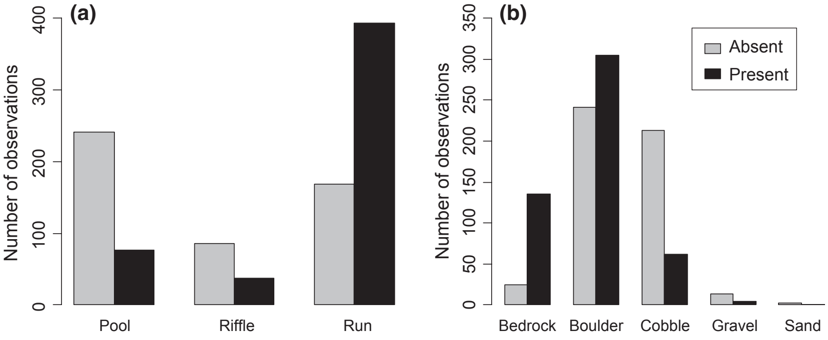

Fig. 2. (a) Habitat units and (b) substrate types where spotfin chubs were present versus absent. Data were pooled across stream sites and seasons.

Table 3. Mean regression coefficients of microhabitat covariates in the generalised linear mixed model. Values whose 95% credible intervals do not overlap with zero are shown in bold. Each covariate was standardised by mean divided by two times standard deviation prior to analysis.

		Depth		Velocity		Distance to bank		Bedrock⁄ boulder
	Intercept	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear
Early summer								
Nemo	–1.11	3.00	–1.54	**4.37**	–2.21	0.11	1.63	**2.39**
Deermont	0.07	0.67	–**3.74**	**2.55**	3.01	–0.67	**3.46**	**1.91**
Oakdale	1.27	–1.43	–**8.14**	**4.13**	1.13	–1.20	1.48	0.58
Old Railroad Bridge	1.84	0.08	–7.19	**2.30**	-2.43	0.69	–2.47	**3.49**
Daddys Junction	2.24	1.14	–**4.48**	**2.20**	0.91	0.08	–1.77	**2.84**
Obed Junction	0.61	–1.33	0.44	**3.79**	–1.15	–0.36	–0.49	**2.32**
Jett Bridge	–0.34	–1.46	–1.55	1.50	–7.59	–0.81	0.80	**3.23**
Lilly Bridge	–0.04	–1.53	–5.74	1.55	–3.26	–1.29	–2.13	1.02
Late summer								
Nemo	1.85	0.97	–**5.58**	1.60	–2.33	–0.78	0.86	**3.10**
Deermont	1.73	0.94	–**2.67**	**3.16**	–**2.88**	0.14	0.61	0.05
Oakdale	2.86	0.11	–**4.57**	**2.47**	–**3.70**	–0.66	–1.14	0.73
Old Railroad Bridge	1.67	–0.85	–4.90	1.21	–4.46	–0.01	–0.84	**3.41**
Daddys Junction	0.78	–1.47	–3.63	1.91	–2.47	0.65	–0.87	**3.38**
Obed Junction	–0.14	–**3.47**	0.00	**3.71**	–4.06	–1.47	0.08	**1.77**
Jett Bridge	–1.31	–0.50	–6.81	**3.15**	–1.26	–0.85	0.79	**2.62**
Lilly Bridge	1.18	–1.57	–**7.39**	1.69	–3.80	–0.85	–1.94	**1.97**
Fall								
Nemo	0.84	**2.39**	–2.71	0.76	–1.85	–1.17	2.11	**2.67**
Deermont	2.20	0.64	–**4.29**	1.53	–2.80	–**1.74**	–0.60	**1.37**
Oakdale	3.34	0.42	–**6.19**	1.35	–**5.34**	–1.23	–0.75	1.02
Old Railroad Bridge	–0.87	**3.19**	–**3.24**	–1.00	–1.95	–1.00	0.78	**2.46**
Daddys Junction	0.31	0.93	–7.21	0.26	–**3.08**	–0.44	0.48	**3.14**
Obed Junction	–0.53	0.04	–2.82	1.88	–4.49	–1.47	–1.05	**3.23**
Jett Bridge	–1.86	0.39	–**10.32**	1.61	1.29	–1.91	0.38	1.26
Lilly Bridge	–0.68	–0.12	–**6.22**	0.24	–4.48	–0.44	–1.01	**3.55**

site × season combinations (Table 3), leading to the dome-shaped relationship between stream depth and predicted probability of spotfin chub presence (Fig. 3). The linear term of stream depth was significantly positive at Nemo and Old Railroad Bridge sites in fall, but it was significantly negative at Obed Junction site in late summer (Table 3).

A seasonal pattern was observed for the effect of current velocity. High-velocity microhabitats were used in early summer, when the linear term of current velocity at 60% depth was significantly positive at six of eight study sites (Table 3 & Fig. 4). Spotfin chubs occupied high-velocity microhabitats in late summer as well (four sites with significantly positive effect size), but no stream sites were characterised with significantly positive coefficients in fall (Table 3). Similar to stream depth, the quadratic term of current velocity was predominantly negative, but only a total of four sites had statistically significant values across seasons (Table 3).

Distance to stream bank was by far the least important microhabitat covariate tested in the GLMM. Only one linear and one quadratic term were statistically significant for this microhabitat covariate, and the direction of coefficients had a mixture of positive and negative signs (Table 3).

The Bayesian GLMM identified a couple of interactions between microhabitat and macrohabitat covariates (Table 4 and Fig. 5). First, the stream size index, a macrohabitat covariate, was significantly negatively correlated with the logistic regression coefficient of bedrock⁄boulder substrate (Table 4). It indicated that

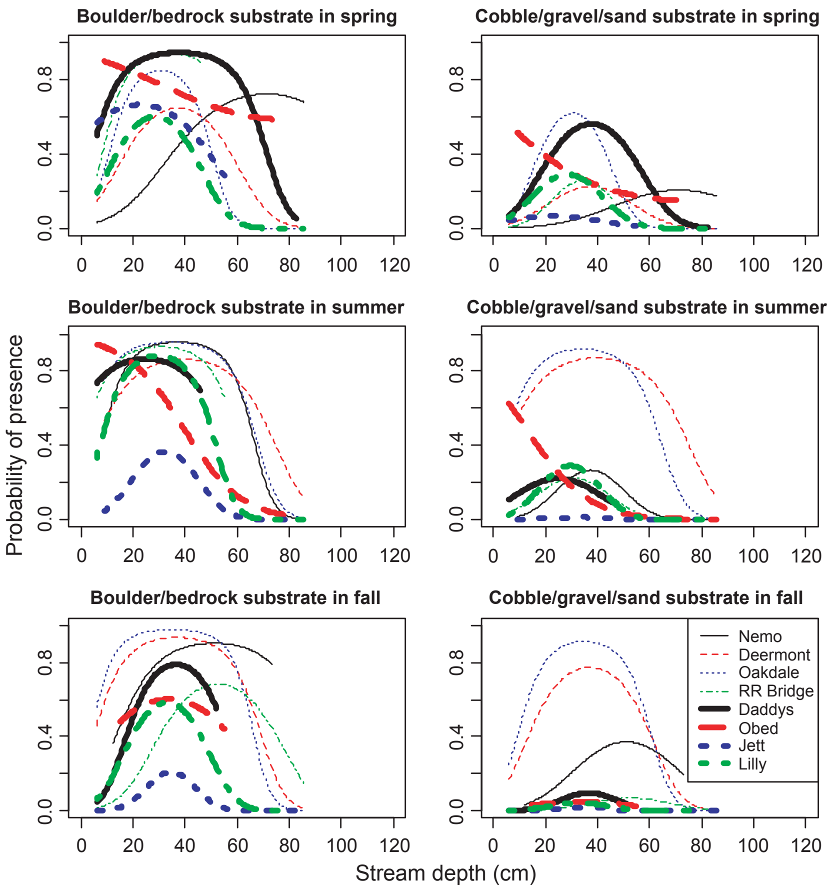

Fig. 3. Effect of stream depth on the spotfin chub presence by season and substrate type.

Table 4. Regression coefficients of macrohabitat covariates (columns) on the effect size of microhabitat covariates (rows) in the generalised linear mixed model (see Eq. 3 in the text). Mean values are shown with 95% credible intervals in parentheses. Values whose 95% credible intervals do not overlap with zero are shown in bold (except the intercept term).

	Intercept	Stream size index	Stream temperature
Depth	0.05 (−1.38, 1.26)	−0.16 (−2.69, 2.48)	−0.91 (−3.32, 1.35)
Velocity at 60% depth	1.99 (1.02, 3.29)	0.59 (−1.13, 2.78)	1.77 (−0.45, 3.85)
Distance to bank	−0.70 (−1.51, 0.04)	−0.77 (−2.40, 0.60)	0.54 (−0.82, 2.00)
Bedrock/boulder	2.23 (1.71, 2.88)	**−1.96** (**−2.97**, **−0.95**)	−0.49 (−1.77, 0.85)

spotfin chubs more likely used bedrock/boulder substrate at smaller stream sites, but substrate type did not influence microhabitat use as strongly at larger stream sites (Fig. 5a). Second, while nearly statistically significant, stream temperature (a macrohabitat covariate) tended to be positively correlated with the effect size of current velocity (Table 4). That is, spotfin chubs tended to select higher velocity microhabitats at warmer stream temperatures (typically early and late summer: see Table 1), but velocity was less important at cooler temperatures (fall) (Fig. 5b).

Discussion

Overall, spotfin chubs were more likely to be present in microhabitats characterised by boulder/bedrock substrates, high to medium velocity, and medium depth. However, our analysis highlighted variation in microhabitat use of spotfin chubs among stream sites and seasons. Spatial and temporal variation in microhabitat use of stream fish, particularly stream salmonids, has been commonly reported (e.g., Heggenes 2002; Ayllón et al. 2010). However, plasticity in microhabitat use has been observed less frequently for rare stream fish (e.g., Labonne et al. 2003; Copp & Vilizzi 2004), although the lack of such findings may be due partly to the sampling issue of rare species such as geographically limited distribution and low detection rates.

The general patterns of microhabitat use identified by the GLMM are congruent with previous findings that spotfin chubs prefer run habitats from spring to fall (Jenkins & Burkhead 1984; Etnier & Starnes 1993; Russ 2006). Relative to riffle and pool habitats, runs

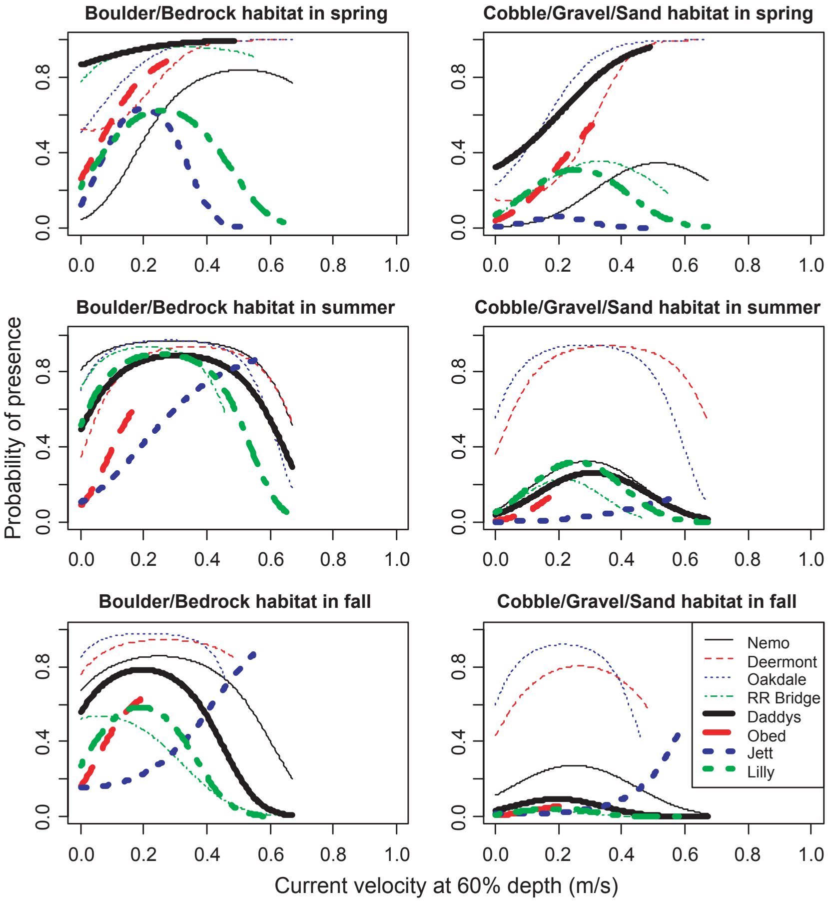

Fig. 4. Effect of current velocity at 60% depth on the spotfin chub presence by season and substrate type.

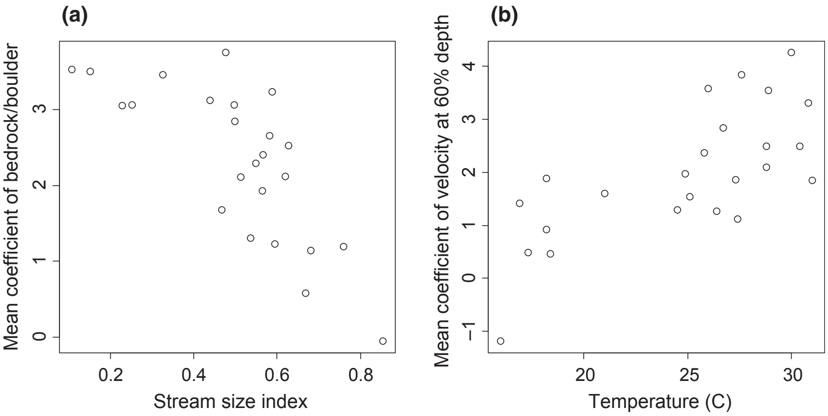

Fig. 5. Relationships between macrohabitat covariates and microhabitat covariates: (a) stream size index versus mean regression coefficient values of stream depth, and (b) stream temperature versus mean regression coefficient values of current velocity at 60% depth.

were inherently characterised by medium depth, high-velocity and bedrock/boulder substrate in the study sites. It is then not surprising that these microhabitat covariates were identified as important in the GLMM. Stream fish typically select microhabitat locations based on a combination of microhabitat characteristics (Mattingly et al. 2003; Hazelton & Grossman 2009) to maximise survival or growth rates during nonspawning seasons (Skalski & Gilliam 2002; Sotiropoulos et al. 2006). It can be assumed that this benthic insectivorous minnow maximises food intake and minimises predation risk by occupying medium depth and mid to high-velocity microhabitats from early summer to fall. In addition, spotfin chubs spawn within narrow slots and fissures on boulder and bedrock substrate between May and August (Jenkins & Burkhead 1984, 1994), which corresponded to early and late summer in this study. During these seasons, spotfin chubs may have preferentially selected boulder and bedrock for spawning as well.

The observed interactions between microhabitat and macrohabitat covariates suggest that research on microhabitat use and other ecological responses of stream fish should consider the 'contextual' effect (Holm et al. 2001; Vilizzi et al. 2004; Xu et al. 2010). Substrate type was the most consistently important microhabitat covariate across stream sites and seasons, but its importance still varied by stream size. Specifically, spotfin chubs were more likely to use bedrock/boulder substrate at smaller stream sites (where they were less common), but they were less selective of substrate type at larger stream sites (where they were abundant). This pattern of microhabitat use may be either active or passive. In smaller stream sites, suitable microhabitats may be inherently limited, and few individuals that remain may select the most suitable microhabitat available associated with bedrock/boulder substrate. In contrast, higher densities of spotfin chubs at larger stream sites may indicate overall favourable environmental conditions, and individuals may occupy seemingly less preferred microhabitats (e.g., cobble and gravel). This may be why the importance of bedrock/boulder substrate was not evident at larger stream sites in our analysis.

Spotfin chubs tended to use higher velocity microhabitats at warmer stream temperatures, although this pattern was not statistically significant. Stream temperature at study sites was nearly comparable between early summer and late summer, but it was noticeably cooler during fall (see Table 1). Early and late summer were the spawning season for spotfin chubs (Jenkins & Burkhead 1984, 1994), and our results suggest that they may select and deposit eggs within bedrock and boulder fissures with high current velocities to avoid egg suffocation from potential sedimentation. In the meantime, the reduced importance of high-velocity microhabitats during fall is likely indicative of lower activity levels under cooler temperatures. Russ (2006) found that spotfin chubs were hard to locate underwater during winter, but they were found in pool habitats over sand substrate on the few observed occasions (stream temperature range: 2.2–3.8 °C). A similar seasonal shift in microhabitat use has been observed in other lotic fish, particularly stream salmonids (Bardonnet & Baglinière 2000; Huusko et al. 2007).

This study aimed to assess as many microhabitat covariates as practicable, but other unmeasured factors may also influence microhabitat use of spotfin chubs. First, interspecific interactions were not assessed in this study, but they may or may not affect microhabitat use of stream fish (Santos et al. 2004; Hazelton & Grossman 2009; Crow et al. 2010). Only occasionally did other species of minnows behaviourally compete with spotfin chubs during the course of this study (C.U. Schmidt, personal observation). However, in the Emory River watershed, spotfin chubs would likely face competition from species such as whitetail shiners (*Cyprinella galactura*), spotfin shiners (*Cyprinella spiloptera*) and warpaint shiners (*Luxilus coccogenis*). These species exhibit more aggressive and flexible feeding behaviour relative to spotfin chubs (Jenkins & Burkhead 1984; Etnier & Starnes 1993). In addition, spotfin chubs would be at greater risk of predation from species such as smallmouth bass (*Micropterus dolomieu*) and could avoid microhabitats with predatory fish. Second, we combined data from adults (>50 mm) and subadults (<50 mm) because the majority of field observations were made on adults, and subadults were not present in all study sites. However, ontogenetic microhabitat shifts are common among stream fish (Copp & Vilizzi 2004; Henderson & Johnston 2010). Subadults were commonly observed to school with adults in this study (C.U. Schmidt, personal observation), but the predominance of adults in our observations in itself may suggest that subadults utilise microhabitats that are difficult to locate underwater.

Microhabitat use data of stream fish are frequently collected to inform stream habitat and flow management decisions. For example, the Physical Habitat Simulation (PHABSIM) combines field-collected data on microhabitat use and physical habitat models to quantify weighted useable habitat areas over a range of discharge levels (Jowett 1997). In this application, microhabitat suitability of stream fish is typically assumed to remain unchanged across space and time, but habitat suitability models often perform poorly when they are applied to different streams or seasons (Holm et al. 2001; Anderson et al. 2006). The varying importance of some microhabitat covariates as a function of macrohabitat characteristics observed in the current study explains partly why such an approach might not be transferable across space and time. The GLMM provided a useful framework for understanding potential interactions between microhabitat and macrohabitat covariates. Without macrohabitat-level predictors (i.e., stream size index and temperature), this important pattern would have been masked and instead it would have been treated as unexplained variation among stream sites and seasons. In fact, conclusions on microhabitat use of stream fish may much depend on study designs. That is, when data are collected in a spatially and temporally extensive manner and when they are analysed to account for patterns across space and time, the researcher may likely find spatial and temporal heterogeneity in microhabitat use and conclude that the studied stream fish is a habitat generalist (e.g., Ayllón et al. 2010). However, studies based on a limited set of locations and times may well highlight site- or time-specific patterns, and the species may be considered as a habitat specialist (e.g., van Snik Gray & Stauffer 1999). We recommend that studies of microhabitat use attempt to understand patterns across space and time where possible and quantify how such patterns are explained.

The federally threatened status and continuing decline of spotfin chubs argues for conservation actions based on the best available science. A major conclusion of this study is that spotfin chubs are associated with microhabitat characteristics most typical of runs between early summer and fall. Therefore, maintaining the integrity of this habitat type is paramount and relevant in this 'water-limited' region of the Cumberland Plateau. Streams in the study region receive little groundwater input because of its well-drained soils, and wadeable streams are typically characterised with series of isolated pools during summer baseflow. Hydrological alteration such as water withdrawals could reduce and degrade suitable run habitats for spotfin chubs.

This study also highlights the importance of seasonal- and site-specific patterns of microhabitat use by spotfin chubs. The somewhat flexible microhabitat use suggests that scientific efforts should focus on identifying and protecting available suitable habitat across space and time. Prudent conservation measures would discourage alteration of the range of microhabitat conditions used by spotfin chubs in our study. It should also be noted that winter habitat use of this species appears to differ; Russ (2006) observed spotfin chubs in pools over sand substrate during winter. Finally, additional research needs to be conducted to evaluate if anthropogenic disturbances (e.g., land development and water withdrawals) impact microhabitat use of spotfin chubs in the Emory River watershed. For example, increased suspended sedimentation is known to affect physiology and growth of spotfin chubs (Sutherland & Meyer 2007; Sutherland et al. 2008), thereby potentially affecting microhabitat use and subsequently altering population-level processes (e.g., survival).

In conclusion, spotfin chubs were, in general, more likely to be present in microhabitats characterised by bedrock and boulder substrates, medium to high velocity, and medium depth (most typically run habitats). The greatest affinity was observed for bedrock and boulder substrate. However, the patterns were not necessarily consistent among the stream sites and seasons, and spotfin chubs appear to exhibit flexible microhabitat use to some extent. This study suggests that some variation in microhabitat use among stream sites and seasons may be explained by macrohabitat characteristics that change spatially and temporally. Quantifying this type of interaction across scales can advance our understanding of complex species-habitat relationships.

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