Development of Habitat Suitability Indices for the Candy Darter, with Cross-Scale Validation across Representative Populations

Corey G. Dunn & Paul L. Angermeier

ARTICLE

Development of Habitat Suitability Indices for the Candy Darter, with Cross-Scale Validation across Representative Populations

Corey G. Dunn*

Department of Fish and Wildlife Conservation, Virginia Tech, 100 Cheatham Hall, Blacksburg, Virginia 24061,USA; and Department of Fisheries and Wildlife Sciences, University of Missouri, 302 Anheuser-Busch Natural Resources Building, Columbia, Missouri 65201, USA

Paul L. Angermeier

U.S. Geological Survey, Virginia Cooperative Fish and Wildlife Research Unit, 100 Cheatham Hall, Virginia Tech, Blacksburg, Virginia 24061, USA

Abstract

Understanding relationships between habitat associations for individuals and habitat factors that limit populations is a primary challenge for managers of stream fishes. Although habitat use by individuals can provide insight into the adaptive significance of selected microhabitats, not all habitat parameters will be significant at the population level, particularly when distributional patterns partially result from habitat degradation. We used underwater observation to quantify microhabitat selection by an imperiled stream fish, the Candy Darter *Etheostoma osburni*, in two streams with robust populations. We developed multiple-variable and multiple-life-stage habitat suitability indices (HSIs) from microhabitat selection patterns and used them to assess the suitability of available habitat in streams where Candy Darter populations were extirpated, localized, or robust. Next, we used a comparative framework to examine relationships among (1) habitat availability across streams, (2) projected habitat suitability of each stream, and (3) a rank for the likely long-term viability (robustness) of the population inhabiting each stream. Habitat selection was characterized by ontogenetic shifts from the low-velocity, slightly embedded areas used by age-0 Candy Darters to the swift, shallow areas with little fine sediment and complex substrate, which were used by adults. Overall, HSIs were strongly correlated with population rank. However, we observed weak or inverse relationships between predicted individual habitat suitability and population robustness for multiple life stages and variables. The results demonstrated that microhabitat selection by individuals does not always reflect population robustness, particularly when based on a single life stage or season, which highlights the risk of generalizing habitat selection that is observed during nonstressful periods or for noncritical resources. These findings suggest that stream fish managers may need to be cautious when implementing conservation measures based solely on observations of habitat selection by individuals and that detailed study at the individual and population levels may be necessary to identify habitat that limits populations.

A clear understanding of habitat requirements is essential for effective species management (Rosenfeld 2003). In stream networks, habitat is hierarchically organized into discrete spatial scales spanning large river basins to microhabitats, which facilitate the persistence of populations as well as the growth and reproduction of individuals (Frissell et al. 1986). Incompatibility between a species' life history requirements and available resources can exclude that species from an area at any spatial scale within the habitat hierarchy (Schlosser and Angermeier 1995). Often, a clear (i.e., mechanistic) understanding of habitat requirements is obtained only after the integration of findings from numerous observational and experimental studies spanning multiple levels of ecological organization (Rosenfeld 2003).

*Corresponding author: cgd7n7@mizzou.edu

The decline of North America's rich freshwater fish fauna over the past century partly reflects an inadequate understanding of basic habitat requirements and how anthropogenic changes to aquatic ecosystems impinge on those requirements (Jelks et al. 2008; Burkhead 2012). Regional declines of many species are characterized by the gradual dissolution of a network of populations. Individual populations are lost due to sudden anthropogenic or natural events or the accumulation of years of population declines owing to altered environmental conditions (Angermeier 1995). This process often results in a distributional pattern of disjunct populations that are scattered across the landscape in locations with sufficient habitat quality and quantity to support positive or neutral growth in the absence of immigration (Schlosser and Angermeier 1995). Predisturbance conditions are usually undocumented, and thus managers tasked with recovering a species are left without (1) a true reference of normal population function within affected areas, (2) critical knowledge of life history that applies across the species' range, or (3) both. However, remaining populations and associated environmental conditions can inform management. Within areas still supporting populations, information that is beneficial to species recovery includes an understanding of available habitat structure, how individuals interact with the environment, and which parameters influence individual fitness and population function.

Population-level metrics (e.g., presence, density, and demographic rates) are normally measured via extensive surveys across the distributional range of focal species. Extensive surveys provide a representative sample of possible physical habitat configurations across the species' range and therefore are less susceptible to site-specific biases (Newcomb et al. 2007). However, researchers must often balance the extent of surveys with the sampling intensity per site. In particular, extensive surveys may be infeasible for nongame species, which have historically received less attention (Loomis and White 1996; White 1996; Gabelhouse 2005). Furthermore, extensive surveys may merely reveal correlative population-level responses across space while missing the underlying mechanisms, particularly when rare habitats at specific life stages ultimately regulate populations (Torgersen et al. 1999; Fausch et al. 2002). Therefore, detailed study of individual habitat use is frequently employed to identify the factors limiting populations.

Fish–habitat relationships are frequently quantified in the form of habitat suitability models. Management uses of these models include characterizing important habitat types (Guay et al. 2000; Haxton et al. 2008; Midway et al. 2010); guiding habitat augmentation (Boavida et al. 2012); and, increasingly, identifying suitable habitat for species reintroduction (Mattingly and Galat 2002; Dixon and Vokoun 2009). Models vary in complexity, but most individual-level models assume that individuals actively select conditions that optimize fitness within the context of specific behavioral modes (e.g., reproduction, foraging, and refuge use). For example, foraging individuals try to maximize the ratio of energy intake to expenditure while minimizing mortality risk (Werner and Gilliam 1984; Grossman 2013). Habitat suitability models frequently use the density of individuals occupying a habitat type as a metric for habitat suitability (Rosenfeld 2003); however, this metric can be affected by plasticity of habitat use (Leftwich et al. 1997), resource availability (Dunham et al. 2002), biotic interactions (Orth 1987), ontogeny (Rosenberger and Angermeier 2003), and behavior mode (Kwak et al. 1992). Thus, individual-level models of habitat use frequently perform poorly outside the spatiotemporal context in which they were developed (Fausch et al. 1988; Leftwich et al. 1997; Hewitt et al. 2009). Further, individual-level habitat studies rarely examine links to population-level responses (Peckarsky et al. 1997). Although recent methodological advances allow researchers to explicitly link individual- and population-level patterns by using individual-based models (Grimm and Railsback 2005), these models may be infeasible—except for well-studied species—due to their extensive data requirements.

The primary goal of this study was to examine whether the predicted microhabitat suitability for an imperiled stream fish, the Candy Darter *Etheostoma osburni*, is consistent with population robustness across four streams. Herein, "robustness" reflects population size, density, and likely long-term viability. To accomplish this, we used a study design that revealed relationships among the three primary factors that are relevant to the development and application of individual-level habitat suitability models: (1) instream habitat gradients, (2) individual habitat selection, and (3) population robustness across streams. First, individual-level habitat selection (i.e., disproportional use) was estimated from two streams with robust populations and that presumably contained optimal habitat (i.e., "reference condition" approach; Stoddard et al. 2006; Newcomb et al. 2007). Next, we validated habitat selection by examining the predicted suitability of available habitat within streams where populations of Candy Darters were robust, localized, or extirpated. By comparing habitat gradients, predicted suitability, and actual population robustness, we examined a seldom-tested assumption of habitat suitability models developed from individual-level habitat selection: that patch quality perceived by individuals at the microhabitat scale can be "scaled up" to reflect population robustness at the stream segment scale.

METHODS

Focal Species

The Candy Darter is endemic to the New River drainage, where the species is patchily distributed across the Appalachian Plateau Physiographic Province and the Valley and Ridge Physiographic Province in Virginia and West Virginia (Chipps et al. 1993; Jenkins and Burkhead 1994). Candy Darters historically inhabited many stream types (Jenkins and Burkhead 1994); currently, however, most populations remain in cool, high-gradient to moderate-gradient streams within forested watersheds. The reduced range may be due to habitat degradation, but this hypothesis has received little investigation. Within streams, adults almost exclusively occupy patches with swift flow and coarse substrates (Chipps et al. 1994). Habitat use by immature

life stages of the Candy Darter has never been described (Supplementary Table S.1 available in the online version of this article) despite the importance of these life stages for population dynamics (Schlosser 1985, 1998), and little is known about habitat use and behavior in early spring during the spawning season. The current management of the species is similar to that of many nongame species, as managers must use a framework that is missing critical pieces of information and also suffers from a lack of cohesiveness among the patchwork of small-scale studies describing individual-level habitat associations from different portions of the species' distributional range. Coherent relationships between individual habitat selection and population robustness are needed to inform managers about which recovery actions are likely to be cost effective.

Field Sites

We selected four streams where Candy Darter populations were robust, localized, or extirpated (Figure 1). Two streams supporting large populations (hereafter, status = "robust populations") were selected to develop habitat suitability models based

FIGURE 1. Map of the New River drainage and study sites: **(A)** South Fork Cherry River (SFC), West Virginia (contains a robust population of Candy Darters); **(B)** East Fork Greenbrier River, West Virginia (robust population); **(C)** Laurel Creek (LC), Virginia (localized population); and **(D)** Sinking Creek, Virginia (extirpated population). Insets depict the survey designs that were used to develop habitat suitability indices within streams supporting robust populations (e.g., SFC) and to systematically measure habitat availability in streams with localized (LC) or extirpated populations. Candy Darters have never been collected in the Blue Ridge province.

on the literature and preliminary sampling. The South Fork Cherry River (SFC) and East Fork Greenbrier River (EFG), West Virginia, are third- and fourth-order streams located in the Gauley River and Greenbrier River subbasins, respectively. Both streams primarily drain forested watersheds at high elevations (>700 m) within the Appalachian Mountains (Messinger and Hughes 2000). To examine microhabitat selection, we selected relatively undisturbed, accessible 5-km sections of stream in both EFG and SFC. Each study section was divided into five 1-km segments, and then 300-m sites from the first (downstream), third, and fifth (upstream) segments were randomly selected for survey. Due to prohibited access in the fifth segment of EFG, we randomly selected a 300-m site between the first and third segments. Randomization ensured that the 900 m of survey effort per stream and season (3.6 km in total) were spatially representative of the study sections within each stream.

Laurel Creek (LC), Virginia, is a third-order stream within the Valley and Ridge Physiographic Province and contains a small, isolated population of Candy Darters (hereafter, status = "localized population"). The population in LC is likely self-sustaining, as the closest known population is approximately 50 fluvial kilometers away and there is no evidence of connectivity between the two populations. Systematic habitat surveys (described below) were conducted beginning at the mouth of LC and extending 4.2 km upstream to a series of small impoundments, encompassing the entire known range of the population in this system.

Sinking Creek (SC), Virginia (hereafter, status = "extirpated population"), is one of five systems in Virginia where Candy Darter are extirpated; it is a candidate site for reintroduction. Burton and Odum (1945) collected one individual over the summers of 1938–1941. However, there are no other records of Candy Darter occurrence in this heavily surveyed system (Jenkins and Burkhead 1994; Hitt and Roberts 2012). The collection of only one specimen in SC is consistent with early records from other streams where the species was extirpated. By the time of the first significant fish surveys in the Virginia portion of the New River drainage (1940s), Candy Darters were localized and always rare in streams where they are now extirpated (Jenkins and Kopia 1995). In the study segment, SC is a fourth-order stream with channel and land cover characteristics that are typical of a large stream in the Valley and Ridge Physiographic Province. Habitat surveys (described below) were conducted in SC at systematically spaced sites within a 5.5-km segment near the original collection locality.
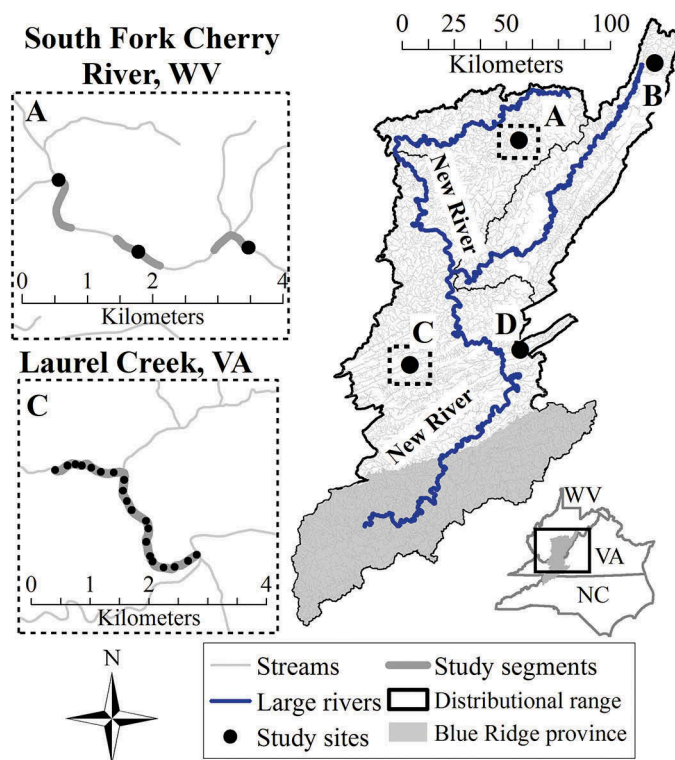
Underwater Observation

We sampled microhabitat use and availability in spring and late summer–fall (hereafter, "fall") to examine possible behavioral changes and differences in habitat availability between seasons. Spring sampling occurred during high flows and the spawning season (May to early June 2011), whereas fall sampling corresponded with low flows and the nonspawning season (August–October 2011). Within EFG and SFC, we used direct underwater observation (snorkeling) during base flow to record the suite of microhabitat conditions that were

immediately associated with each individual. We ensured sufficient water clarity by only sampling when turbidity was less than 5.00 NTU. Beginning at the downstream-most point of a study site, the stream was longitudinally divided into two halves, and a snorkeler was assigned to survey each half. Snorkelers proceeded upstream at the same pace while searching under rocks and moving laterally between the center of the stream and the bank. When a snorkeler spotted a Candy Darter, the snorkeler used a ruler to estimate the fish's TL by either directly measuring the individual or by measuring a nearby rock of comparable size. Nearly all lengths were estimated less than 1 m from individual fish. If a snorkeler influenced an individual's initial position, the observation was omitted. While spring habitat use by adults reflected the areas occupied during the spawning season (staging areas for spawning), the exact microhabitat patches used for spawning within staging areas were not quantified because spawning followed courtship behavior, and observations of habitat use were restricted to the first sighting of individual darters.

Snorkelers classified each fish as belonging to one of three life stages based on the lengths at maturity reported by Jenkins and Burkhead (1994:827–830), our own observations of lengths at maturity from collected individuals, and pigmentation differences among life stages and sexes. Visual estimation of fish lengths and attribution of life stages during underwater observation have been previously used when examining habitat use by darters (Mattingly and Galat 2002; Ashton and Layzer 2010), including the Candy Darter (Chipps et al. 1994). Females that were 60 mm TL or larger and males that were 65 mm TL or larger were classified as adults. Individuals that were 46–59 mm TL were considered juveniles. Some individuals (60–64 mm TL) that were clearly juvenile males based on their pigmentation were classified as juveniles. All 45-mm-TL and smaller individuals were classified as age 0 regardless of the season. All age-0 individuals were postlarvae and ranged from 17 to 45 mm TL. A length frequency histogram constructed from estimated lengths contained three modes corresponding to the three life stages that we monitored (Figure S.1). After recording TL, the snorkeler placed a weighted fluorescent flag at the exact location of each fish and guided the individual downstream to prevent double counting.

After snorkelers finished flagging darter locations, five microhabitat variables were recorded at each flag. We measured depth with a top-setting wading rod and measured the average water column velocity at 60% depth by using a Marsh–McBirney Model 2000 flow meter. The nearest substrate particle was classified (based on its intermediate axis) into one of nine ordered substrate size categories according to a modified Wentworth scale: silt (<0.06 mm), sand (0.07–2.0 mm), gravel (3.0–16 mm), pebble (17–64 mm), small cobble (65–128 mm), large cobble (129–256 mm), boulder (257–1,000 mm), large boulder (>1,000 mm), and bedrock. Finally, within the 0.5-m^2 area surrounding each flag, we visually estimated the average depth of rocks that were embedded by fine substrates (hereafter,

"embeddedness") and the surface area that was covered by silt (hereafter, "silt cover"). Percentages of both metrics were subsequently coded into five ordered categories (Newcomb et al. 2007:846): 0 = ≤5%, 1 = 6–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%. Category 0 (≤5%) represented observations with no perceived embeddedness or silt cover.

Microhabitat Availability

Immediately after habitat use was recorded, the availability of microhabitats in EFG and SFC was measured by placing transects perpendicular to flow spanning the study site. In spring, 30 transects were placed every 10 m; in fall, 15 transects were placed every 20 m. We used data from spring to determine that the number of transects could be reduced to 15 per site without affecting the relative frequencies of available habitat categories. Beginning 1 m from the right descending bank, a field crew member recorded depth, average water velocity, substrate size, embeddedness, and silt cover every 2 m along each transect by using the same protocols employed for microhabitat use (described above). This ensured that the available habitat points were proportional to the area of each stream so as to reduce error when pooling observations across all sites and both streams (EFG and SFC). Observations were not pooled across seasons. Post hoc inspection of frequency distributions and multivariate space representing available microhabitats showed that physical habitats in the two streams were similar; therefore, error resulting from pooling across sites and streams was likely minimal. Furthermore, habitat selection (described below) showed no clear bimodality, which would have likely resulted from stream-specific differences in habitat availability rather than consistent responses to measured habitat gradients from separate streams.

We applied habitat suitability models developed from EFG and SFC to the available instream habitat in LC and SC to assess the ability of the models to predict suitable habitat for populations in a region outside the context of original model development. We employed a sampling design that systematically quantified available microhabitats throughout the 4.2-km section of LC, and we sampled a comparable extent (5.5 km) in SC. For LC and SC, we delineated sites by randomly selecting one of the first four riffles at the downstream-most point in each stream, and we systematically selected sites beginning at every fourth riffle extending upstream throughout the section (Dolloff et al. 1993). Therefore, sites in LC and SC consisted of all channel units between the bases of two consecutive riffles, and the number of sites per stream depended on the number of riffles within study sections. At each site, we placed transects perpendicular to flow and spaced every 10 m, beginning within the first 3 m of the base of the riffle and extending upstream to the base of the next upstream riffle. At five equidistant points along each transect, the same aforementioned microhabitat variables were recorded. We also snorkeled sites in LC to determine whether the microhabitats used there by Candy Darters were similar to those used in EFG and SFC.

The designs we used to quantify habitat availability in LC and SC differed from those used in EFG and SFC for multiple reasons. First, habitat data from LC and SC were not used for habitat suitability models, so it was not necessary to ensure that the observations of habitat availability were proportional to the area of each stream (discussed above). Second, although survey extents were similar across all streams (3.0–5.5 km), we distributed effort more evenly (i.e., more sites) in LC and SC to accomplish multiple management objectives (not reported here). The use of more systematically spaced sites in LC helped to clarify the distribution of Candy Darters within the stream, which could potentially be related to site-level habitat attributes, including rare—yet critical—habitat patches within LC (Torgersen et al. 1999; Fausch et al. 2002). A similar approach was used in SC to identify specific sites for potential restoration and reintroduction efforts. Because additional management objectives within LC and SC were focused at the site level (i.e., specific riffle–pool sequences), we used a fixed number of points for each transect in LC and SC to provide greater sample sizes and to improve estimates of habitat availability at each site.

Data Analysis

Habitat suitability criteria.—We estimated habitat suitability by first developing habitat selection curves. We use "selection" to refer to disproportionate use relative to availability across a single microhabitat gradient in an uncontrolled environment (i.e., a natural stream setting); in contrast, "preference," refers to disproportionate use in a controlled experimental setting (Rosenfeld 2003). Predictions from habitat selection curves are referred to as habitat suitability criteria (HSC), which were developed for each variable at each life stage within each season (hereafter, single-variable suitability unit = HSC). Habitat suitability criteria reflect the ratio of habitat use to availability for habitat bins or categories spanning each gradient (Newcomb et al. 2007:857–872). First, depth observations were categorized into 10-cm bins, and velocity observations were categorized into 0.2-m/s bins. To ensure that each bin contained at least one observation, all observations greater than 70 cm were combined into a single bin for depth, and all observations greater than 1.0 m/s were combined into a single bin for velocity. Bins for substrate, embeddedness, and silt cover were the same as the original categories described above. Bins for habitat use and availability were subsequently relativized and standardized so that a value of l corresponded with the most selected possible value, while a value of 0 corresponded with the least selected possible value. Finally, we used generalized additive models with a Gaussian error distribution to regress HSC values against the corresponding midpoint of each bin to aid visual interpretation of habitat selection. However, all estimates of suitability are from the original HSC.

Habitat suitability indices.—After developing HSCs from habitat use and availability in EFG and SFC, the HSCs were combined into multivariable and multiple-life-stage habitat

suitability indices (HSIs; Newcomb et al. 2007). An HSI is a type of habitat suitability model that can be easily deconstructed to investigate the contributions of each life stage and variable to species-level estimates of instream suitability. Habitat suitability criteria and HSIs were used to predict the suitability of available habitat within each focal stream. Seasonal habitat suitability for each stream was the arithmetic mean of HSCs for each life stage (life stages l to L, where $L = 3$) based on the five habitat variables (variables v to V, where $V = 5$) for each habitat observation in a stream (n to N, where $N =$ the total number of habitat availability observations per stream and season):

$$\text{HSI}_{\text{stream－season}} = \frac{\sum_{n=1}^{N} \sum_{l=1}^{L} \sum_{v=1}^{V} (\text{HSC})_{nlv}}{N \times L \times V}. \quad (1)$$

Therefore, streamwide suitability within each season was the average HSI value of all measured 0.5-m^2 microhabitat patches based on habitat selection by multiple life stages. Overall habitat suitability for each stream (HSI$_{\text{stream}}$) was calculated as the arithmetic mean of spring and fall HSI values:

$$\text{HSI}_{\text{stream}} = \frac{\text{HSI}_{\text{stream－spring}} + \text{HSI}_{\text{stream－fall}}}{2}. \quad (2)$$

Finally, because life-stage-specific and variable-specific suitability values were nested within the calculation of seasonal suitability for each stream, we deconstructed stream-level HSIs into values for each combination of life stage, season, and habitat variable.

Multivariate habitat use and suitability.—We used nonmetric multidimensional scaling (NMDS) and biplots to visualize how microhabitat use and availability corresponded to predicted HSI values. For each season, microhabitat measurements from the four streams were organized into a Euclidian distance matrix, and the multivariate configuration with the lowest stress value after 20 runs was plotted using two axes. Convex-hull polygons were drawn around all observations of habitat availability in each stream. Next, each observation of habitat availability was color coded to reflect its HSI value. We also added NMDS points corresponding to the microhabitats used by Candy Darters in LC to examine the consistency of microhabitat use across streams. Finally, highly correlated Pearson's product-moment correlation coefficients (Pearson's $r \geq |0.50|$) between axes and instream habitat variables were added to biplots. The coefficients are also provided in Table S.2.

Cross-scale relationships.—We used a framework that examined the relationships between (1) instream habitat gradients across streams currently or formerly containing Candy Darter populations, (2) predicted individual-level suitability within each stream, and (3) observed population robustness across streams. The framework organized these components into a 3 × 3 correlation matrix in which each component was the heading of a single row and column (Figure 2). Analytically, the framework used the regional

	Stream-habitat gradient	Predicted individual suitability	Population robustness
Stream-habitat gradient	$r = 1.0$	r = Predicted individual response	ρ = Observed population relationship
Predicted individual suitability		$r = 1.0$	ρ = Cross-scale relationship
Population robustness			$\rho = 1.0$

FIGURE 2. Framework for examining relationships among stream habitat gradients, predicted individual habitat suitability, and observed robustness of Candy Darter populations across streams (r = Pearson's product-moment correlation coefficient; ρ = Spearman's rank-order correlation coefficient). The relationship between columns 1 and 3 is the observed population relationship to a habitat gradient. The relationship between columns 1 and 2 is the predicted response of individuals to a habitat gradient across streams. The relationship between columns 2 and 3 is the cross-scale relationship between predicted individual suitability and observed population robustness.

pattern of decline to generate a gradient of population robustness that could be compared to other columns of the matrix, thereby imposing the context of localization on relationships between the individual level and the population level.

The first relationship was Spearman's rank-order correlation (ρ) between the mean of each instream habitat variable within each stream and a rank corresponding with population robustness within each stream (i.e., columns 1 and 3). These relationships are typically the focus of distributional surveys aimed at observing population-level responses (e.g., site-level occupancy and abundance) across environmental gradients, which typically must sacrifice site-level intensity for larger spatial extents (i.e., more sites). Possible values of ρ ranged from 1 to –1, indicating positive and negative relationships, respectively, between population robustness and environmental gradients.

The second relationship was Pearson's product-moment correlation between the mean of each instream habitat variable within each stream and the predicted habitat suitability (i.e., HSC and HSI values) of each stream (i.e., columns 1 and 2). Correlations represented the predicted individual responses to habitat gradients at the stream level, and Pearson's r-values could range from 1 to –1. To trust these correlations is to assume that individual-level habitat selection reflects stream-level habitat suitability for populations—an assumption that is frequently not validated (Rosenfeld 2003).

Finally, the relationship between predicted stream-level suitability based on individual-level HSI values (column 2) and a rank of population robustness (column 3) represented the relationship between the individual and population levels. This

correlation, the cross-scale relationship (CSR), will always be positive if individual-level HSIs can be scaled up to reflect population robustness. The CSR served as a form of validation in that negative or weak CSR coefficients indicated disconnects between the two ecological levels. The EFG, SFC, LC, and SC were given ranks of 3 (robust), 3 (robust), 2 (localized), and 1 (extirpated), respectively, which were corroborated by the observed population densities (Table S.3). The framework was inherently qualitative and was designed to facilitate detailed comparisons within and across representative systems. Correlation coefficients provided simple, objective measures of the strength of relationships. Different correlation coefficients were used because the estimated suitability and environmental gradients were ratio scale and normally distributed (i.e., appropriate for Pearson's r), whereas ranks for population status were ordinal and nonparametric (i.e., appropriate for ρ).

Hereafter, we use the term "CSR" to refer to consistent relationships observed at the microhabitat (individual) and stream segment (population) spatial scales. The concept of spatial scaling is well established in ecology (Wiens 1989; Levin 1992) and has catalyzed the proliferation of multiple-scale approaches aimed at identifying relationships among ecological levels of organization and the spatial scales at which habitat is organized (Schneider 2001). Rather than a top-down approach, which is frequently used in habitat suitability investigations, we used a bottom-up approach to examine the ability of microhabitat models to predict the suitability of habitat in stream segments. Figure 2 demonstrates important relationships among scales that are often overlooked when scaling up microhabitat suitability models to the spatial scales necessary to support populations.

RESULTS

Seasonal Habitat Availability across Streams (Columns 1 and 3 in Figure 2)

Streams with extant Candy Darter populations had similar instream habitat. The EFG, SFC, and LC contained many shallow areas (i.e., riffles and shallow runs), whereas SC had a meandering, lower-gradient channel with fewer and more isolated riffles composed of gravel, pebble, and cobble (Table 1). Embeddedness was consistently lower in streams with robust populations (<6%) than in LC or SC (6–25%).

Decreased rain and higher evapotranspiration throughout summer and fall resulted in shallower depths and slower water velocities for all streams in the fall. Seasonal differences in habitat availability were most apparent in EFG, where discharge was reduced by 92% from spring to fall. Despite being a heavily spring-fed system, the reduction in discharge in SC (–79%) was similar to that in LC (–82%) and greater than that in SFC (–73%). However, base flow (i.e., depth and velocity) remained higher in SC, likely due to greater groundwater contributions. Substrate size was the most constant of all variables. There were slightly higher levels of embeddedness and silt cover for most streams during fall, likely due to

TABLE 1. Means (±95% confidence interval in parentheses) and counts of observations of habitat availability for Candy Darters in four streams (EFG = East Fork Greenbrier River; SFC = South Fork Cherry River; LC = Laurel Creek; SC = Sinking Creek) and two seasons. Substrate categories are 1 = silt, 2 = sand, 3 = gravel, 4 = pebble, 5 = small cobble, 6 = large cobble, 7 = small boulder, 8 = large boulder, and 9 = bedrock. Embeddedness and silt categories are 0 = ≤5%, 1 = 6–25%, 2 = 26–50%, 3 = 51–75%, and 4 = >75%.

Stream	Population status	N	Depth (cm)	Velocity (m/s)	Substrate (rank)	Embeddedness (rank)	Silt cover (rank)
				Spring			
EFG	Robust	620	28.8 (1.5)	0.41 (0.02)	5.3 (0.1)	0.3 (0.0)	0.8 (0.1)
SFC	Robust	693	25.9 (1.3)	0.28 (0.02)	5.5 (0.1)	0.3 (0.1)	0.6 (0.1)
LC	Localized	435	26.2 (1.5)	0.35 (0.02)	5.6 (0.2)	1.1 (0.1)	1.0 (0.1)
SC	Extirpated	490	47.5 (2.3)	0.43 (0.02)	4.7 (0.1)	1.5 (0.1)	0.8 (0.1)
				Fall			
EFG	Robust	212	16.2 (2.2)	0.11 (0.02)	5.1 (0.2)	0.4 (0.1)	1.1 (0.1)
SFC	Robust	277	19.1 (1.7)	0.15 (0.02)	5.3 (0.2)	0.5 (0.1)	0.8 (0.1)
LC	Localized	440	17.6 (1.2)	0.15 (0.02)	5.1 (0.2)	1.0 (0.1)	0.9 (0.1)
SC	Extirpated	515	32.5 (1.6)	0.19 (0.02)	4.5 (0.1)	1.8 (0.1)	1.3 (0.1)

deposition of suspended sediment coinciding with reduced stream discharge. Higher fine-sediment levels from spring to fall were most pronounced for SC (embeddedness in spring = 1.5, ±95% confidence interval [CI] = 0.1; embeddedness in fall = 1.8, 95% CI = 0.1; silt in spring = 0.8, 95% CI = 0.1; silt in fall = 1.3, 95% CI = 0.1), which was relatively turbid in spring but clear in the fall (C.G.D., personal observation).

Relationships between population status and environmental gradients (columns 1 and 3 in Figure 2) tended to be strong and consistent across seasons (Table 2). We interpreted large coefficients ($\rho \geq |0.50|$) as indicators of strong population relationships with environmental gradients; consistency in the direction of coefficients across seasons indicated few seasonal effects on these relationships. Streams with more robust populations tended to be shallower ($\rho = -0.63$), to have less-embedded and less-silted substrates (embeddedness: $\rho = -0.95$, silt cover: $\rho = -0.42$), and to have slower water velocities ($\rho = -0.79$). The negative relationship between average stream-level water velocity and population robustness was higher in fall during low-flow conditions (spring: $\rho = -0.63$; fall: $\rho = -0.95$). Finally, there was a positive, albeit weak, correlation between substrate size and population robustness ($\rho = 0.47$).

Individual Habitat Selection

We recorded 290 (EFG = 115; SFC = 175) and 508 (EFG = 286; SFC = 222) microhabitat use observations for multiple life stages in the spring and fall, respectively. Among the three life stages, counts of adults were the most consistent across systems (EFG = 135; SFC = 151) and seasons (spring = 137; fall = 149). Counts of subadult life stages (age-0 and juvenile fish) were higher in the fall, coinciding with new recruitment. We also observed Candy Darters at 8 (spring) and 14 (fall) of 20 total sites throughout LC. Adults and juveniles were observed during spring, and all three life stages were detected during fall.

Selection curves for all life stages across seasons were either approximately monotonic or unimodal, indicating that observed curves were consistent with the selection of habitat across environmental gradients (Figure 3). Clear, biologically sensible selection patterns aid interpretation of habitat associations and obviate the need to rely on P-values from tests of nonrandom habitat selection (Cherry 1998). Generally, most life stages selected microhabitats with at least moderate flow (>0.19 m/s), shallow depths (<0.5 m), coarse substrates (>sand), and nonembedded and nonsilted substrates (<26%). However, each life stage demonstrated more nuanced habitat selection patterns corresponding with age and body size. The most pronounced ontogenetic differences were for water velocity, with adults selecting the swiftest water velocities

TABLE 2. Spearman's rank-order correlation coefficients (ρ) between habitat variables and a rank representing the population status of Candy Darters in four study streams and two seasons (columns 1 and 3 in Figure 2). Streams with the highest Candy Darter densities were given the highest rank (East Fork Greenbrier River = 3 [robust]; South Fork Cherry River = 3 [robust]; Laurel Creek = 2 [localized]; Sinking Creek = 1 [extirpated]). Predictions are based on historical accounts of habitat use (Table S.1). The predicted relation for embeddedness was not applicable (NA) because there were no historical accounts. Values of ρ that were $\geq |0.50|$ and consistent with predictions are shown in bold to emphasize the strength of the relationship; ρ values that were $\geq |0.50|$ and inconsistent with predictions are italicized. The "Combined" column presents the averages of spring and fall values.

Variable	Predicted relationship	Spring	Fall	Combined
Depth	–	**–0.63**	**–0.63**	**–0.63**
Velocity	+	*–0.63*	*–0.95*	*–0.79*
Substrate	+	0.32	**0.63**	0.47
Embeddedness	NA	**–0.95**	**–0.95**	**–0.95**
Silt cover	–	–0.21	**–0.63**	–0.42

available in spring (>1.20 m/s) and fall (>0.60 m/s). Juveniles selected intermediate water velocities (0.40–1.20 m/s) in both seasons, while age-0 fish selected slower water velocities (0.0–0.80 m/s). Similar ontogenetic patterns occurred for substrate, embeddedness, and silt cover. Adults selected larger substrates and avoided areas with fine sediments, resulting in near-zero HSC values for all microhabitats with embeddedness or silt cover scores greater than 25% (rank = 2). Younger life stages selected smaller substrates and were less averse to fine sediments.

Ontogenetic habitat selection patterns were similar across seasons. The most pronounced difference was that of juveniles, which selected velocities more similar to those of adults in fall than in spring (Figure 3). When individual variables were collectively viewed, the observed ontogenetic differences were attributable to habitat shifts from the pool margins and runs occupied by age-0 fish to the swift, turbulent riffles occupied by adults. Juveniles tended to select run channel units or riffle margins in spring and shifted to riffles by fall (i.e., intermediate habitat selection).

Our underwater observations enabled us to document the behavior underlying habitat selection patterns (Jordan et al. 2008). Individuals tended to segregate by life stage rather than behavior mode. For example, in spring, adults foraged, used cover, and displayed behavior associated with spawning (e.g., antagonistic behavior or courtship) within the most selected habitats. None of the habitat use observations revealed the exact locations selected by females for egg deposition, but spawning was observed during surveys and occurred near areas that were strongly selected by adults in spring.

Most of the Candy Darters inhabiting LC used habitat patches that were similar to those used in EFG and SFC (Figure 4; Figure S.2). Low sample sizes prevented us from developing selection curves for each life stage based on observations of habitat use and availability within LC; however, nearly all observations of habitat use were consistent with projected highly suitable habitat.

Individual-Level Habitat Suitability within and across Streams (Columns 1 and 2 in Figure 2)

In spring, two distinct groups of suitability values were apparent: streams with robust Candy Darter populations (EFG: HSI = 0.68, 95% CI = 0.01; SFC: HSI = 0.66, 95% CI = 0.02) and streams where Candy Darters were localized (LC: HSI = 0.58, 95% CI = 0.02) or extirpated (SC: HSI = 0.56, 95% CI = 0.01; Table S.4). The EFG had the highest overall HSI value as a result of having the highest HSC values for depth, velocity, and substrate size. Lower HSI values for LC and SC were attributable to their low HSC values for embeddedness and substrate size (Table S.4).

Habitat suitability values were lower in fall than in spring due to less-suitable depths, velocities, and fine-sediment levels. In the fall, HSI values also separated into two tiers; however, unlike spring, the highest tier comprised streams with extant populations (EFG: HSI = 0.51, 95% CI = 0.02; SFC: HSI = 0.56, 95% CI = 0.02; LC: HSI = 0.52, 95% CI = 0.02), while SC had markedly lower HSI values (HSI = 0.44, 95% CI = 0.01; Table S.4). Sinking Creek remained the least suitable stream due to its relatively low HSC values for embeddedness and silt cover.

These results were corroborated by NMDS plots of projected habitat suitability for each observation of available microhabitat within the four streams (Figure 4 for spring; Figure S.2 for fall). In both spring and fall, the most suitable microhabitats occurred in high-velocity areas composed of coarse substrates and few fine sediments. Although highly suitable habitat was within the environmental space enveloped by all four streams, SC contained more areas with low suitability, representing slower, more embedded, or more silted habitat patches.

Predicted habitat suitability across streams mirrored individual-level habitat selection. The strongest correlations between predicted suitability and habitat availability at the stream scale (i.e., Pearson's $r \geq |0.50|$) across seasons were negative relationships with embeddedness and silt cover for all life stages (Table 3). In other words, predicted individual-level suitability (i.e., selection) decreased with greater average embeddedness and silt cover across the four streams. Strong positive relationships with increasing water velocity were also observed for adults and juveniles across streams. Predicted suitability for both depth and substrate tended to be either weakly consistent or inconsistent with predicted relationships based on historical accounts of habitat selection by Candy Darters (Table S.1). Inconsistencies reflected seasonal differences in depth selection (spring: Pearson's $r = -0.42$; fall: Pearson's $r = 0.01$) and ontogenetic differences between adults and younger life stages for depth (adult: Pearson's $r = 0.21$; juvenile: Pearson's $r = -0.12$; age 0: Pearson's $r = -0.37$) and substrate (adult: Pearson's $r = 0.21$; juvenile: Pearson's $r = -0.34$; age 0: Pearson's $r = -0.66$), which demonstrates that temporal or ontogenetic habitat shifts can generate conflicting habitat suitability predictions across a species' life cycle.

Relationships between Predicted Individual-Level Habitat Suitability and Population Robustness across Streams (Columns 2 and 3 in Figure 2)

Overall, when averaged across two seasons and three life stages, predicted habitat suitability was positively correlated with population robustness (studywide CSR coefficient [ρ_{CSR}] = 0.95; Figure 5), indicating that the proportion of suitable microhabitats within a stream was related to population robustness. However, the strength of these relationships varied with life stage, season, and habitat variable. The HSIs had higher CSR coefficients in spring (ρ_{CSR} = 0.95) than in fall (ρ_{CSR} = 0.63) owing to weaker relationships for velocity and substrate size in the fall. All life stages had equal CSR coefficients after averaging HSI values across seasons (ρ_{CSR} = 0.95); however, coefficients for adults were consistently the
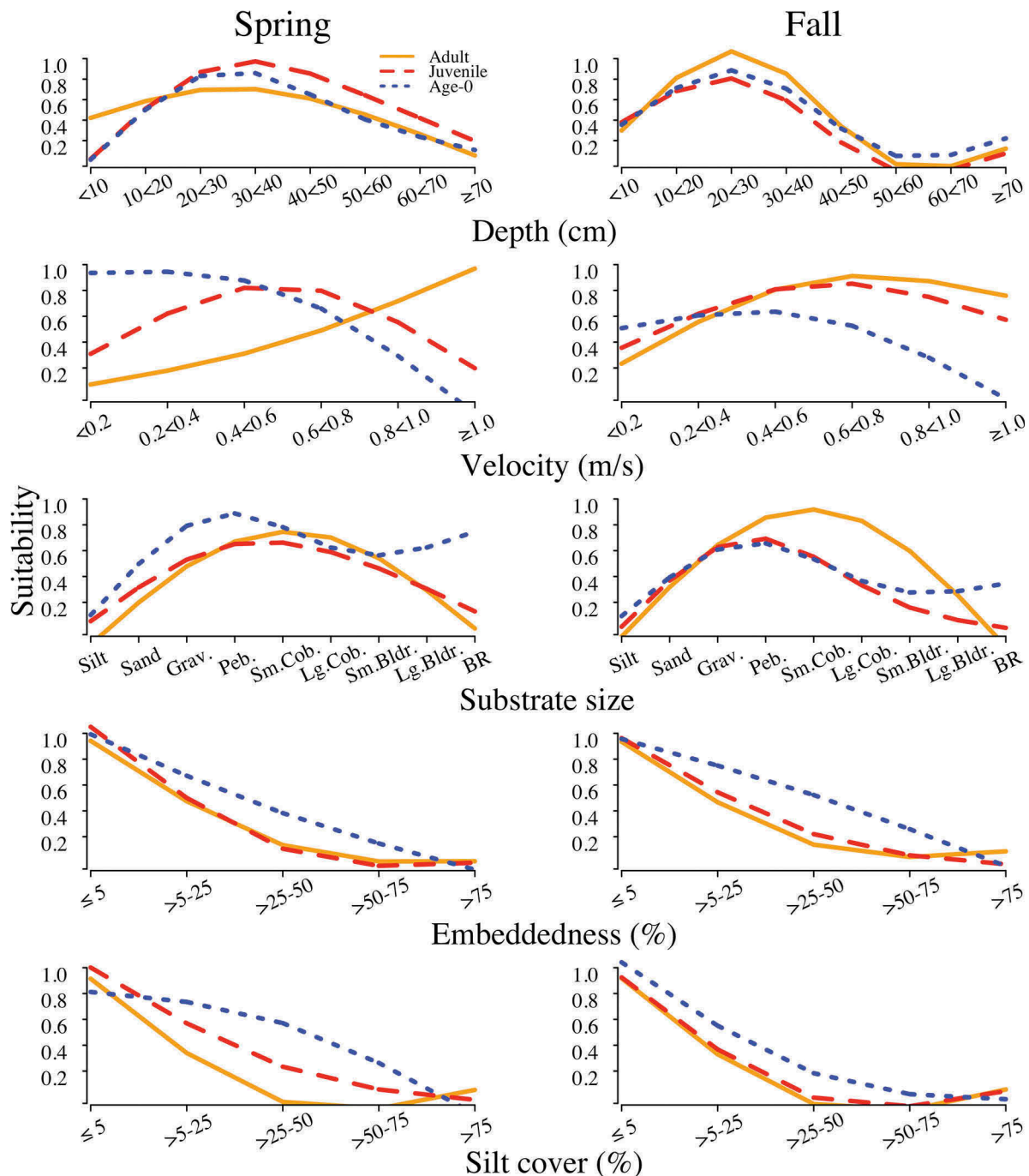
FIGURE 3. Habitat selection curves developed from habitat used by Candy Darters during three life stages and two seasons and the available habitat in two streams. Continuous curves, presented as visual aids, were obtained by regressing suitability values against the midpoint of each bin using generalized additive regression models (substrate abbreviations: Grav. = gravel; Peb. = pebble; Sm. Cob. = small cobble; Lg. Cob. = large cobble; Sm. Bldr. = small boulder; Lg. Bldr. = large boulder; BR = bedrock).

highest for both seasons (spring: $\rho_{CSR} = 0.96$; fall: $\rho_{CSR} = 0.96$), which may be due to the greater microhabitat specificity of adults. Coefficients for depth and substrate size were the most inconsistent, which indicates that these variables may only be important at the population level during certain life stages or seasons. In contrast, velocity consistently had the most negative CSR for all scenarios ($\rho_{CSR} = -1.0$), which indicates that despite strong selection of high-velocity habitat, streams with more high-velocity habitat patches did not support more robust populations. Velocity CSR coefficients were
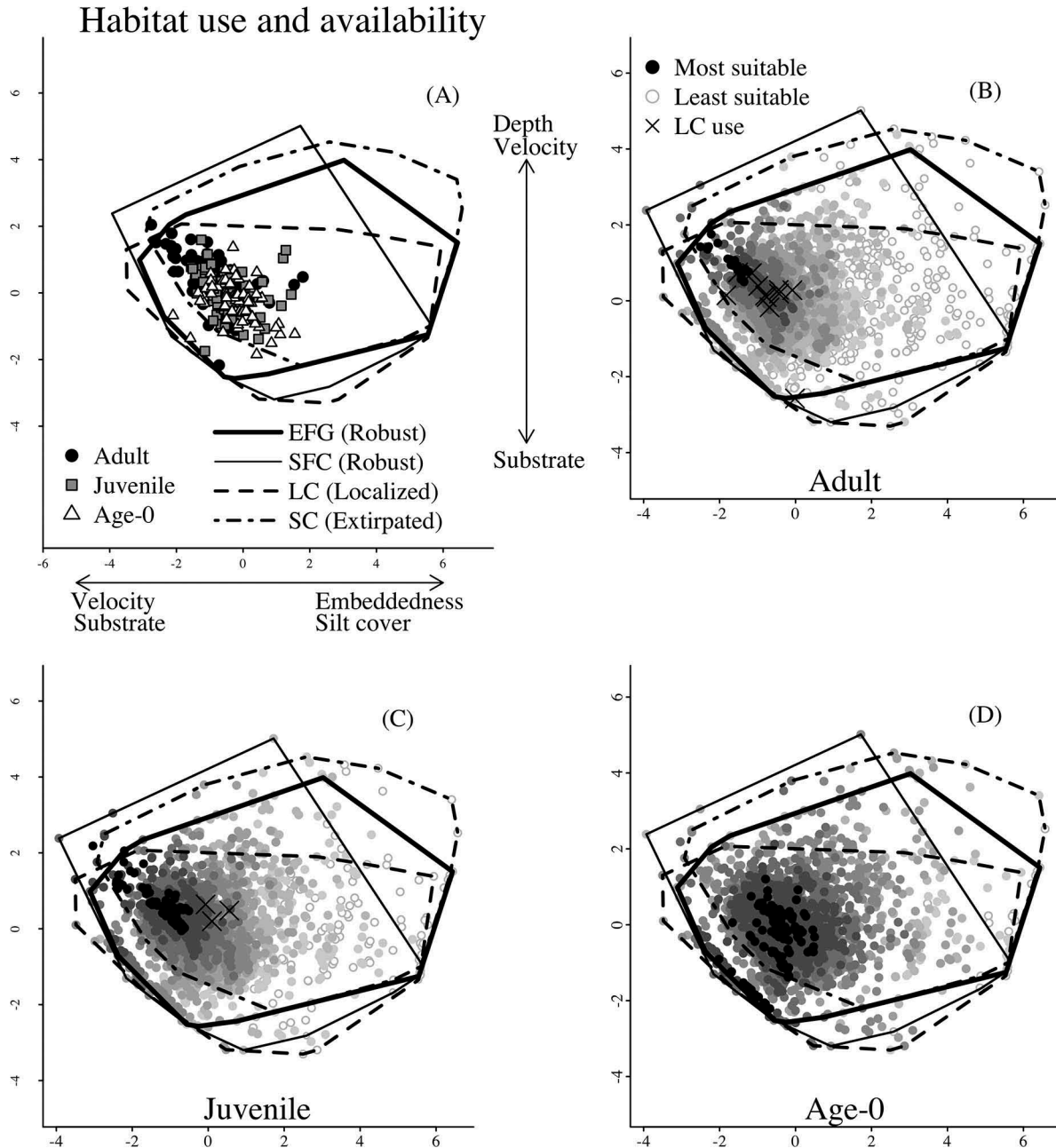
Habitat use and availability

FIGURE 4. Nonmetric multidimensional scaling (NMDS) plots of habitat use, availability, and suitability for Candy Darters in spring: **(A)** habitat use by three life stages and availability in four streams (polygons; EFG = East Fork Greenbrier River; LC = Laurel Creek; SC = Sinking Creek; SFC = South Fork Cherry River); **(B)** predicted habitat suitability for adults; **(C)** predicted habitat suitability for juveniles; and **(D)** predicted habitat suitability for age-0 fish. Symbols for "LC use" represent locations used by Candy Darters in Laurel Creek during the spring. Variables that are highly correlated (Pearson's product-moment correlation coefficient [Pearson's r] ≥ 0.50) with axes are shown. All Pearson's r-values are presented in Table S.2. The NMDS stress value was 0.17. See Figure S.2 for NMDS plot for fall data.

more negative in fall, when all streams with extant populations had slower average velocities than SC, where Candy Darters were extirpated (spring: $\rho_{CSR} = -0.39$; fall: $\rho_{CSR} = -0.50$). The CSR correlations for silt cover were season specific and life stage specific but were positive overall ($\rho_{CSR} = 0.63$). Finally, embeddedness HSI values were highly correlated with population robustness regardless of season (spring: $\rho_{CSR} = 0.95$; fall: $\rho_{CSR} = 0.63$) or life stage (adult: $\rho_{CSR} = 0.95$; juvenile: $\rho_{CSR} = 0.95$; age 0: $\rho_{CSR} = 0.95$), which indicates that embeddedness is consistently the most important parameter for both the selection of microhabitats by individuals and the robustness of populations.

TABLE 3. Pearson's product-moment correlation coefficients (Pearson's r) between predicted individual habitat suitability for Candy Darters and averages of five instream habitat variables across four streams that varied in Candy Darter population status (columns 1 and 2 in Figure 2). Predictions are based on prior accounts of habitat use (Table S.1). The predicted relation for embeddedness was not applicable (NA) because there were no historical accounts. "Multi-stage" is the correlation between average suitability across multiple life stages (Table S.4) and habitat gradients (Table 1). Pearson's r-values that were $\geq |0.50|$ and consistent with predictions are shown in bold to emphasize the strength of the relationship. Pearson's r-values that were $\geq |0.50|$ and inconsistent with predictions are italicized. The "Combined" column presents the averages of spring and fall values.

Variable	Predicted relationship	Adults	Juveniles	Age 0	Multi-stage
Spring					
Depth	–	–0.10	0.21	**–0.74**	–0.42
Velocity	+	**0.97**	**0.99**	–0.19	**0.99**
Substrate	+	0.15	0.25	*–0.54*	0.06
Embeddedness	NA	**–1.00**	**–1.00**	**–1.00**	**–1.00**
Silt cover	–	–0.36	**–0.92**	**–0.69**	**–0.98**
Fall					
Depth	–	*0.52*	–0.45	–0.01	0.01
Velocity	+	**0.93**	**0.78**	**0.74**	**0.77**
Substrate	+	0.28	*–0.93*	*–0.78*	*–0.71*
Embeddedness	NA	**–1.00**	**–1.00**	**–1.00**	**–1.00**
Silt cover	–	**–0.91**	**–0.90**	**–0.99**	**–0.93**
Combined					
Depth	–	0.21	–0.12	–0.37	–0.20
Velocity	+	**0.96**	**0.89**	0.28	**0.88**
Substrate	+	0.21	–0.34	*–0.66*	–0.32
Embeddedness	NA	**–1.00**	**–1.00**	**–1.00**	**–1.00**
Silt cover	–	**–0.63**	**–0.91**	**–0.84**	**–0.96**

DISCUSSION

Much of North America's imperiled fish fauna has an unnatural distributional pattern marked by disjunct yet viable populations occupying a fraction of their historical range (Jelks et al. 2008). A key to fish conservation is the identification of habitat that promotes the resistance or resiliency of these populations to factors that diminish habitat quality. We used a comparative approach aimed at directly contrasting systems that are currently supporting Candy Darter populations and those that formerly supported populations. Often, the processes underlying the localization of populations are anthropogenic; therefore, this comparative approach may reflect the gradients leading to the decline of the Candy Darter.

Scaling Up Individual Habitat Selection to Populations

Many fishes exhibit complex life cycles that are marked by the use of distinctive habitat patches through ontogeny, yet much of the existing management is based solely on adult
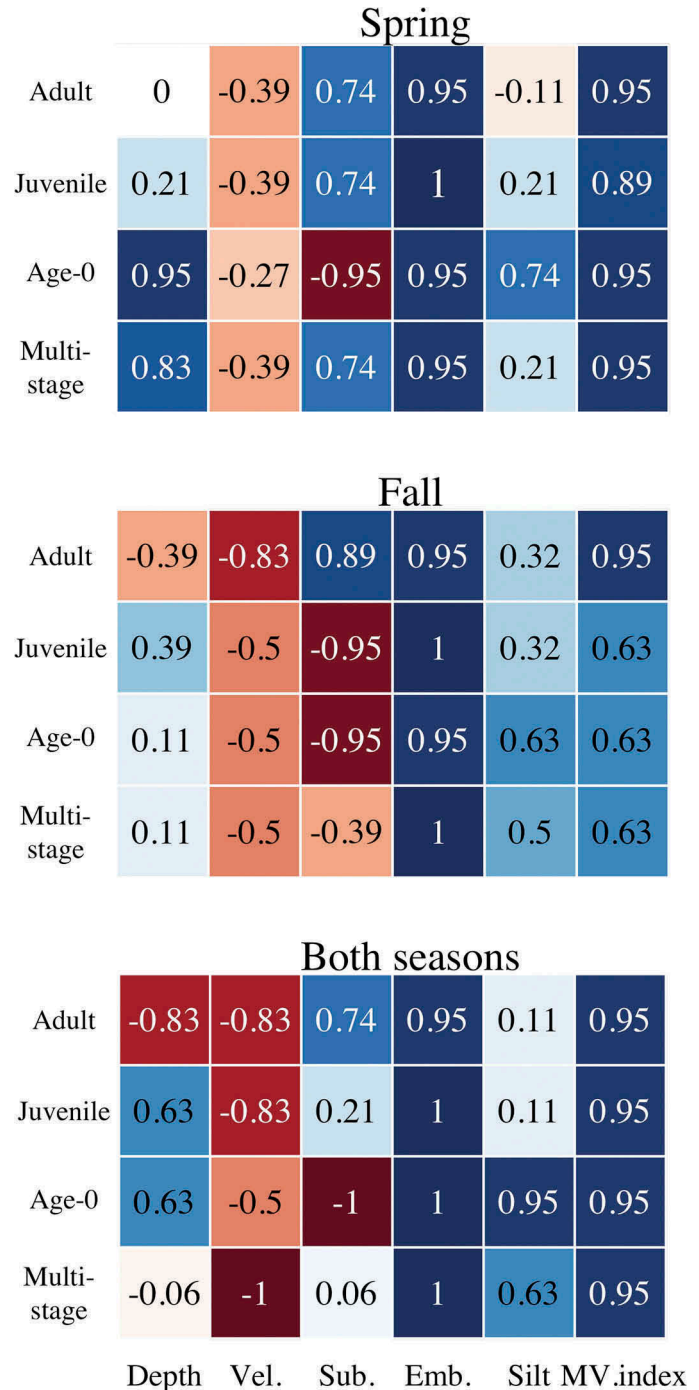

FIGURE 5. Spearman's rank-order correlation coefficients (ρ) between predicted individual microhabitat suitability and Candy Darter population robustness in four streams during two seasons (columns 2 and 3 in Figure 2; Multi-stage = multiple-life-stage habitat suitability index; MV.index = multiple-variable habitat suitability index; Vel. = velocity; Sub. = substrate size; Emb. = embeddedness). Positive coefficients (blue) indicate that individual habitat selection predicts population robustness, whereas negative coefficients (red) indicate disconnects between the individual level and the population level. Darker shading reflects greater absolute value of ρ. The lower far-right cell (ρ = 0.95) represents the studywide cross-scale relationship.

habitat use (Copp and Vilizzi 2004; King 2004), which may not be as limiting as habitat for subadults. Although our observations of habitat selection by adult Candy Darters were largely consistent with previous accounts of adults, clear ontogenetic differences were documented, most notably for depth, substrate, and velocity. The most apparent ontogenetic shift by Candy Darters was for velocity, with age-0 fish selecting low-velocity to moderate-velocity areas and adults being largely restricted to high-velocity areas. These observations are consistent with habitat shifts observed in other species of darter (Rosenberger and Angermeier 2003; Skyfield and Grossman 2008; Ashton and Layzer 2010). For Candy Darters, it is unclear whether these shifts are structured by predation risk (Schlosser 1987; Werner and Hall 1988; Labeelund et al. 1993), the energetic costs of maintaining position in fast flows (Lobb and Orth 1991; Mann and Bass 1997; Moore and Thorp 2008), differing food sources (Schlosser 1990; King 2004), or intraspecific competition (Davey et al. 2005; Petty and Grossman 2007). In spring, adult males were highly territorial near areas where spawning occurred (i.e., staging areas), and this may have excluded juveniles from the high-velocity habitats. Our observations of adult male territoriality and lower spatial overlap between adults and juveniles in spring during the spawning season tentatively support intraspecific competition as one potential mechanism structuring observed ontogenetic habitat shifts.

Microhabitat use is a product of complex interactions among fish size, behavior mode, physiological state, intraspecific and interspecific interactions, and habitat availability. As a result of this complexity, selection could be a measure of the most suitable habitat available for individuals, but alternative habitat may be substitutable and therefore the resource may be less influential at the population level (Rabeni and Sowa 1996). If so, individual selection could mislead managers to incorporate nonessential resources into their guiding image of suitable habitat. For example, adult Candy Darters are flow specialists based on their specificity for high-velocity, shallow microhabitats (Chipps et al. 1994); however, the negative CSR coefficients for water velocity and depth demonstrated that suitable microhabitats for these variables were more available or similarly available in SC compared to the streams with viable populations. Seasonal decreases in suitable velocity and depth microhabitats were greatest in EFG, where discharge was the most reduced from spring to fall. Rather than observing lower abundances of adults as a result of mortality or emigration in fall during low-flow conditions, we found that all life stages—especially adults—compensated by shifting locations to the most suitable flows available. A hypothesis warranting further testing is that low fall flows enhance the survival of age-0 individuals, which frequent shallow, slow microhabitats, as observed in other stream fishes (Schlosser 1982; Rosenberger and Angermeier 2003). Additionally, periods of drought or low flows can have a disproportionate negative influence on the large piscivorous

fishes that prey upon age-0 fish (Schlosser 1987). Low fall flows may have created nursery habitat that was unsuitable for predators, in turn allowing for expanded foraging in warmer, more productive habitat (Moore and Gregory 1988; Henderson and Johnston 2010) and less density dependence among the large age-0 year-classes we observed in both of the streams with robust populations (Schlosser 1990). Similarly, individuals selected certain substrate sizes during specific life stages, but the near-zero CSR coefficient indicated that substrate size may not be limiting at the population level within the context of localization. Overall, our results suggest that ontogenetic shifts and seasonal habitat plasticity may limit the management utility of a simple, generalized image of suitable habitat for a species based solely on the selection of habitat by a single life stage during a single season or potentially even a single year.

The largest and most consistent CSR coefficients indicated that both individual habitat selection and population robustness were negatively related to embeddedness. Embeddedness can profoundly alter the function of stream ecosystems and has been implicated in the declines of most imperiled fishes in North America (Jelks et al. 2008). The specific pathways through which elevated embeddedness may influence individuals and populations of Candy Darters remain unexamined. Potential hypotheses are the filling of interstitial spaces, which can alter food webs by reducing the microhabitats used by macroinvertebrates (Ryan 1991; Henley et al. 2000) and can eliminate structure used for cover and refugia. Alternatively, observed negative relationships could covary with life stages not studied herein, such as a loss of rearing habitat that is suitable for eggs and larvae. Future studies specifically aimed at identifying relationships among the characteristics, placement, and abundance of the exact habitat patches needed for egg incubation and larval survival would help to determine the role of these life stages in the population dynamics within each stream.

Additional research on several topics would help to clarify the mechanisms underlying the decline of the Candy Darter. For example, documenting fish responses to experimental manipulations of habitat would help to reduce any observational biases associated with habitat selection patterns and to control for multicollinearity among variables (Rosenfeld 2003). Moreover, the present study quantified habitat availability at a spatial scale that was large enough to be germane to Candy Darter population dynamics (i.e., stream segment), but additional research spanning relevant temporal scales (i.e., multiple years) could provide a better understanding of the consistency of habitat availability and the stability of predicted suitability under different conditions. However, until specific mechanisms influencing individual fitness and population dynamics are understood, managers could utilize our HSIs, and especially the embeddedness selection curves, to help identify sites with suitable habitat for the translocation or restoration of Candy Darters.

Hypothesized Ecological Processes among Instream Habitat Patches

Instream habitat, as perceived by small aquatic organisms, represents a landscape of microhabitat patches with varying quality (Palmer et al. 2000). For continued occupation within a region, nonsubstitutable resources must be abundant enough, accessible, and in harmony with the life cycle of a species. On average, habitat patches in SC had the lowest suitability for each of the three life stages investigated. However, the NMDS plots and HSIs indicated that highly suitable habitat patches existed in SC. It is unclear whether the prevalence of these patches, particularly unembedded substrate, is too low to support a Candy Darter population. Poor habitat suitability may interact with other population threats and further diminish population resistance to altered conditions. When suitable habitat is proportionally low and spatially diffuse, the suitability of habitat patches is likely reduced by neighborhood effects from the surrounding poor-quality habitat (Dunning et al. 1992; Schlosser 1995). Moreover, when navigating corridors between suitable patches, individuals may be exposed to fitness-reducing factors, such as an elevated risk of predation, which may be exacerbated by a reduction in benthic complexity in embedded systems (Roberts and Angermeier 2007). Many nonnative fishes, including piscivores, have colonized SC (Hitt and Roberts 2012) and now occupy likely corridor habitat. Although no information exists on the movements of Candy Darters among suitable patches or the predation rates by introduced piscivores, Labbe and Fausch (2000) found that nonnative piscivores influenced the demographic rates of the Arkansas Darter *E. cragini* due to predation in corridor habitat. Understanding interactions between multiple-scale habitat suitability and other factors, such as predation and movement, will require detailed demographic investigation. Nonetheless, findings suggest that the prevalence and harshness of the matrix of nonsuitable habitat could be as important as the presence of suitable habitat within an area. These findings are consistent with other multiple-scale investigations of darter habitat, which have reported that the presence of suitable habitat nested within a matrix of poor-quality habitat may not be enough to sustain populations (Freeman and Freeman 1994; Davis and Cook 2010; Compton and Taylor 2013). For species with uncertain habitat requirements, the incorporation of multiple scales into investigations may help to identify consistencies across populations and individuals (Torgersen et al. 1999; Fausch et al. 2002) and to refine hypotheses related to limiting habitat parameters.

Application to Imperiled Species Management and Recovery

Frameworks that employ realistic and validated benchmarks are staples of stream restoration and biomonitoring (Stoddard et al. 2006; Whittier et al. 2007) but are less common when defining fish habitat suitability at the site and population levels. We estimated the habitat suitability of streams where Candy Darter populations were robust (EFG and SFC) or localized (LC). Consequently, segment-scale suitability values for EFG and SFC are also ecologically derived benchmarks of optimal instream habitat conditions among known populations of the Candy Darter, while values for LC may meet the minimum habitat conditions necessary to sustain a population. In contrast, HSI values are often categorized into levels of suitability (e.g., "optimal" habitat ≥ 50th percentile of HSI [Thomas and Bovee 1993], HSI ≥ 0.40 [Freeman et al. 1997]), which may be meaningful benchmarks for predicting the habitats selected by individuals but may not have significance for populations. Attempts at validating individual habitat selection often examine the consistency of habitat selection across systems (Newcomb et al. 2007). However, even if habitat use is consistent, the approach still does not establish relationships between individual habitat selection and population function. Alternatively, the approach used here effectively rescaled suitability based on individual habitat selection to represent segment-level suitability for a population, which will likely be more meaningful for conservation efficacy.

Validating individual habitat selection at the stream scale may be particularly applicable for imperiled species management. For example, recovery plans often aim to re-establish extirpated populations (George et al. 2009), yet historical conditions within streams are rarely documented. Managers could reference suitability values from streams with robust populations when identifying streams with suitable habitat for reintroduction. Alternatively, potentially more realistic criteria may be the suitability values from streams supporting small populations (e.g., LC) given that additional (albeit small) populations can dilute the risk of regional extirpation.

Conclusions

The HSIs for Candy Darters should not be considered infallible or definitive. A correlative framework is no substitute for detailed study of the mechanisms influencing individuals or populations. However, more direct measures of individual fitness (e.g., growth and fecundity) or population function (e.g., demographic rates) across additional streams could be incorporated within the general framework described herein (Figure 2). A clear understanding of habitat requirements is typically gained through a progression of detailed investigation at multiple ecological levels (Rosenfeld 2003). Our approach is likely helpful in identifying the limiting habitat types at the beginning of this progression, which could potentially assist in directing future investigations and conservation measures.

Candy Darters are highly selective of specific instream habitat patches within occupied streams, and habitat selection varies through ontogeny. Habitat specificity may reflect adaptive benefits of certain patch types for growth, survival, and reproduction under natural conditions. However, when viewed across populations that were affected by anthropogenic disturbances, habitat specificity did not always indicate limiting conditions. Similar to many nongame species, the only habitat information available for Candy Darters prior to this investigation were descriptions of adult habitat use within short reaches (<150 m) in a few streams during a single season. While our findings

support some of these descriptions, ontogenetic shifts and seasonal habitat plasticity make habitat selection more complex than previously described. Such complexity demonstrates that studies aimed at the individual level could be potentially misleading when identifying habitat that is suitable to facilitate population persistence. This finding underscores the potential inadequacy of the information guiding management decisions for many of North America's freshwater fishes. Until rigorous study of relationships between individuals and populations becomes the norm for species with lower management priority, the framework used here may be a viable approach to identifying habitat parameters that are important at both levels of ecological organization.

ACKNOWLEDGMENTS

REFERENCES

Angermeier, P. L. 1995. Ecological attributes of extinction-prone species: loss of freshwater fishes of Virginia. Conservation Biology 9:143–158.

Ashton, M. J., and J. B. Layzer. 2010. Summer microhabitat use by adult and young-of-the-year Snail Darters (*Percina tanasi*) in two rivers. Ecology of Freshwater Fish 19:609–617.

Boavida, I., J. M. Santos, R. Cortes, A. Pinheiro, and M. T. Ferreira. 2012. Benchmarking river habitat improvement. River Research and Applications 28:1768–1779.

Burkhead, N. M. 2012. Extinction rates in North American freshwater fishes, 1900–2010. Bioscience 62:798–808.

Burton, G. W., and E. P. Odum. 1945. The distribution of stream fish in the vicinity of Mountain Lake, Virginia. Ecology 26:182–194.

Cherry, S. 1998. Statistical tests in publications of The Wildlife Society. Wildlife Society Bulletin 26:947–953.

Chipps, S. R., W. B. Perry, and S. A. Perry. 1993. Status and distribution of *Phenacobius teretulus, Etheostoma osburni*, and "*Rhinichthys bowersi*" in the Monongahela National Forest, West Virginia. Virginia Journal of Science 44:48–58.

Chipps, S. R., W. B. Perry, and S. A. Perry. 1994. Patterns of microhabitat use among four species of darters in three Appalachian streams. American Midland Naturalist 131:175–180.

Compton, M., and C. Taylor. 2013. Spatial scale effects on habitat associations of the Ashy Darter, *Etheostoma cinereum*, an imperiled fish in the southeast United States. Ecology of Freshwater Fish 22:178–191.

Copp, G. H., and L. Vilizzi. 2004. Spatial and ontogenetic variability in the microhabitat use of stream-dwelling Spined Loach (*Cobitis taenia*) and Stone Loach (*Barbatula barbatula*). Journal of Applied Ichthyology 20:440–451.

Davey, A. J. H., S. J. Hawkins, G. F. Turner, and C. P. Doncaster. 2005. Size-dependent microhabitat use and intraspecific competition in *Cottus gobio*. Journal of Fish Biology 67:428–443.

Davis, J. G., and S. B. Cook. 2010. Habitat use of the Tuxedo Darter (*Etheostoma lemniscatum*) at macrohabitat and microhabitat spatial scales. Journal of Freshwater Ecology 25:321–330.

Dixon, C. J., and J. C. Vokoun. 2009. Burbot resource selection in small streams near the southern extent of the species range. Ecology of Freshwater Fish 18:234–246.

Dolloff, C. A., D. G. Hankin, and G. H. Reeves. 1993. Basinwide estimation of habitat and fish populations in streams. U.S. Forest Service, Report SE-93, Asheville, North Carolina.

Dunham, J. B., B. S. Cade, and J. W. Terrell. 2002. Influences of spatial and temporal variation on fish–habitat relationships defined by regression quantiles. Transactions of the American Fisheries Society 131:86–98.

Dunning, J. B., B. J. Danielson, and H. R. Pulliam. 1992. Ecological processes that affect populations in complex landscapes. Oikos 65:169–175.

Fausch, K. D., C. L. Hawkes, and M. G. Parsons. 1988. Models that predict standing crop of stream fish from habitat variables: 1950–1985. U.S. Forest Service Report PNW-GTR-213.

Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. Bioscience 52:483–498.

Freeman, B., and M. Freeman. 1994. Habitat use by an endangered riverine fish and implications for species protection. Ecology of Freshwater Fish 3:49–58.

Freeman, M. C., Z. H. Bowen, and J. H. Crance. 1997. Transferability of habitat suitability criteria for fishes in warmwater streams. North American Journal of Fisheries Management 17:20–31.

Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley. 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. Environmental Management 10:199–214.

Gabelhouse, D. W. 2005. Staffing, spending, and funding of state inland fisheries programs. Fisheries 30(2):10–17.

George, A. L., B. R. Kuhada, J. D. Williams, M. A. Cantrell, P. L. Rakes, and J. R. Shute. 2009. Guidelines for propagation and translocation for freshwater fish conservation. Fisheries 34:529–545.

Grimm, V., and S. F. Railsback. 2005. Individual-based modeling and ecology. Princeton University Press, Princeton, New Jersey.

Grossman, G. 2013. Not all drift feeders are trout: a short review of fitness-based habitat selection models for fishes. Environmental Biology of Fishes 97:465–473.

Guay, J. C., D. Boisclair, D. Rioux, M. Leclerc, M. Lapointe, and P. Legendre. 2000. Development and validation of numerical habitat models for juveniles of Atlantic Salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 57:2065–2075.

Haxton, T. J., C. S. Findlay, and R. W. Threader. 2008. Predictive value of a Lake Sturgeon habitat suitability model. North American Journal of Fisheries Management 28:1373–1383.

Henderson, A. R., and C. E. Johnston. 2010. Ontogenetic habitat shifts and habitat use in an endangered minnow, *Notropis mekistocholas*. Ecology of Freshwater Fish 19:87–95.

Henley, W., M. Patterson, R. Neves, and A. D. Lemly. 2000. Effects of sedimentation and turbidity on lotic food webs: a concise review for natural resource managers. Reviews in Fisheries Science 8:125–139.

Hewitt, A. H., T. J. Kwak, W. G. Cope, and K. H. Pollock. 2009. Population density and instream habitat suitability of the endangered Cape Fear Shiner. Transactions of the American Fisheries Society 138:1439–1457.

Hitt, N. P., and J. H. Roberts. 2012. Hierarchical spatial structure of stream fish colonization and extinction. Oikos 121:127–137.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372–407.

Jenkins, R. E., and N. M. Burkhead. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, Maryland.

Jenkins, R. E., and B. L. Kopia. 1995. Population status of the Candy Darter, *Etheostoma osburni*, in Virginia 1994–1995, with historical review. Roanoke College, Department of Biology, Final Report, Salem, Virginia.

Jordan, F., H. L. Jelks, S. A. Bortone, and R. M. Dorazio. 2008. Comparison of visual survey and seining methods for estimating abundance of an endangered, benthic stream fish. Environmental Biology of Fishes 81:313–319.

King, A. J. 2004. Ontogenetic patterns of habitat use by fishes within the main channel of an Australian floodplain river. Journal of Fish Biology 65:1582–1603.

Kwak, T. J., M. J. Wiley, L. L. Osborne, and R. W. Larimore. 1992. Application of diel feeding chronology to habitat suitability analysis of warmwater stream fishes. Canadian Journal of Fisheries and Aquatic Sciences 49:1417–1430.

Labbe, T. R., and K. D. Fausch. 2000. Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. Ecological Applications 10:1774–1791.

Labeelund, J. H., A. Langeland, B. Jonsson, and O. Ugedal. 1993. Spatial segregation by age and size in Arctic Charr: a trade-off between feeding possibility and risk of predation. Journal of Animal Ecology 62:160–168.

Leftwich, K. N., P. L. Angermeier, and C. A. Dolloff. 1997. Factors influencing behavior and transferability of habitat models for a benthic stream fish. Transactions of the American Fisheries Society 126:725–734.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology 73:1943–1967.

Lobb, M. D., and D. J. Orth. 1991. Habitat use by an assemblage of fish in a large warmwater stream. Transactions of the American Fisheries Society 120:65–78.

Loomis, J. B., and D. S. White. 1996. Economic values of increasingly rare and endangered fish. Fisheries 21(11):6–10.

Mann, R. H. K., and J. A. B. Bass. 1997. The critical water velocities of larval Roach (*Rutilus rutilus*) and Dace (*Leuciscus leuciscus*) and implications for river management. Regulated Rivers: Research and Management 13:295–301.

Mattingly, H. T., and D. L. Galat. 2002. Distributional patterns of the threatened Niangua Darter, *Etheostoma nianguae*, at three spatial scales, with implications for species conservation. Copeia 2002:573–585.

Messinger, T., and C. Hughes. 2000. Environmental setting and its relations to water quality in the Kanawha River basin. U.S. Geological Survey, Water-Resources Investigations Report 00-4020, Reston, Virginia.

Midway, S. R., T. J. Kwak, and D. D. Aday. 2010. Habitat suitability of the Carolina Madtom, an imperiled, endemic stream fish. Transactions of the American Fisheries Society 139:325–338.

Moore, K. M. S., and S. V. Gregory. 1988. Summer habitat utilization and ecology of Cutthroat Trout fry (*Salmo clarki*) in Cascade Mountain streams. Canadian Journal of Fisheries and Aquatic Sciences 45:1921–1930.

Moore, S. L., and J. H. Thorp. 2008. Coping with hydrogeomorphic variations in a prairie river: resiliency in young-of-the-year fishes. River Research and Applications 24:267–278.

Newcomb, T. J., D. J. Orth, and D. F. Stauffer. 2007. Habitat evaluation. Pages 843–886 *in* C. S. Guy and M. L. Brown, editors. Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, Maryland.

Orth, D. J. 1987. Ecological considerations in the development and application of instream flow habitat models. Regulated Rivers: Research and Management 1:171–181.

Palmer, M. A., C. M. Swan, K. Nelson, P. Silver, and R. Alvestad. 2000. Streambed landscapes: evidence that stream invertebrates respond to the type and spatial arrangement of patches. Landscape Ecology 15:563–576.

Peckarsky, B. L., S. D. Cooper, and A. R. McIntosh. 1997. Extrapolating from individual behavior to populations and communities in streams. Journal of the North American Benthological Society 16:375–390.

Petty, J. T., and G. D. Grossman. 2007. Size-dependent territoriality of Mottled Sculpin in a southern Appalachian stream. Transactions of the American Fisheries Society 136:1750–1761.

Rabeni, C. F., and S. P. Sowa. 1996. Integrating biological realism into habitat restoration and conservation strategies for small streams. Canadian Journal of Fisheries and Aquatic Sciences 53:252–259.

Roberts, J. H., and P. L. Angermeier. 2007. Movement responses of stream fishes to introduced corridors of complex cover. Transactions of the American Fisheries Society 136:971–978.

Rosenberger, A., and P. L. Angermeier. 2003. Ontogenetic shifts in habitat use by the endangered Roanoke Logperch (*Percina rex*). Freshwater Biology 48:1563–1577.

Rosenfeld, J. 2003. Assessing the habitat requirements of stream fishes: an overview and evaluation of different approaches. Transactions of the American Fisheries Society 132:953–968.

Ryan, P. A. 1991. Environmental effects of sediment on New Zealand streams —a review. New Zealand Journal of Marine and Freshwater Research 25:207–221.

Schlosser, I. J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. Ecological Monographs 52:395–414.

Schlosser, I. J. 1985. Flow regime, juvenile abundance, and the assemblage structure of stream fishes. Ecology 66:1484–1490.

Schlosser, I. J. 1987. The role of predation in age-related and size-related habitat use by stream fishes. Ecology 68:651–659.

Schlosser, I. J. 1990. Environmental variation, life history attributes, and community structure in stream fishes: implications for environmental management and assessment. Environmental Management 14:621–628.

Schlosser, I. J. 1995. Critical landscape attributes that influence fish population dynamics in headwater streams. Hydrobiologia 303:71–85.

Schlosser, I. J. 1998. Fish recruitment, dispersal, and trophic interactions in a heterogeneous lotic environment. Oecologia 113:260–268.

Schlosser, I. J., and P. L. Angermeier. 1995. Spatial variation in demographic processes of lotic fishes: conceptual models, empirical evidence, and implications for conservation. Pages 392–401 *in* J. L. Nielsen, editor. Evolution and the aquatic ecosystem: defining unique units in population conservation. American Fisheries Society, Symposium 17, Bethesda, Maryland.

Schneider, D. C. 2001. The rise of the concept of scale in ecology. Bioscience 51:545–553.

Skyfield, J. P., and G. D. Grossman. 2008. Microhabitat use, movements and abundance of Gilt Darters (*Percina evides*) in southern Appalachian (USA) streams. Ecology of Freshwater Fish 17:219–230.

Stoddard, J. L., D. P. Larsen, C. P. Hawkins, R. K. Johnson, and R. H. Norris. 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16:1267–1276.

Thomas, J. A., and K. D. Bovee. 1993. Application and testing of a procedure to evaluate transferability of habitat suitability criteria. Regulated Rivers: Research and Management 8:285–294.

Torgersen, C. E., D. M. Price, H. W. Li, and B. A. McIntosh. 1999. Multiscale thermal refugia and stream habitat associations of Chinook Salmon in northeastern Oregon. Ecological Applications 9:301–319.

Werner, E. E., and J. F. Gilliam. 1984. Ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics 15:393–425.

Werner, E. E., and D. J. Hall. 1988. Ontogenetic habitat shifts in Bluegill: the foraging rate-predation risk trade-off. Ecology 69:1352–1366.

White, R. J. 1996. Growth and development of North American stream habitat management for fish. Canadian Journal of Fisheries and Aquatic Sciences 53:342–363.

Whittier, T. R., J. L. Stoddard, D. P. Larsen, and A. T. Herlihy. 2007. Selecting reference sites for stream biological assessments: best professional judgment or objective criteria. Journal of the North American Benthological Society 26:349–360.

Wiens, J. A. 1989. Spatial scaling in ecology. Functional Ecology 3:385–397.