

# Quantifying microhabitat availability: stratified random versus constrained focal-fish methods

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**Abstract** During a study of microhabitat use by gilt darters (*Percina evides*), we compared two methods for quantifying microhabitat availability in a southern Appalachian stream (USA). The first method used stratified random sampling throughout the site and the second involved taking constrained random measurements within a 2-m radius of the focal fish. Darters were generally over-represented in microhabitats with higher average velocities, greater amounts of erosional substrata, and lower amounts of depositional and large substrata. The two methods generally yielded similar patterns of microhabitat use. Nonetheless, of the seven microhabitat categories in which differential microhabitat use occurred in summer, four were present in both data sets, but three differed between methods. We observed no differences between methods for autumn data. Finally, the standard deviations of the summer-stratified random data set were significantly greater (sign test,  $P < 0.05$ ) than those of the constrained data set. Our results suggest that either method for quantifying microhabitat availability can be used to quantify the general habitat use patterns of this species, but constrained analyses yielded a more restricted view

of the total habitat available. Nonetheless, if the fishes range over a site, clearly stratified habitat availability analysis is preferred.

**Keywords** Darter · Habitat · *Percina* · Habitat selection · Fish habitat · Habitat availability · Stream fish · *Percina evides*

## Introduction

Quantification of habitat use by fishes is an important research area for both stream ecologists and fisheries biologists (Rosenfeld, 2003). Typically, researchers study habitat use using a variety of methods, including both direct observational and indirect capture-based methods such as nets or electrofishing, over several possible spatial scales (e.g., macrohabitat, mesohabitat, and microhabitat). With the advent of snorkeling as a stream sampling technique, more researchers are using direct observation of individual fish to quantify microhabitat use (see references in Grossman & Ratajczak, 1998). The general methodology for these studies involves quietly approaching an undisturbed fish from downstream, marking its position, and then recording a series of habitat measurements at the fish's position (e.g., distance from the surface, substratum and shelter, water column depth, average and focal point velocity, and substratum composition). In general, sampling continues until an adequate sample size is reached (15–50 individuals) and it is repeated on a

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seasonal or multi-year basis (Rosenfeld, 2003). Microhabitat use data are then compared statistically to a microhabitat ‘availability’ data set, which typically consists of a series of randomly or systematically sampled microhabitat measurements containing the same data collected for the fish. If significant differences are found, the investigator may conclude that the fish displays habitat selection (Rosenfeld, 2003), although we have previously used the term non-random habitat use for this behavior (Grossman & Freeman, 1987; Grossman & Ratajczak, 1998). We use the term non-random microhabitat use because selection implies an active choice, which cannot be demonstrated using strictly descriptive studies.

To our knowledge, there have been few comparisons of the techniques used to quantify microhabitat availability for underwater observational studies of microhabitat use by fishes. Microhabitat availability data can be collected using several methods including systematic sampling, typically via transects (Thompson et al., 2001), random or stratified random sampling (Grossman & Ratajczak, 1998), and constrained random sampling (Petty & Grossman, 1996; Henry & Grossman, 2007; Skyfield & Grossman, 2008). In this article, we compare the patterns of non-random habitat use by the gilt darter (*Percina evides*) using stratified random and constrained random microhabitat availability data sets. Our analysis should aid stream ecologists in choosing an appropriate method for the quantification of microhabitat availability for benthic stream fishes.

## Methods

### Species and study site

Gilt darters are medium-sized benthic darters that inhabit microhabitats with higher velocities and cobble substrata, although these patterns are affected by size and sex (Skyfield & Grossman, 2008). They occur in three disjunct populations inhabiting the southern Appalachians, upper Midwest, and Ozarks (Near et al., 2001). Gilt darters feed on benthic macroinvertebrates and are declining in abundance in several areas including the southeastern United States (Hatch, 1985; Margulies et al., 1980).

Because the study site and methods were described in the previous article (Skyfield & Grossman, 2008),

we only will describe them briefly here. Our study site was a 110-m section of Tellico Creek, located in Macon County, North Carolina, USA. Tellico Creek is a third order tributary of the Little Tennessee River, with an average discharge of 0.63 m<sup>3</sup>/s (Sutherland et al., 2002). The study site physiognomy was typical of relatively undisturbed, small streams in the southern Appalachian Mountains and comprised riffle, run, and pool sections. Bankside vegetation consisted of rhododendron (*Rhododendron maxima*) and mountain laurel (*Kalmia latifolia*) and the overstory was typical of southern Appalachian mixed hardwood conifer forest (Grossman et al., 1998). Although we did not sample for other species, it is likely that the fish assemblage resembled that described in Grossman et al. (1998).

### Microhabitat measurements

Darter microhabitat use methods are presented in Skyfield & Grossman (2008) and were based on the techniques of Grossman & Freeman (1987). We made fish microhabitat measurements between August 9–28, 2005 (Summer) and October 21–November 5 (Autumn). Darter observations were made in daylight hours (~0900 to ~1800) by entering the study site at a random location and snorkeling slowly upstream. When an undisturbed darter was sighted, its exact position was recorded and a painted lead weight was placed at that location (Petty & Grossman, 2004). After the entire site had been sampled, we returned to each darter location and measured: mean water velocity ( $\pm 0.01$  cm/s, electronic velocity meter, 60% depth, Bovee & Milhous, 1978), focal point velocity, bottom velocity (at the substratum), depth, distance from the substratum, and substratum composition using the methods of Grossman & Freeman (1987) and Petty & Grossman (1996). Substratum composition was measured in a 20 cm  $\times$  20 cm quadrat directly under the fish. We estimated the percent composition of seven substratum categories based on maximum particle diameters and a modified Wentworth particle scale: bedrock (>30 cm no exposed edges), boulder (>30 cm), cobble (<30 cm & >2.5 cm), gravel (<2.5 cm & >0.2 cm), sand (<0.2 cm), silt (material that could be suspended in the water column), and debris (organic material) (Grossman & Freeman, 1987; Grossman & Ratajczak, 1998).

## Microhabitat availability

We used two different techniques to quantify microhabitat availability for comparison with fish microhabitat use data: (1) stratified random sampling and (2) constrained random sampling. We collected the same data using the same techniques for both types of availability samples and fish samples (see above) with the exception of fish focal point velocity and distance from the substratum, which required the presence of a fish. For stratified random sampling, we collected five random samples per 10 m of bank for a minimum of 55 samples (range 57–59). We identified the locations using a random number table to select a longitudinal position (meter mark) and a percent distance across the stream. We collected constrained random samples by using a random number table to choose a distance between 20 and 200 cm from each darter's location (range 54–88). We used the random number table to determine the direction of the constrained sample by randomly choosing a number between 0 and 24, which corresponded to 30-min intervals on a clock face. An availability sample was then collected at that position. We have previously shown that between 30 and 42 availability measurements are adequate to detect changes in the major (>10%) substratum categories in a nearby similar stream (Grossman & Freeman, 1987); hence, our availability sample sizes should have been adequate to describe the microhabitat availability in the site.

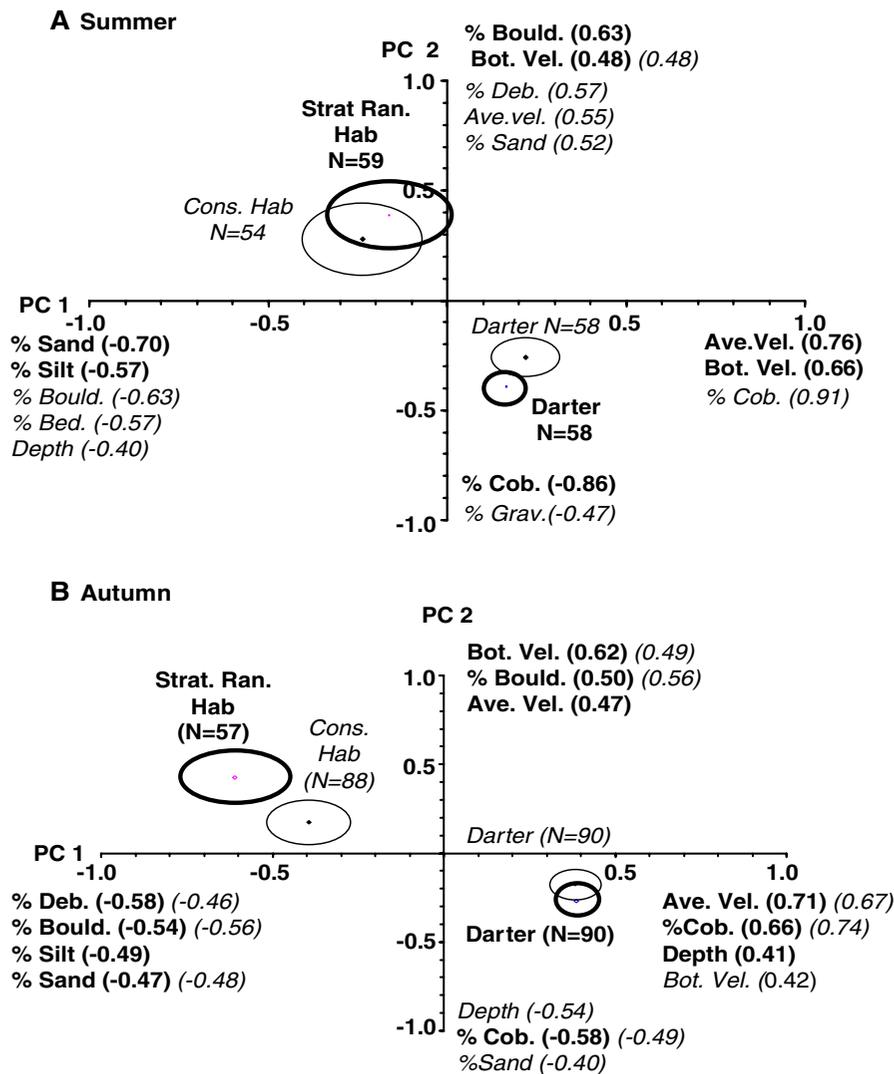
## Statistical analyses

We tested for differences between darter microhabitat use and availability using the principle component analysis (PCA) technique of Grossman & Freeman (1987) and Grossman & deSostoa (1994). Both linear and percentage data were transformed using  $\ln(x + 1)$  and arcsine-square root transforms, respectively. We only reported ecologically interpretable components with eigenvalues greater than one (Grossman & Freeman, 1987). We conducted a PCA on each set of microhabitat use and availability data, and because results for both constrained and stratified methods were extremely similar, we plotted data for both types of availability measurements on the same axes for each season. This produced two sets of fish use and availability centroids per PCA graph. We then

identified the differences in variables and variable loadings for the different availability methods in different typeface (see Fig. 1). In order to test for significant differences between availability and fish data, we plotted means and 95% confidence ellipses for availability and use data, with no overlap representing significance at the 0.05 level (Grossman & deSostoa, 1994). Differences between ellipses were verified by comparison with raw means for each data set, and substratum differences that did not differ by >2% were not considered different unless mean values were below 15%. Finally, we tested for significant differences in both means and standard deviations between each seasonal pair of constrained and stratified random data sets using a sign test. Because our a priori expectation was that stratified random data would show higher standard deviations than constrained data, we used an one-tailed test with  $P = 0.05$ . Spearman's  $s-r$  indicated that standard deviations were not significantly correlated with means ( $P$ 's > 0.05), so these comparisons were appropriate.

## Results

Microhabitat use comparisons yielded generally similar results regardless of the manner in which microhabitat availability data were collected. During summer, comparison of constrained microhabitat availability and darter use data indicated that darters were over-represented in microhabitats that had lower bottom velocities, more cobble and gravel, and less boulder, bedrock, and sand. Similarly, when darter microhabitat use was compared with stratified random microhabitat availability data, darters were over-represented in microhabitats having higher average but lower bottom velocities, more cobble, and less boulder and sand (Fig. 1A, Table 1). In autumn, both stratified random and constrained microhabitat availability comparisons indicated that darters were over-represented in deeper microhabitats with higher average velocities, more cobble, and less boulder, sand and, debris (Fig. 1B, Table 1). The two availability methods only produced differences in microhabitat use by darters in the summer samples. For example, the stratified random data set indicated that darters used microhabitats with higher average velocities than available at random, whereas the



**Fig. 1** Comparison of non-random microhabitat use patterns by darters during summer **A** and Autumn **B**. Centroids (mean and 95% confidence ellipse) for both stratified random and constrained random microhabitat availability data are presented in each graph, as well as centroids for darters using both methods (see Skyfield & Grossman, 2008). Figures depict principle components 1 and 2 and only variables with loadings  $\geq 0.40$ , and interpretable differences (substratum differences  $>2\%$ , when means are  $>15\%$ ) are presented. Because PCA's

for both stratified and constrained availability sampling yielded very similar results, we plotted them on a single set of principle components and denoted the different variables and loading with different text (stratified random sampling—bold, constrained sampling—italics). Loadings are given in parentheses. Sample sizes follow: (1) Summer stratified random  $N = 59$ , Summer constrained random  $N = 54$ , Summer darters  $N = 58$ , Autumn stratified random  $N = 57$ , Autumn constrained random  $N = 88$ , and Autumn darters  $N = 90$

constrained data set produced differences in gravel and bedrock substrata. Interestingly, the constrained data set yielded more categories for non-random use than stratified random data, although this difference only amounted to one category. Both techniques appeared capable of identifying the general patterns of microhabitat use for this darter.

Comparisons of both means and standard deviations of the microhabitat availability estimation techniques (Table 1) only yielded one significant difference and the standard deviations of the summer-stratified random data set were significantly greater ( $P < 0.05$ ) than those of the constrained data set.

**Table 1** Univariate mean and standard deviation (SD) of stratified random and constrained microhabitat availability and darter microhabitat use data (after Skyfield and Grossman, 2008)

Season, Method	<i>N</i>	Depth (cm)	Ave. vel. (m/s)	Bot. vel. (m/s)	% Bedrock	% Boulder	% Cobble	% Gravel	% Sand	% Debris
Tellico Summer constrained	54	36 (11)	0.39 (0.18)	0.17 (0.12)	3 (16)	22 (31)	56 (32)	14 (17)	5 (13)	0 (1)
Tellico Summer stratified random	59	31 (18)	0.35 (0.32)	0.17 (0.20)	6 (21)	23 (32)	46 (33)	12 (20)	10 (21)	2 (7)
Tellico Summer use	58	39 (9)	0.40 (0.16)	0.14 (0.13)	1 (6)	6 (11)	71 (22)	20 (19)	2 (5)	0 (0)
Tellico Autumn constrained	88	29 (13)	0.27 (.19)	0.11 (0.11)	1 (9)	20 (29)	47 (30)	14 (14)	11 (17)	5 (14)
Tellico Autumn stratified random	57	25 (11)	0.27 (0.24)	0.12 (0.14)	5 (20)	24 (33)	38 (29)	17 (17)	11 (15)	5 (9)
Tellico Autumn use	90	33 (10)	0.32 (0.18)	0.10 (0.10)	0 (2)	8 (13)	69 (22)	17 (15)	5 (8)	1 (3)

Categories that did not differ between availability and use data sets or were within the range of measurement error ( $\pm 2\%$  for substratum data) are not presented

## Discussion

We have shown that two different methods of quantifying microhabitat availability generally yielded similar patterns of microhabitat use for gilt darters in a southern Appalachian stream. We expected that stratified random availability data would yield more significant habitat categories than constrained analyses, because they encompass the entire range of microhabitats present in the site. This was not the case, however, and both data sets produced unique habitat categories in the summer sample. Nonetheless, the standard deviations of the microhabitat variables in the stratified random data set for summer were significantly greater than the constrained data set, confirming our hypothesis that a greater amount of microhabitat variation would be encompassed by this method. In addition, our study was conducted during a drought year, and this may have reduced the microhabitat variation in the site. Finally, it is possible that the lack of differences in Autumn comparisons may have been affected by differential sample sizes (stratified random  $N = 57$ , constrained  $N = 88$ ); however, we have previously shown that much smaller sample sizes are sufficient for quantifying microhabitat availability in a similar, nearby stream (Coweeta Creek, Grossman & Freeman, 1987).

We previously have used constrained and stratified random microhabitat availability techniques for different purposes. For general studies of microhabitat use, we have employed stratified random techniques (Grossman & Freeman, 1987), whereas, when testing

whether benthic stream fishes occupied patches that have significantly higher amounts of prey than random patches (Petty & Grossman, 1996; Henry & Grossman, 2007), we have used constrained techniques. In the latter studies, we were most interested in the relationship between the characteristics of local patches rather than the reach-scale habitat availability. Nonetheless, cogent arguments can be made for the use of either technique in general microhabitat or patch-use studies, and constrained methods are simpler logistically. In addition, the biological characteristics of the species should be considered when choosing a method. For example, if the movement patterns of the study species are such that they travel throughout the entire site, then stratified methods would be more appropriate on an a priori basis. Conversely, if movements are restricted, then constrained methods might be more appropriate. Finally, depending on the spatial heterogeneity of the site, constrained samples have a higher probability of being spatially autocorrelated with fish samples. This bias would tend to obscure the identification of non-random use; however, this was not a problem in our study (see Fig. 1). In conclusion, our results suggest that both methods were adequate for characterizing the general patterns of microhabitat use for a benthic darter, but stratified random analyses may depict a broader range of available habitat and is preferable when fish displays substantial movement within a site.

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