

ABSTRACT

MIDWAY, STEPHEN RUSSELL. Habitat Ecology of the Carolina Madtom, *Noturus furiosus*, an Imperiled Endemic Stream Fish. (Under the direction of D. Derek Aday and Thomas J. Kwak.)

The Carolina madtom *Noturus furiosus* is an imperiled stream catfish (Ictaluridae) endemic to the Tar and Neuse river basins in North Carolina. The species is listed as State Threatened, and whereas the Tar Basin population resembles its historical distribution, the Neuse Basin population has shown recent significant decline. Quantifying habitat use and availability is critical for effective management and subsequent survival of the species. This study combined field and laboratory research to investigate habitat use and suitability, as well as efficacy of an artificial cover unit. To assess habitat suitability, we investigated six reaches (three in each river basin) to (1) quantify Carolina madtom microhabitat use, availability, and suitability, (2) compare suitable microhabitat availability between the two basins, and (3) examine the effectiveness of an instream artificial cover unit. We also conducted laboratory experiments to examine madtoms' use of the same artificial cover unit relative to three natural cover types.

Carolina madtom were located and their habitat use was quantified at four of six survey reaches; the species appeared to be absent at two reaches in the impacted Neuse Basin. Carolina madtom most frequently occupied shallow to moderate depths (0.5 m) of swift moving water over a sand substrate using cobble for cover. Univariate and principal components analyses both showed Carolina madtom use of instream habitat to be selective, or nonrandom. Interbasin comparisons suggested that most suitable microhabitats (particularly water depth and velocities) were more prevalent in the Neuse

than in the Tar Basin, which is interesting considering that the Neuse population appears to be the more impacted of the two. Consequently, we suggest that other physical or biotic factors must be responsible for the decline in the Neuse Basin population.

Our instream artificial cover units were occupied mainly by Carolina madtom (25% of the time), and rarely by a suite of other stream animals. Comparing areas with the artificial cover units ('treated areas') to those without them ('control areas'), Carolina madtom abundance among all treated areas was statistically higher than the controls, demonstrating that madtoms will use suitable artificial cover when available. Microhabitat characteristics of occupied artificial cover units closely resembled those of natural microhabitat use. Results from the field component of the study provide habitat suitability criteria that can inform management and conservation of the Carolina madtom, and the artificial cover units present a cost-effective conservation and restoration option if increased management is deemed necessary.

In the laboratory component of the study, Carolina madtom were placed in an experimental stream tank (44 cm x 88 cm in area and about 45 cm deep) and given 24 hours to make a selection among four cover options, three natural (one each of rock, leaf pack, and mussel shell) and the artificial cover unit. Among 30 experimental trials, Carolina madtom preferred the artificial cover unit, selecting it 63% of the time. Rock was selected 23% and leaf pack 13% of the time. Contrary to previous anecdotal observations, mussel shells were not selected during any trials. Results from the laboratory experiments, coupled with similar findings from instream work, indicate that artificial cover may be a viable option for species conservation and restoration.

Given the State Threatened status and limited distribution, our results have implications for conservation and restoration of this native and endemic southeastern catfish. Successful management and conservation of declining Carolina madtom populations is dependent upon preserving Tar Basin habitat, identifying Neuse Basin impacts, and restoring Neuse Basin populations.

Habitat Ecology of the Carolina Madtom, *Noturus furiosus*,
an Imperiled Endemic Stream Fish

by
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BIOGRAPHY

Steve Midway was born in Bethlehem, Pennsylvania, on November 10, 1982. He graduated from Liberty High School in 2001 and started his undergraduate work at the University of Vermont of that year. While at Vermont, professors such as Drs. J. Ellen Marsden, Larry Forcier, and Robert Costanza made lasting impressions, cementing the notion that studying the natural world would be a worthwhile career. Steve also gained valuable experience as a fisheries technician working on issues involving Lake Champlain. A semester abroad in the Caribbean also helped foster intrigue with marine systems.

After graduation, Steve accepted jobs in California and Hawaii teaching environmental education to a variety of ages. While this work provided him with additional pedagogical insight, it also provided time to explore new ecologies and ask new questions. Also during this time, Steve was able to, with an old friend, develop a small business focused on planning and implementing resource and energy efficiency for homes and businesses.

In the fall of 2006, Steve found an opportunity to study a rare and endemic North Carolina stream fish with Drs. Aday and Kwak. The content of this thesis dominated the following two years of his life.

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**Chapter I – Habitat Suitability of the Carolina Madtom,
an Imperiled Endemic Stream Fish**

Abstract

The Carolina madtom *Noturus furiosus* is an imperiled stream catfish endemic to the Tar and Neuse river basins in North Carolina. The species is listed as State Threatened, and whereas the Tar Basin population resembles its historical distribution, the Neuse Basin population has shown recent significant decline. Quantifying habitat requirements and availability is critical for effective management and subsequent survival of the species. To assess habitat suitability, we investigated six reaches (three in each basin) to (1) quantify Carolina madtom microhabitat use, availability, and suitability, (2) compare suitable microhabitat availability between the two basins, and (3) examine use of an instream artificial cover unit. Carolina madtom were located and their habitat was quantified at four of six survey reaches; it was absent at two reaches in the impacted Neuse Basin. Carolina madtom most frequently occupied shallow to moderate depths (0.5 m) of swift moving water over a sand substrate using cobble for cover. Univariate and principal components analyses both show Carolina madtom use of instream habitat to be selective, or nonrandom. Interbasin comparisons suggest that most suitable microhabitats (particularly water depth and velocities) were more prevalent in the impacted Neuse than in the Tar Basin, which was unexpected given our difficulty in locating fish in the Neuse and its documented recent population declines. As such, we suggest that other physical or biotic effects are responsible for the decline in the Neuse Basin populations. Our instream artificial cover units were occupied mainly by Carolina madtom (25% of the time) and by a suite of other stream animals on rare occasion. Carolina madtom abundance among all areas treated with the artificial cover unit was

statistically higher than the control areas, demonstrating that madtoms will use artificial cover when available. Microhabitat characteristics of occupied artificial cover units closely resembled those of natural, instream microhabitat use and present a conservation and restoration option if increased management is deemed necessary. Results from the field component of the study provide suitability criteria and artificial cover information that can inform management and conservation of the Carolina madtom.

Introduction

Warmwater streams in the southeastern United States support substantial biological diversity on broad spatial scales (Meffe and Sheldon 1988; Lydeard and Mayden 1995). Because these systems are dynamic, management becomes a challenging task, compounded by the fact that fish often require different conditions (e.g., flow) than other aquatic species (Hubert and Rahel 1989; Aadland 1993). Southeastern U.S. streams have recently seen a dramatic increase in imperiled species due to habitat loss, exotic species, and pollution (Wilcove et al. 1998; Jelks et al. 2008). In particular, a disproportionate rate of imperilment and extirpation is occurring among benthic fishes (e.g., sculpins, darters, and madtoms) as stream bottoms are often the first impacted habitat type (Angermeier 1995; Etnier 1997; Warren et al. 1997). Aadland (1993) also noted higher rates of imperilment for non-game species, as they are generally less managed than their commercial and recreational counterparts. Endemic species are particularly susceptible to extirpation, as their isolation increases vulnerability to both human activity and natural catastrophic events (Warren and Burr 1994; Burkhead et al. 1997). Habitat quality and quantity influence species diversity; a greater diversity of quality correlates to higher fish diversity (Gorman and Karr 1978; Schlosser 1982; Reeves et al. 1993; Ricciardi and Rasmussen 1999). The difficulty from a conservation and management standpoint is selecting appropriate habitat metrics to quantify, particularly because of myriad species-specific habitat requirements and life history strategies (Pajak and Neves 1987; Aadland 1993).

The Carolina madtom *Noturus furiosus* is a small, nongame, endemic stream-dwelling catfish and one of 25 described madtom species (genus *Noturus* Burr and Stoeckel 1999). To date, only one publication exists outlining Carolina madtom ecology (Burr et al. 1989). The species is presently on the IUCN's Red List of Threatened Species, but is considered data deficient (Baillie et al. 2004), and most information for its management has been inferred from studies of congeners. The native range of the Carolina madtom includes only two North Carolina drainage basins, the Tar and Neuse rivers (Burr et al. 1989). Within these basins, the species inhabits clear to tannin-stained, free-flowing streams in both the piedmont and coastal plain physiographic regions (Burr et al. 1989). The Neuse River Basin is considered an impacted basin, showing a recent decline in the Carolina madtom population (C. Wood, North Carolina Wildlife Resources Commission, personal communication). The Tar River Basin has historically supported greater numbers of Carolina madtom (Burr et al. 1989), with some of the densest subpopulations located in the piedmont region just above the fall line (North Carolina Wildlife Resources Commission, unpublished data).

Habitat associations of the Carolina madtom appear to be similar to those of most of its congeners (Taylor 1969; Burr and Stoeckel 1999). Suitable stream microhabitats have been anecdotally described as riffles, runs, and pools, with highest occurrences observed in swift current during warm months at depths of 0.3 to 1.0 m (Burr et al. 1989). Due to its benthotaxic behavior, stream substrate composition is of particular importance. Leaf-litter, sand, gravel, and small cobble are all common substrates associated with the species. Burr et al. (1989) noted frequent occurrence in sand mixed with gravel in leaf

litter. Areas of moderate to slow flow with abundant cover are the typical habitat during reproduction that occurs principally between May and July (Burr et al. 1989), though substrate suitability of the Carolina madtom may change seasonally in relation to life history behaviors. Population densities are, for the most part, unknown and assumed to be low. Based on years of sampling, Burr and Stoeckel (1999) noted that, *Noturus* spp. densities never reach those associated with most other stream-dwelling fishes.

Additionally, because the Carolina madtom has a restricted range and produces relatively small clutches, it is thought to be particularly sensitive to environmental changes, much like other endemic freshwater species (Angermeier 1995; Burr and Stoeckel 1999).

A number of investigators have studied other madtom species, often focusing on life history (Mayden et al. 1980; Mayden and Walsh 1984; Starnes and Starnes 1985; Gagen et al. 1998) or habitat use (Wildhaber et al. 2000; Orth and Maughan 1982). The federally endangered Neosho madtom has been most intensively studied, including quantification of habitat use and population structure (Fuselier and Edds 1994; Wildhaber et al. 2000; Bulger and Edds 2001). Habitat suitability functions have also been developed for the now federally endangered freckled madtom (Orth and Maughan 1982; Simonson and Neves 1992). To date, however, there has been no quantification of habitat use, suitability, or preference for the Carolina madtom. This information is fundamental for understanding the ecology of the species and guiding management decisions.

Given the general decline in suitable habitat for madtoms (Robison and Harp 1985; Etnier and Starnes 1991), management aimed at conserving or restoring species

must often consider habitat augmentation. Any documented interaction of *Noturus* spp. with artificial habitat has been primarily anecdotal. Indeed, few studies exist of any nongame stream-dwelling fish and associations with artificial habitat. Kottkamp and Moyle (1972) investigated use of beverage cans and documented six stream fishes – including two catfish species – inhabiting discarded cans. Although Burr et al. (1989) noted anecdotal use of human refuse by Carolina madtom, their conclusions were limited. Given the potential utility of artificial habitat augmentation, such devices could be used to enhance Carolina madtom populations if protective shelter or spawning cavities were a limiting population factor (Burr and Stoeckel 1999). If it can be shown that Carolina madtom readily use artificial habitat, habitat augmentation efforts, combined with suitable flow regimes, could work to return Carolina madtom populations toward more robust, historic levels.

Our study was designed to quantify instream habitat associations of the Carolina madtom. Our primary objectives were to (1) determine instream habitat use and suitability for the species, (2) compare suitable habitat between an impacted basin and a rural basin, and (3) quantify instream use of an artificial cover unit. Results of this habitat evaluation may assist stream and fisheries managers in understanding habitat requirements for an endemic, imperiled stream fish as well as supplement current knowledge of biologically diverse southeastern U.S. streams.

Study Area

Our study took place in the Tar and Neuse river basins in eastern North Carolina (Figure 1), including Franklin, Halifax, Nash, Wilson, Wayne, and Johnston Counties. Historical occurrences of Carolina madtom are documented in these basins around the fall line, in the lower piedmont, and upper coastal plain physiographic regions. Streams in these areas range from low gradient with sluggish pools and intermittent riffles to blackwater streams and low-lying swamps (NCDENR 2002).

The Tar River Basin (14,429 km²) covers a relatively rural part of the state, and a recent assessment found 55% of the basin to be forested or wetland, 28% agricultural, and only 1% urban (NCDENR 2004). Though the Neuse Basin (16,149 km²) has a comparable composition of forest or wetland (56%) and agriculture (23%), much more of the basin (8%) is urban (NCDENR 2002; Whitall et al. 2003). The Neuse is routinely considered to be an endangered basin (American Rivers Foundation 2007) with impacts such as urban wastewater, fertilizer, industrial development and animal operations all contributing to eutrophication (Pinckney et al. 1997; Paerl et al. 1998).

We studied three reaches in both the Tar and Neuse river basins for a total of six reaches, effectively covering the Carolina madtom's range (Table 1; Figure 1). The three Tar Basin reaches were sampled in 2007, and the three Neuse Basin reaches were sampled in 2008. In the Tar River Basin, we sampled the mainstem Tar River (Tar 1), Swift Creek (Tar 2), and Little Fishing Creek (Tar 3). In the Neuse River Basin, we sampled Contentnea Creek (Neuse 1), Little River (Neuse 2), and Swift Creek (Neuse 3). Reaches varied from 60 to 100 m in length, and were delineated based on snorkelable

habitat. All reaches also had historical documentation of Carolina madtom presence (W. C. Starnes, North Carolina Museum of Natural Sciences, unpublished data).

Methods

Habitat Use, Availability, and Suitability

We identified Carolina madtom microhabitats over two spring and summer seasons, ranging from 5 May 2007 to 18 July 2008. During both years, drought conditions were present in both basins. We sampled three reaches in each basin for a total of six reaches. All six reaches were surveyed using snorkeling techniques. Specifically, each reach was sampled twelve times, with each sampling event lasting two person-hours per survey (for a total effort of 24 hours per reach) to quantify Carolina madtom occurrence and instream habitat use. Two snorkelers began at the downstream limit of the reach and proceeded upstream, visually surveying the entire stream bottom. Observed Carolina madtom locations were marked by placing a small weight attached to a float at the exact location. Upon conclusion of each survey, water depth (m), bottom velocity (m/s), mean column velocity (m/s), substrate composition, cover, and location within the reach were recorded for each Carolina madtom point location. Depth, bottom velocity and mean column velocity were measured with a topset wading rod and a Marsh–McBirney Model 2000 digital flowmeter. Mean column velocity was measured at 60% of the total depth from the surface (depths ≤ 0.80 m) or was calculated as the average of measurements at 20% and 80% of the total depth (depths > 0.80 m). Substrate was determined as the greatest percent coverage of a substrate type according to a

modified Wentworth particle-size classification (Table 2) at the exact location of the fish. Cover was recorded as either the physical object under which the Carolina madtom was found, or if the fish was not under cover, the closest cover in a 1-m² quadrat with the Carolina madtom serving as the center point. Cover categories included none (no cover in the 1-m² quadrat), leaf pack, woody debris, cobble, boulder, and mussel shell. Fish were not handled during sampling.

We quantified available stream microhabitat for each reach under base flow conditions in June after half (i.e., six) of the snorkel surveys were complete. Within each study reach, cross-sectional transects were delineated at 5-m intervals (12-20 transects per reach). The location of the first transect was selected randomly. Along each transect water depth, bottom velocity, mean column velocity, substrate, and cover were recorded at 1-m intervals using methods described above. Depth and velocity measurements were taken in the middle of the 1-m² quadrat, while substrate and cover included the entire quadrat.

Habitat use was analyzed both by univariate and multivariate approaches in an effort to gain insight into individual microhabitat parameters (e.g., depth, substrate) as well as overall habitat type (e.g., thalweg, riffle). We pooled all Carolina madtom observations and calculated arithmetic means for water depth, bottom velocity, and mean column velocity. Microhabitat suitability was estimated to identify optimal ranges within each habitat parameter. Suitability was calculated by dividing microhabitat use by availability for a range of the variable or category, standardizing to a maximum of 1, summing the values for each category among all reaches, and again standardizing to 1.

Analyzing individual reaches prior to pooling allowed us to develop a composite suitability function for the species by avoiding comparisons of one reach's use to a different reach's availability. The most suitable, or optimal, range or category was that with a value of 1. In cases where more than one range or category was equivalently high, the combined range was considered optimal (i.e., suitability = 1.0).

To determine univariate microhabitat selectivity (nonrandom microhabitat use), we compared microhabitat use to availability for each parameter. For all analyses, categorical substrate was transformed into a continuous variable based on the appropriate range of substrate sizes (Table 2). A Kolmogorov-Smirnov (K-S) two-sample test was used for continuous variables (water depth, bottom velocity, mean column velocity, and substrate) and a log-likelihood ratio *G*-test for independence was used for the categorical cover variable. Significant *P*-values (< 0.05) indicated microhabitat selectivity or nonrandom microhabitat use.

We also analyzed habitat using a multivariate principal components analysis (PCA) of the four continuous microhabitat variables. Cover was not incorporated into this analysis because it could not be converted into a continuous variable. Principal components were developed based on the correlation matrix of these variables from habitat availability surveys. The PCA extracted linear descriptions of the combined univariate parameters that explained the maximum amount of variation within the data. Two principal components were retained in each analysis and generally conformed to the recommendation to retain components with eigenvalues greater than 1.0 (Kwak and Peterson 2007). Microhabitat-use component scores were then calculated using the

coefficients derived from the availability components. Dimensions (linear components) were described by two or more of the variables based on significant component loadings. Microhabitat use and availability scores were plotted, and a K-S two-sample test was performed on each component to test for statistically different distributions. Significant P -values (< 0.05) indicated nonrandom habitat use for that component's combination of variables.

Interbasin Habitat Comparison

We compared microhabitat availability distributions between basins (of which sample sizes were comparable $N = 828$ Tar Basin, $N = 797$ Neuse Basin survey points) to assess whether suitable habitat was lacking in the Neuse River Basin, where the fish is rare and declining. By testing for differences in microhabitat parameter distributions (K-S test, G -test), we were able to discern whether available microhabitat varied significantly between basins. Then, by quantifying the amount of optimal habitat in the Neuse River reaches, we were able to determine if suitable habitat was lacking and potentially contributing to population decline. Significant P -values (< 0.05) indicated different distributions of available microhabitat. Comparisons of suitable habitat ranges (from previously calculated suitability) between basins provided further insight regarding the quantity of suitable habitat in Neuse Basin streams.

Artificial Cover Assessment

Artificial cover units were constructed by cutting a small opening (approximately 25 mm) and vent slots into an upside down 100-mm clay flowerpot saucer (Figure 2). This saucer was then glued to an upside down 150-mm flowerpot saucer. Commercially-

available landscaping river rocks, approximately 10–30 mm in diameter, were glued to the underside of the larger saucer to provide additional weight and stability. Upon conclusion of the microhabitat availability surveys (after the sixth snorkel survey was complete), artificial cover units were deployed in a randomly selected treatment half of each study reach. Artificial cover units were distributed uniformly in a grid pattern, with a single unit occupying the middle of a 6-m wide by 5-m long quadrat. The total number of artificial cover units per reach was determined based on the size of the reach, so that comparisons among reaches would be standardized to a uniform artificial cover unit density. After a soak period of 10–14 days, artificial cover units were observed for fish occupancy as part of the final six snorkel surveys. When stream snorkeling conditions were poor (e.g., high turbidity), artificial cover units were removed from the water to be checked and gently placed back in the original stream location with minimal stress conveyed to any organism inside. In addition to documenting fish use, all continuous microhabitat parameters were measured each time an artificial cover unit was sampled.

A before-after-control-impact (BACI) statistical analysis (Underwood 1994) was used to determine if artificial cover units increased abundance of Carolina madtom in our six study reaches. The before-impact period included the six surveys prior to application of the treatment (cover units), and the after-impact period included the final six surveys during which the treatment was in place. Surveys were treated as sub-samples within each reach to produce mean abundance estimates before and after impact for both the control and treatment reach halves. For each reach, a *D*-statistic was calculated to be the difference of differences; that is, a comparison between the treatment half before and

after to the control half before and after. All *D*-statistics were combined to calculate a mean and standard error, which was then used to calculate a *t*-statistic and corresponding *P*-value. Significant *P*-values (< 0.05) indicated that artificial cover units were effective in increasing Carolina madtom abundance in stream reaches where they were uniformly deployed.

Results

We sampled a total of 274 Carolina madtom (including 154 observed using artificial cover units) from May 2007 to July 2008. Carolina madtom were observed in 4 of 6 sampled reaches; all reaches in the Tar River Basin and one site (Neuse 1) in the Neuse River Basin supported populations. No individuals were detected at the Neuse 2 and Neuse 3 study reaches. Water temperatures during instream sampling ranged from 20 – 28°C.

Habitat Use, Availability, and Suitability

Overall, Carolina madtom occupied instream microhabitats with a mean water depth of 0.42 m, mean bottom velocity of 0.14 m/s, and mean column velocity of 0.22 m/s. The most frequently used substrate and cover were sand and cobble. Instream microhabitat use varied by reach, but mean values did not deviate substantially from the overall means (Table 3). Microhabitat availability also varied among reaches, and because they were recorded at baseflow conditions, they represent a range of microhabitats that fish are likely to experience. Overall, Carolina madtom instream densities were low, averaging 1.1 – 1.5 fish/reach detected per survey (Table 4).

Univariate analysis of habitat selectivity pooled from all Tar River Basin reaches shows that for all five microhabitat parameters, Carolina madtom select habitat nonrandomly (Table 5). A wide range of depth was available, but fish tended to occupy shallower (<0.50 m) microhabitats (Figure 3). The available bottom velocity distribution was positively skewed, with the majority of microhabitats available in the slowest waters (<0.05 m/s). Bottom velocity of microhabitats occupied was distributed evenly up to about 0.20 m/s, at which point use diminished, displaying selection of slow to moderate bottom velocities compared to much more slow-moving water available. The distribution of available mean column velocity was also positively skewed, much like that of bottom velocity. Mean column velocity microhabitat use approximated a normal distribution, suggesting an abundance of slow water with fish selecting for moderately flowing water. Available substrate was dominated by sand and silt, while use occurred primarily over sand and gravel substrates (Figure 4). Silt was clearly avoided. Cover associations were nonrandom, showing selection for cobble and boulder (though sample sizes were limited) with woody debris more marginally selected, but used widely in reaches where cobble substrate was scarce.

Habitat suitability was calculated based on microhabitat use and availability data from the Tar Basin ($N = 95$). The small number of Neuse samples ($N = 25$) were withheld so that habitat suitability would be based on a non-impacted basin and any potential Neuse Basin habitat effects would be avoided. Suitability distributions were developed for each of the three Tar Basin reaches, and then combined and standardized for a composite basin distribution. The range of optimal water depth was 0.10–0.19 m,

optimal bottom velocity 0.10–0.24 m/s, and optimal mean column velocity 0.20–0.29 m/s (Figure 5). The optimal substrate was gravel and the optimal cover included woody debris, cobble and boulders (Figure 6). Generally, the most suitable microhabitats were also the most used (Figures 3 and 4). For the continuous variables depth, bottom velocity, and mean column velocity, all of the most suitable ranges were also the most frequently occupied. Microhabitat use of the categorical variables substrate and cover differed slightly from suitabilities. Substrate use was highest for sand, with slightly less use of gravel, although gravel was clearly the most suitable substrate. The frequent use of sand substrate is likely related to the extremely high availability of sand in these systems. Cover use skewed slightly towards woody debris. As was the case with substrate, more woody debris was available for use, and woody debris and cobble were equally suitable cover types.

Availability of suitable habitat varied among the four reaches where Carolina madtom were present (Table 6). All reaches contained suitable depths, but less than 10% of available depth in Tar 3 and Neuse 1 were in the suitable range. Availability of suitable bottom velocities was low for all reaches (<10%). Suitable mean column velocities were also limited, with only one reach exhibiting availability >10%. Although suitable velocities were low, this might be expected when investigating a rheotactic species in low-velocity systems. Except for one reach, Tar 2, suitable substrates were all less than 5% available. Suitable cover was highly available ($\geq 28\%$) Tar 1 and Tar 3, but not at other reaches.

Habitat use and suitability were also analyzed using a multivariate principal components analysis that provided further evidence that Carolina madtom use habitat nonrandomly. For each of the analyses among four reaches, two components were sufficient to describe stream habitat (Table 7). Components were based on microhabitat loadings and described microhabitat gradients from eddy to thalweg, riffle to pool, or scour pool to run habitats. For all reaches, Carolina madtom occupied habitat nonrandomly in component 1 and nonrandomly in two of four reaches for component 2 (K-S two-sample test, Table 8).

In all analyses, component 1 demonstrated that Carolina madtom habitat use is nonrandom among those microhabitats available. Carolina madtom disproportionately occupied areas of high velocity and coarse substrate, frequently associated with a thalweg or riffle complex (Figures 7 and 8). Habitat use described in component 2 was nonrandom in two of four analyses, Tar 3 and Neuse 1. Trends were similar to those of component 1; Carolina madtom selected habitat characterized by medium depth and high velocity, or areas associated with a run (Figure 8).

Interbasin Habitat Comparison

Microhabitat availability between basins was significantly different for all parameters (Table 5). In addition, as much or more suitable habitat was present in the impacted Neuse River Basin (Figures 9 and 10), where the Carolina madtom is rare and populations have declined. The Tar Basin displayed a more even distribution of available depths than that of the Neuse Basin, which had a distribution skewed with a higher frequency of shallow depths (Figure 9). The Neuse Basin had over twice as much

optimal depth (0.10–0.19 m), as defined by the suitability indices. Both basins had the greatest frequency of bottom velocities in the slowest interval. Bottom velocity availabilities in the Tar Basin quickly diminished after the first interval, while the Neuse Basin had a small amount of moderate bottom velocities. This represented the optimal range of bottom velocities (0.10–0.24 m/s) and, as with depth, much more was available in the Neuse Basin (Figure 9). Optimal mean column velocity (0.20–0.29 m/s) was slightly more abundant in the Neuse, but overall values were more similar than those of bottom velocities. Gravel, the optimal substrate, was more widely available in the Neuse Basin (Figure 10). Three cover types were equally optimal, woody debris, boulder, and cobble. Trends in cover availability varied between basins; boulder was available at about the same proportion in each basin, the Tar Basin contained more woody debris, and the Neuse Basin had more cobble. Together, these available habitat trends provide strong evidence that instream microhabitat is not limiting in the Neuse River Basin and is likely not the primary limitation for the associated species decline.

Artificial Cover Assessment

Six surveys at each of the six reaches resulted in a total sample of 606 artificial cover units. We observed a total of 154 Carolina madtom using the artificial cover unit, which resulted in a 25% occupancy rate. While other species were found using the artificial cover units, their presence was extremely low and did not suggest any significant interference with Carolina madtom use. The BACI analysis showed that within the four reaches occupied by Carolina madtom, the species was more abundant in reach halves where artificial cover units were deployed (Table 4). After the treatment

was applied (i.e., artificial cover units deployed in one-half of the reach), all treated areas showed an increase in fish abundance (mean increase of 6.2 fish), while overall reach abundances also increased. Tar 2 showed the greatest increases in abundance, averaging 13 fish in the treated reach. Tar 1 also showed a large increase in abundance, while Tar 3 and Neuse 1 increased at a smaller rate. Whereas three of four control reaches showed a slight increase in abundance after the treatment, these increases were small compared to the treatment reach increases. This finding provides clear experimental evidence that artificial cover units significantly ($t = 2.62$, $df = 3$, $P = 0.04$) increase the number of Carolina madtom in a treated area relative to controls. Artificial cover units were deployed at both Neuse 2 ($N = 24$) and the Neuse 3 ($N = 15$) and attracted no Carolina madtoms after the full treatment period.

We were also interested in looking at the similarities and differences between microhabitat variables among occupied and unoccupied artificial cover units, as well as instream fish locations (Table 9). For instream microhabitat use and occupied artificial cover units, mean bottom velocities overlapped with 95% confidence intervals, and sand was the most used substrate for both. Also, unoccupied artificial cover units were most commonly located over silt substrate, which was previously shown to be the most sub-optimal substrate category.

Discussion

Carolina madtom are found under cover in moderately flowing, sand and gravel-lined streams and rivers in the Tar and Neuse river basins of North Carolina. We found

cobble to be the most frequently used cover structure for the species, although woody debris was also employed when rock cover was limited or did not exist. The streams in the native range of this fish contain very few boulders, but the Carolina madtom demonstrated a tendency to use them as cover objects if the boulders were small enough that larger, predatory species did not inhabit them. The species also occupied microhabitats with a moderate amount of bottom velocity; however, the velocities of the occupied, interstitial spaces may vary widely. We also demonstrated that Carolina madtom do not use stream habitat randomly, but rather select a narrow suite of instream conditions. Results of our multivariate analysis identified these conditions as riffle or thalweg macrohabitats.

Our work is the first to describe instream habitat suitability criteria for this species. Suitability functions are the only biological input in most stream flow models and are useful tools for stream managers to implement flow regimes or to otherwise manage a desired condition (Bovee 1986; Annear et al. 2004). Such indices are also important in impacted basins; the Neuse Basin has been modified with numerous impoundments and is experiencing increasing land development, which makes it prone to quickly-developing drought conditions and widely fluctuating flows.

One of our most relevant but counterintuitive findings was the Neuse Basin's abundance of suitable habitat yet lack of Carolina madtom. Recent work by North Carolina Wildlife Resources Commission (NCWRC) biologists found Carolina madtom abundance in the Neuse Basin to be much lower than historical records indicate, even suggesting local extirpations of the species. The Tar Basin, conversely, has retained

nearly all of its populations (C. Wood, NCWRC, unpublished data). One of the underlying assumptions regarding the basin-wide population decline in the Neuse Basin was degradation of suitable habitat, as instream habitat has been both degraded and lost by deforestation, urban and residential development, impoundments, and effluent (NCDENR 2002). Because we have demonstrated that suitable habitat exists – with twice the frequency as in the Tar Basin for some parameters – the next steps in Carolina madtom research are to investigate other influential factors. A study of historic and present water quality in the impacted basin should be carried out in the framework of Carolina madtom tolerance. In addition to formerly minimally-regulated agricultural and farming practices in the basin, the catchment has seen considerable development recently, and the 2002 claim of 8% urban land-use is probably an underestimate. The Neuse Basin averages 53 more humans per square kilometer than the Tar Basin, which is also a source of considerable impact for area water use (NCDENR 2002, 2004).

Though not quantified in our study, a second potential cause of Carolina madtom decline in the Neuse Basin is the recent introduction of flathead catfish *Pylodictis olivaris*. North Carolina Wildlife Resource Commission biologists working in these systems have noted Carolina madtom declines in the basin's larger river segments that have historically held populations. Flathead catfish typically inhabit these large rivers and have been documented to forage on *Noturus* spp. (Guier et al. 1981; Brewster 2007); in some cases near eradication of native ictalurid species have been recorded (Thomas 1993). Further, simulation modeling suggests that flathead catfish suppress native fish abundance in streams by 5–50% through predatory and competitive interactions (Pine et

al. 2007).

We found visual snorkel surveying to be an effective method of Carolina madtom instream detection and recommend it for similar studies of cover-associated benthic fishes where conditions are suitable. Although there are concerns inherent to visual snorkel surveying (Ensign et al. 1995; Thompson 2003), similar studies of benthic species have suggested visual detection to be as good as traditional methods (Hankin and Reeves 1988) and preferable for threatened and endangered species (Jordan et al. 2008). Burr et al. (1989) employed kick seining to sample Carolina madtom, a viable method, but one that would have prevented us from identifying microhabitat occupancy. Other traditional fish sampling methods (e.g., electrofishing or other netting gears) would have posed similar problems. Past and present work with this fish by biologists at the NCWRC suggested visual snorkeling to be the most effective method, and after familiarizing ourselves with a reach, we were able to thoroughly and confidently survey the entire delineated reach. Concurrent surveys in 2007 by NCWRC state biologists found similar Carolina madtom abundances to those documented in our study, further illustrating the accuracy of the method. Limitations to the technique were almost exclusively imposed when streams quickly increased in flow and turbidity, as is typical in low-gradient, impacted streams.

Management Implications

The Carolina madtom was recently listed as a State Threatened species (LeGrand 2008). The IUCN also includes the Carolina madtom on its Red List of Threatened Species, but the status is considered data deficient (Baillie et al. 2004). With apparently

declining populations in approximately half of the native range and general life history questions still unanswered, additional conservation measures may be necessary for Carolina madtom in the near future to ensure the long-term existence of the species.

Our design and deployment of an artificial cover unit significantly increased the abundance of fish in a treatment area. Because we didn't quantify reproductive behaviors, we cannot comment on their ability as reproductive structures beyond anecdotal observations. We did note occasional Carolina madtom egg guarding and occurrence of *Noturus* spp. young of the year within the artificial cover units. Between sampling years, eastern North Carolina rivers experienced no catastrophic flooding or serious rainfall events (e.g., hurricanes), so we cannot unequivocally predict their retention under extreme flows. Perhaps the most pragmatic aspect of the artificial cover units we designed is that they are quickly and inexpensively produced; an individual unit can be assembled in less than two hours with approximately US\$2 in materials.

Uniform placement of artificial cover units in our study allowed identification of the most effective instream locations should they be applied on a larger scale. Microhabitat parameters associated with occupied artificial cover units closely resembled those of fish occupying natural, instream habitat. Because bottom velocity and substrate were particularly important microhabitat parameters for occupying fish, we suggest an artificial cover unit distribution concentrated in areas of most suitable natural, instream habitat, focusing specifically on both velocity and substrate, would be most effective. While stream restoration is a much larger undertaking than the addition of cover units or fish aggregation devices, these cover units show promise as a short-term, spatially-

restricted component of improvements designed to restore stream cover and support viable Carolina madtom populations.

Carolina madtom play an important role in stream ecosystems, whether in more traditional ecological roles or as part of the suite of Tar-Neuse endemics that make these rivers biologically diverse and distinct. The Swift Creek (Tar 2) and Fishing Creek (Tar 3) tributaries within the Tar Basin are the most biologically diverse in the state (NCNHP 1997), and Swift Creek may be the most significant lotic ecosystem remaining along the Atlantic Seaboard (Alderman et al. 1993). Due to the specific microhabitat requirements and ecological sensitivity of Carolina madtom, the possibility exists to use them as an indicator of overall stream health. Urban land use can severely degrade stream ecosystems (Booth and Jackson 1997; Wang et al. 2000; Roy et al. 2003), and it is likely that Carolina madtom abundances may be negatively influenced as stream degradation increases, both on basinwide and stream-reach scales. Another possible ecological role for Carolina madtom is in a symbiotic or commensal relationship with the rare mussel species found in the Tar River Basin (e.g., the federally endangered Tar River spiny mussel *Elliptio steinstansana*). Mussel glochidium larvae are known to use fish hosts for part of their life (Neves et al. 1985; Yeager and Saylor 1995). With nearly the same habitat requirements for Carolina madtom and rare mussel species, protecting and enhancing Carolina madtom populations could yield positive effects on sympatric freshwater mussels, another imperiled group being actively managed. The application of our results in a management framework will allow the informed actions to protect and enhance the instream habitat of this imperiled endemic fish.

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Table 1. Six Carolina madtom study sites in the Tar (spring and summer 2007) and Neuse (spring and summer 2008) river basins.

Site	Basin	Latitude (N)	Longitude (W)
Tar 1	Tar River	36°08'37.01"	78°22'30.99"
Tar 2	Tar River	36°04'24.65"	78°52'07.63"
Tar 3	Tar River	36°11'10.26"	78°52'50.75"
Neuse 1	Neuse River	35°41'47.99"	78°03'34.92"
Neuse 2	Neuse River	35°26'43.56"	78°02'49.50"
Neuse 3	Neuse River	35°31'12.68"	78°22'38.27"

Table 2. Categories used to describe substrate composition based on a modified Wentworth particle size scale (Bovee and Milhous 1978). Continuous variable values aggregated among substrate categories for microhabitat analyses.

Particle category	Size class (mm)	Continuous variable
Large boulder	>1000	5
Medium boulder	500-1000	5
Small boulder	250-500	5
Large cobble	130-250	4
Small cobble	64-130	4
Very coarse gravel	32-64	3
Coarse gravel	16-32	3
Medium gravel	8-16	3
Fine gravel	4-8	3
Pea gravel	2-4	3
Very coarse sand	1-2	2
Sand	0.062-1	2
Silt/clay	<0.062	1

Table 3. Carolina madtom microhabitat use and availability statistics for Tar 1–3 and Neuse 1 study reaches. SE = Standard Error.

Stream and Variable	<i>N</i>	Mean/mode	SE	Range
Tar 1 microhabitat use				
Depth (m)	21	0.27	0.03	0.12–0.68
Bottom velocity (m/s)	21	0.16	0.02	0.02–0.37
Mean column velocity (m/s)	21	0.27	0.03	0.12–0.52
Substrate	21	sand		
Cover	21	woody debris		
Tar 1 microhabitat availability				
Depth (m)	273	0.23	0.01	0–0.72
Bottom velocity (m/s)	273	0.13	0.01	0–0.46
Mean column velocity (m/s)	273	0.19	0.01	0–0.61
Substrate	273	sand		
Cover	273	woody debris		
Tar 2 microhabitat use				
Depth (m)	37	0.51	0.03	0.01–0.86
Bottom velocity (m/s)	37	0.10	0.01	0–0.23
Mean column velocity (m/s)	37	0.17	0.01	0.04–0.40
Substrate	37	gravel		
Cover	37	cobble		
Tar 2 microhabitat availability				
Depth (m)	278	0.45	0.01	0–0.90
Bottom velocity (m/s)	278	0.03	0.00	0–0.20
Mean column velocity (m/s)	278	0.10	0.01	0–0.34
Substrate	278	silt		
Cover	278	woody debris		

Table 3 (continued).

Variable	<i>N</i>	Mean/mode	SE	Range
Tar 3 microhabitat use				
Depth (m)	37	0.48	0.03	0.24–0.92
Bottom velocity (m/s)	37	0.11	0.01	0–0.25
Mean column velocity (m/s)	37	0.18	0.01	0–0.31
Substrate	37	gravel		
Cover	37	cobble		
Tar 3 microhabitat availability				
Depth (m)	277	0.55	0.02	0–1.14
Bottom velocity (m/s)	277	0.03	0.00	0–0.20
Mean column velocity (m/s)	277	0.08	0.01	0–0.40
Substrate	277	silt		
Cover	277	woody debris		
Neuse 1 microhabitat use				
Depth (m)	25	0.29	0.02	0.12–0.44
Bottom velocity (m/s)	25	0.21	0.02	0.04–0.43
Mean column velocity (m/s)	25	0.34	0.02	0.19–0.58
Substrate	25	gravel		
Cover	25	cobble		
Neuse 1 microhabitat availability				
Depth (m)	330	0.14	0.01	0–0.40
Bottom velocity (m/s)	330	0.10	0.01	0–0.46
Mean column velocity (m/s)	330	0.14	0.01	0–0.65
Substrate	330	gravel		
Cover	330	cobble		

Table 4. Carolina madtom mean reach densities for pre- and post-artificial cover unit treatment. Reach densities (treatment and control) are extrapolated to a standard area unit (fish/ha).

Stream	Cover units	Pre-treatment				Post-treatment			
		Control		Treatment		Control		Treatment	
		fish/reach	fish/ha	fish/reach	fish/ha	fish/reach	fish/ha	fish/reach	fish/ha
Tar 1	36	0.3	5.1	1.7	25.6	1.2	17.9	10.2	156.4
Tar 2	28	1.5	21.7	2.3	32.5	1.7	24.1	13.0	187.7
Tar 3	24	1.3	20.6	0.8	12.3	3.4	56.4	4.7	77.6
Neuse 1	29	1.3	17.2	1.2	15.1	0.8	10.8	3.0	38.7
Mean	29.3	1.1	16.1	1.5	21.4	1.8	27.3	7.7	115.1

Table 5. Statistical comparisons of Carolina madtom microhabitat use and availability and microhabitat availability between the Tar and Neuse river basins. Continuous variables were tested using a K-S two-sample test, and categorical variables were tested with a *G*-test.

Variable	Use vs availability		Interbasin availability	
	Statistic	<i>P</i>	Statistic	<i>P</i>
Depth	$D = 0.156$	0.032	$D = 0.353$	<0.001
Bottom velocity	$D = 0.452$	<0.001	$D = 0.256$	<0.001
Mean column velocity	$D = 0.373$	<0.001	$D = 0.112$	<0.001
Substrate	$D = 0.377$	<0.001	$D = 0.268$	<0.001
Cover	$G = 22.34$	<0.001	$G = 167.96$	<0.001

Table 6. Comparison of suitable microhabitat ranges and percent of suitable microhabitat available for Carolina madtom during the spring and summer of 2007 and 2008 based on four reaches where the species was present.

Variable and reach	Suitable range	Percent available
Depth (m)		
Tar 1	0.10–0.19	19%
Tar 2	0.0–0.19	12%
Tar 3	0.40–0.49	9%
Neuse 1	0.30–0.39	5%
Bottom velocity (m/s)		
Tar 1	0.10–0.14	9%
Tar 2	0.20–0.24	1%
Tar 3	0.15–0.24	5%
Neuse 1	0.20–0.24	7%
Mean column velocity (m/s)		
Tar 1	0.20–0.24	7%
Tar 2	0.25–0.29	4%
Tar 3	0.20–0.34	12%
Neuse 1	0.35–0.39	2%
Substrate		
Tar 1	gravel	1%
Tar 2	gravel	12%
Tar 3	cobble	3%
Neuse 1	cobble	2%
Cover		
Tar 1	woody debris	32%
Tar 2	boulder	4%
Tar 3	cobble	28%
Neuse 1	cobble	8%

Table 7. Retained component loadings (based on a correlation matrix) from principal components analysis on Tar 1–3 and Neuse 1 study reaches. Significant loadings are found in bold.

Reach and Variable	Component 1	Component 2
Tar 1 (<i>N</i> = 273)		
Depth	0.30	0.86
Bottom velocity	0.59	-0.16
Mean column velocity	0.62	0.06
Substrate	0.43	-0.48
Eigenvalue	2.35	0.95
Variance explained	59%	25%
Tar 2 (<i>N</i> = 278)		
Depth	0.08	0.96
Bottom velocity	0.61	-0.03
Mean column velocity	0.63	0.11
Substrate	0.47	-0.27
Eigenvalue	1.95	1.02
Variance explained	49%	26%
Tar 3 (<i>N</i> = 277)		
Depth	0.26	0.88
Bottom velocity	0.58	-0.37
Mean column velocity	0.61	-0.19
Substrate	0.47	0.23
Eigenvalue	2.33	0.99
Variance explained	58%	25%
Neuse 1 (<i>N</i> = 330)		
Depth	0.35	0.93
Bottom velocity	0.57	-0.30
Mean column velocity	0.59	-0.12
Substrate	0.46	-0.18
Eigenvalue	2.55	0.80
Variance explained	64%	20%

Table 8. Statistical comparisons (K-S two-sample test) of microhabitat use and availability scores for individual components of the reach-specific principal components analyses.

Component	<i>D</i> Statistic	<i>P</i> -value
Tar 1		
Component 1	0.337	0.024
Component 2	0.183	0.530
Tar 2		
Component 1	0.530	<0.001
Component 2	0.236	0.057
Tar 3		
Component 1	0.610	<0.001
Component 2	0.338	0.001
Neuse 1		
Component 1	0.745	<0.001
Component 2	0.487	<0.001

Table 9. Statistics for Carolina madtom in 1) natural microhabitats, 2) occupied artificial cover units, and 3) unoccupied artificial cover units.

Variable	<i>N</i>	Mean/mode	95% CI	Range
Instream cover use				
Depth (m)	120	0.42	(0.38, 0.46)	0.01–0.43
Bottom velocity (m/s)	120	0.14	(0.12, 0.16)	0–0.43
Mean column velocity (m/s)	120	0.12	(0.10, 0.14)	0–0.58
Substrate	120	sand		
Artificial cover unit use				
Depth (m)	139	0.34	(0.30, 0.38)	0.06–0.94
Bottom velocity (m/s)	139	0.12	(0.10, 0.14)	0–0.53
Mean column velocity (m/s)	139	0.19	(0.17, 0.21)	0–0.53
Substrate	139	sand		
Artificial cover unit non-use				
Depth (m)	466	0.36	(0.34, 0.38)	0–1.04
Bottom velocity (m/s)	466	0.06	(0.05, 0.07)	0–0.41
Mean column velocity (m/s)	466	0.12	(0.11, 0.13)	0–0.61
Substrate	466	silt		

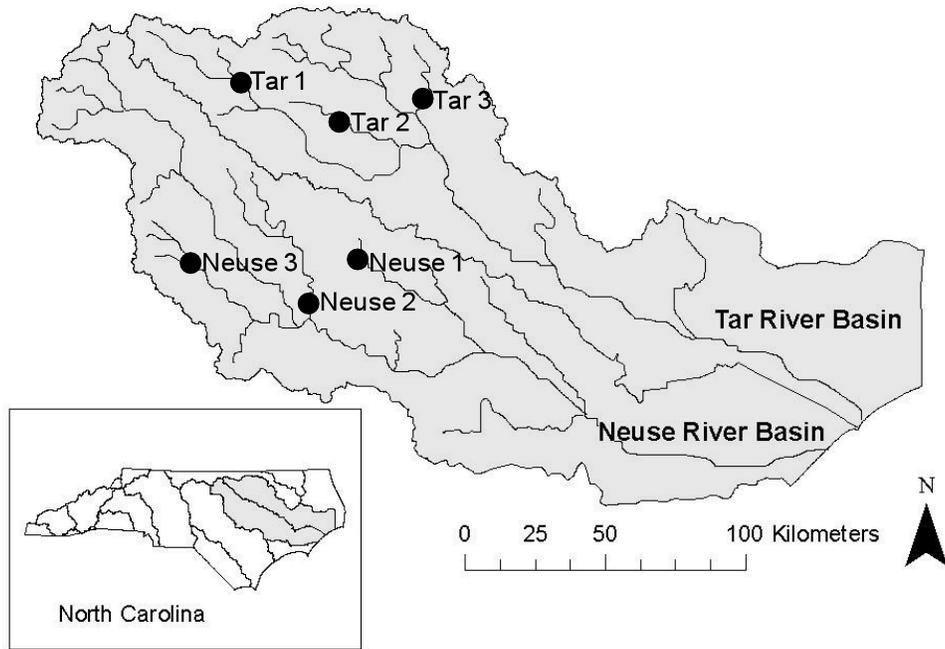


Figure 1. Map of Carolina madtom study reaches in the Tar and Neuse river basins in North Carolina. See Table 1 for site location descriptions.



Figure 2. Artificial cover unit used in the study.

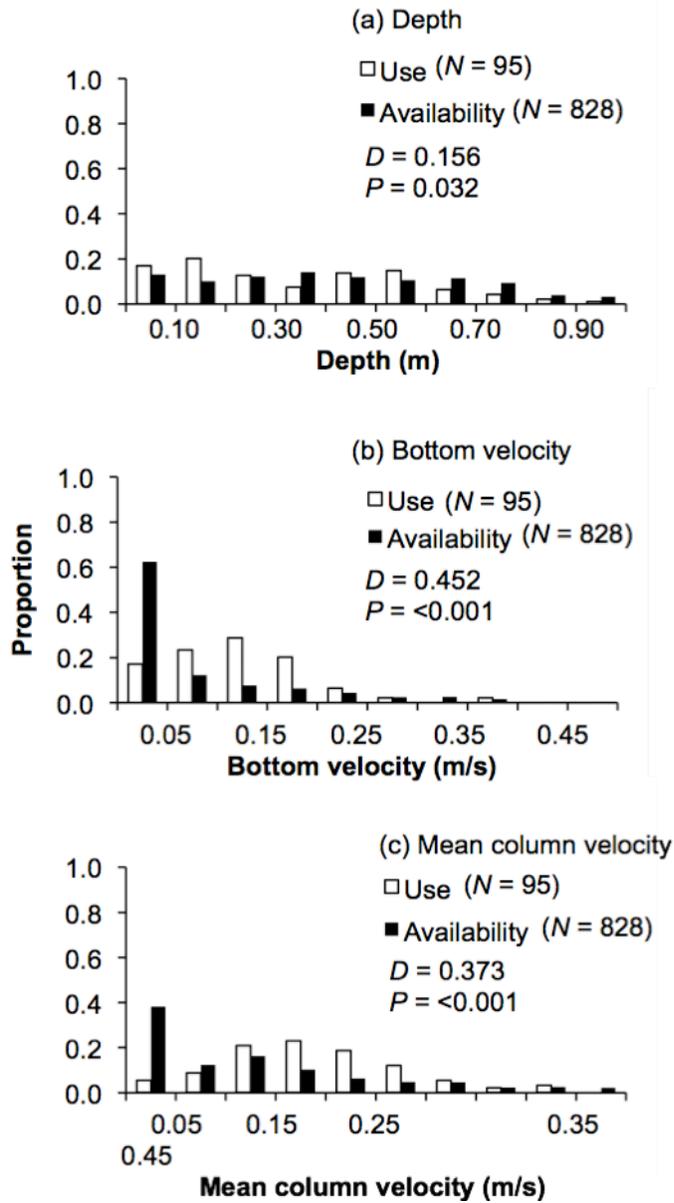


Figure 3. Frequency distributions of (a) depth, (b) bottom velocity, and (c) mean column velocity for Carolina madtom microhabitat use and availability in the Tar River Basin (2007). Use and availability were compared using a K-S two-sample test.

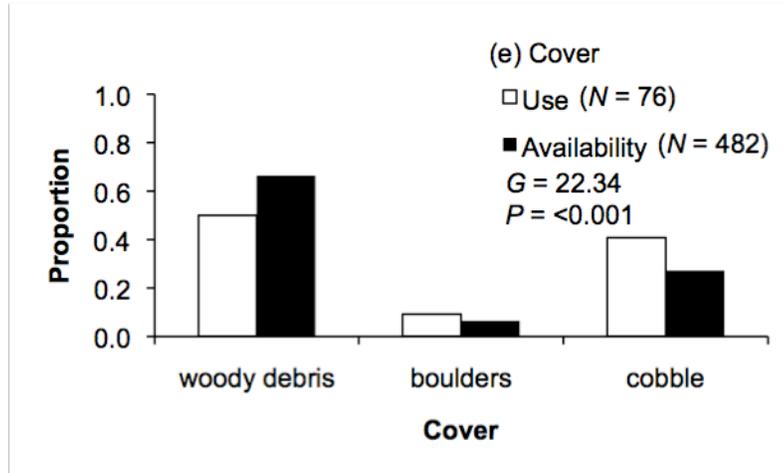
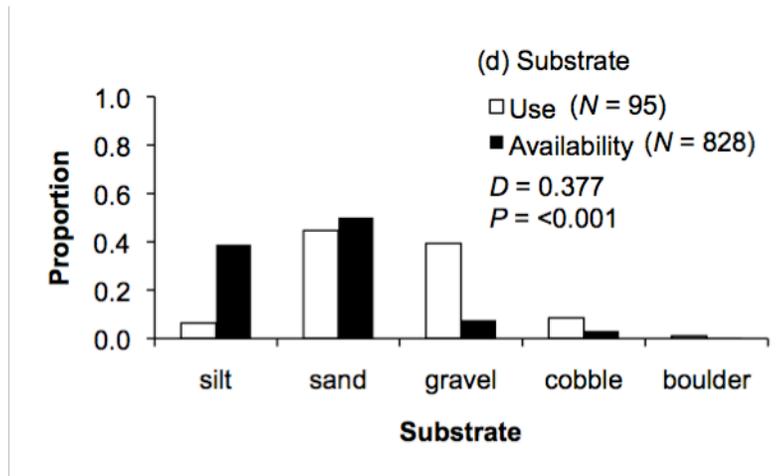


Figure 4. Frequency distributions of (d) substrate and (e) cover for Carolina madtom microhabitat use and availability in the Tar River Basin (2007). Use and availability were compared using a K-S two-sample test for substrate and a *G*-test for cover.

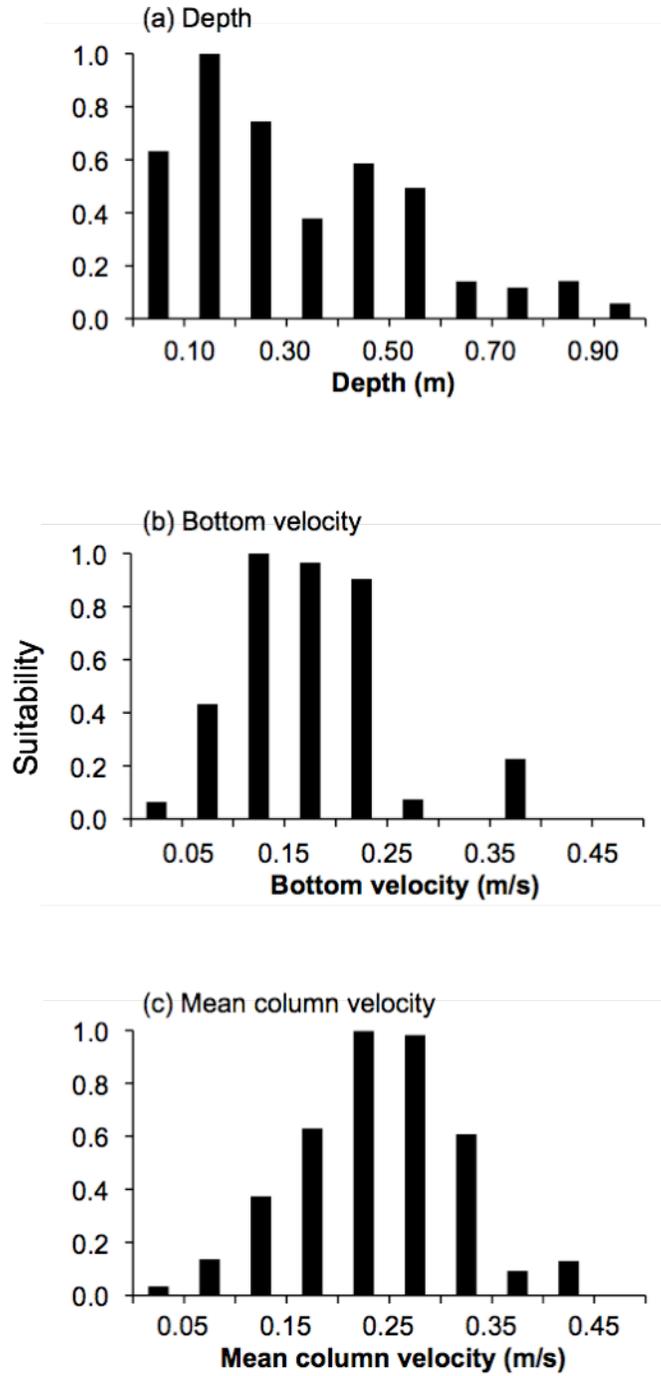


Figure 5. Carolina madtom microhabitat suitability for (a) depth, (b) bottom velocity, and (c) mean column velocity, based on data collected from the Tar River Basin (2007).

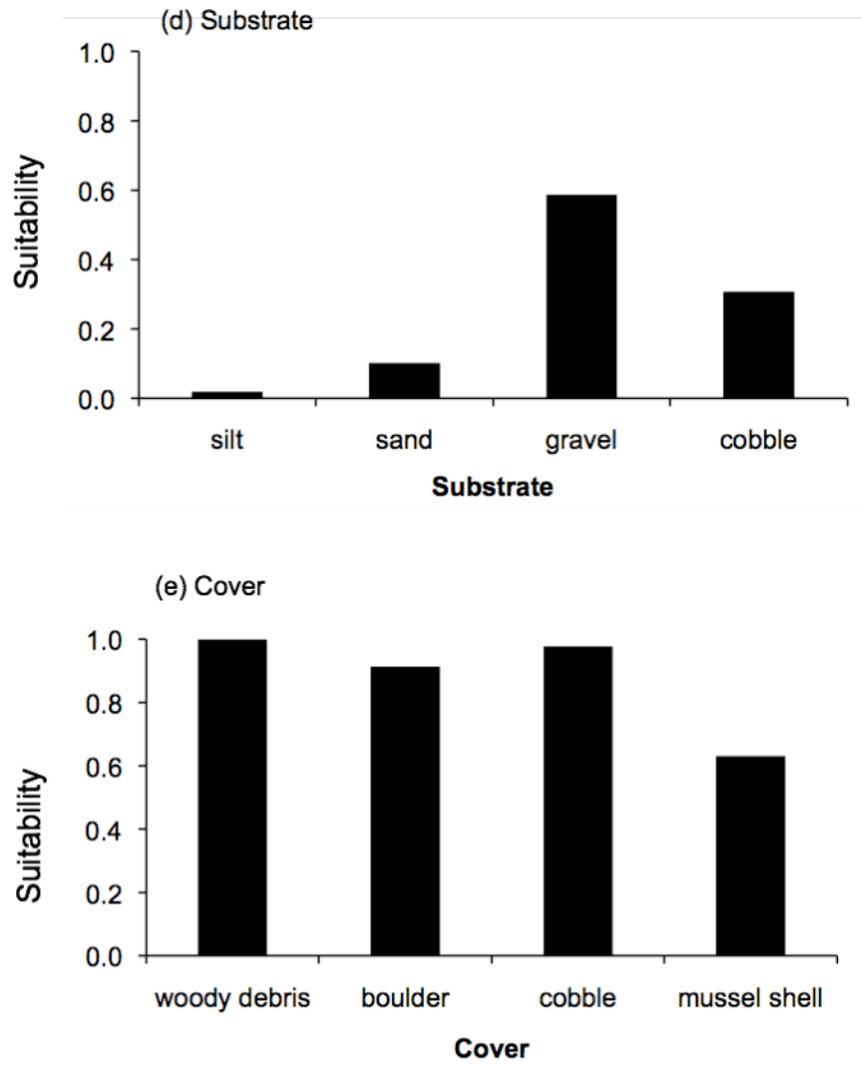


Figure 6. Carolina madtom microhabitat suitability for (d) substrate, (e) cover, based on data collected from the Tar River Basin (2007).

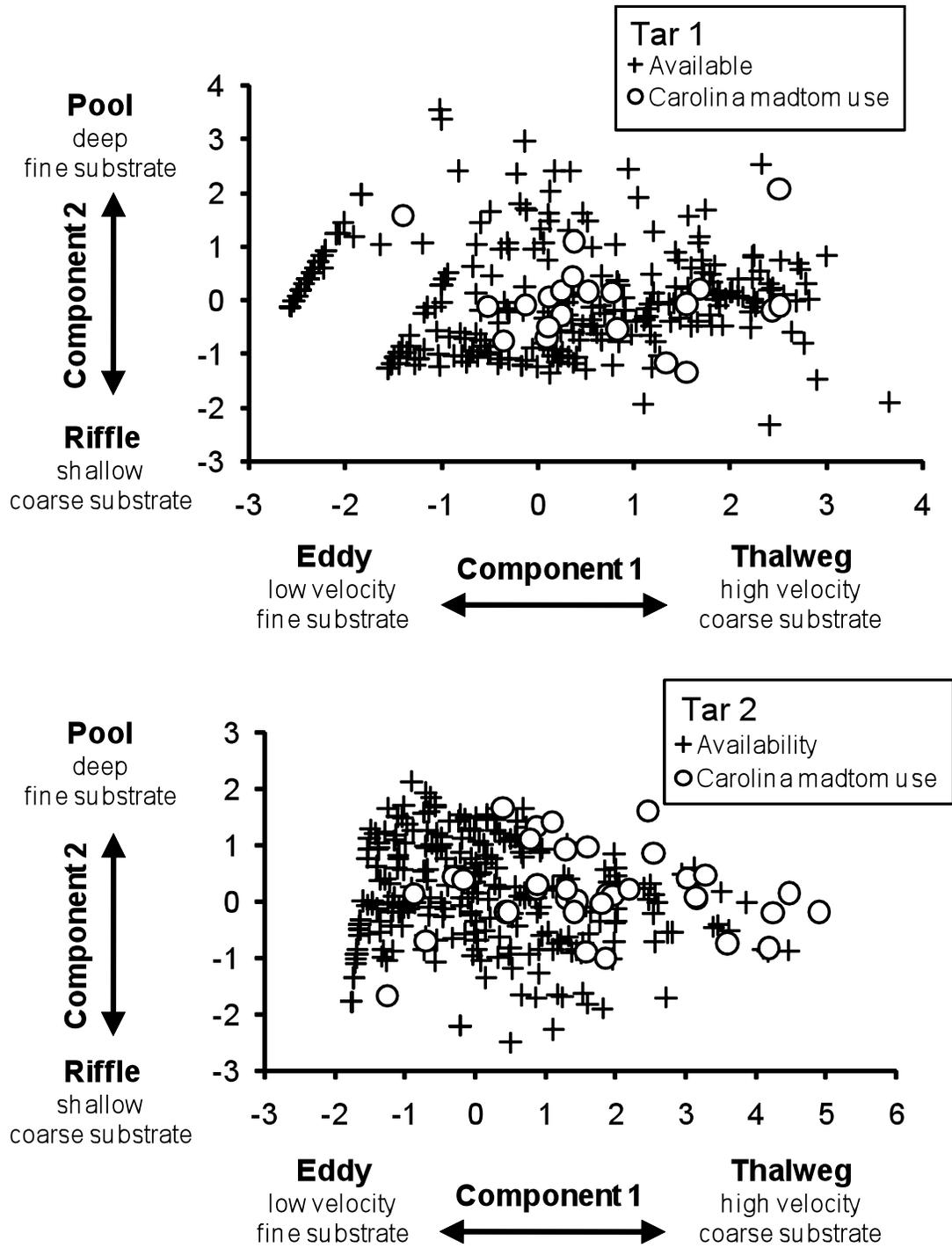


Figure 7. Plots of Carolina madtom microhabitat use and available habitat principal component scores for Tar 1 and Tar 2 study reaches. Component loadings appear in Table 7, and statistical comparisons appear in Table 8.

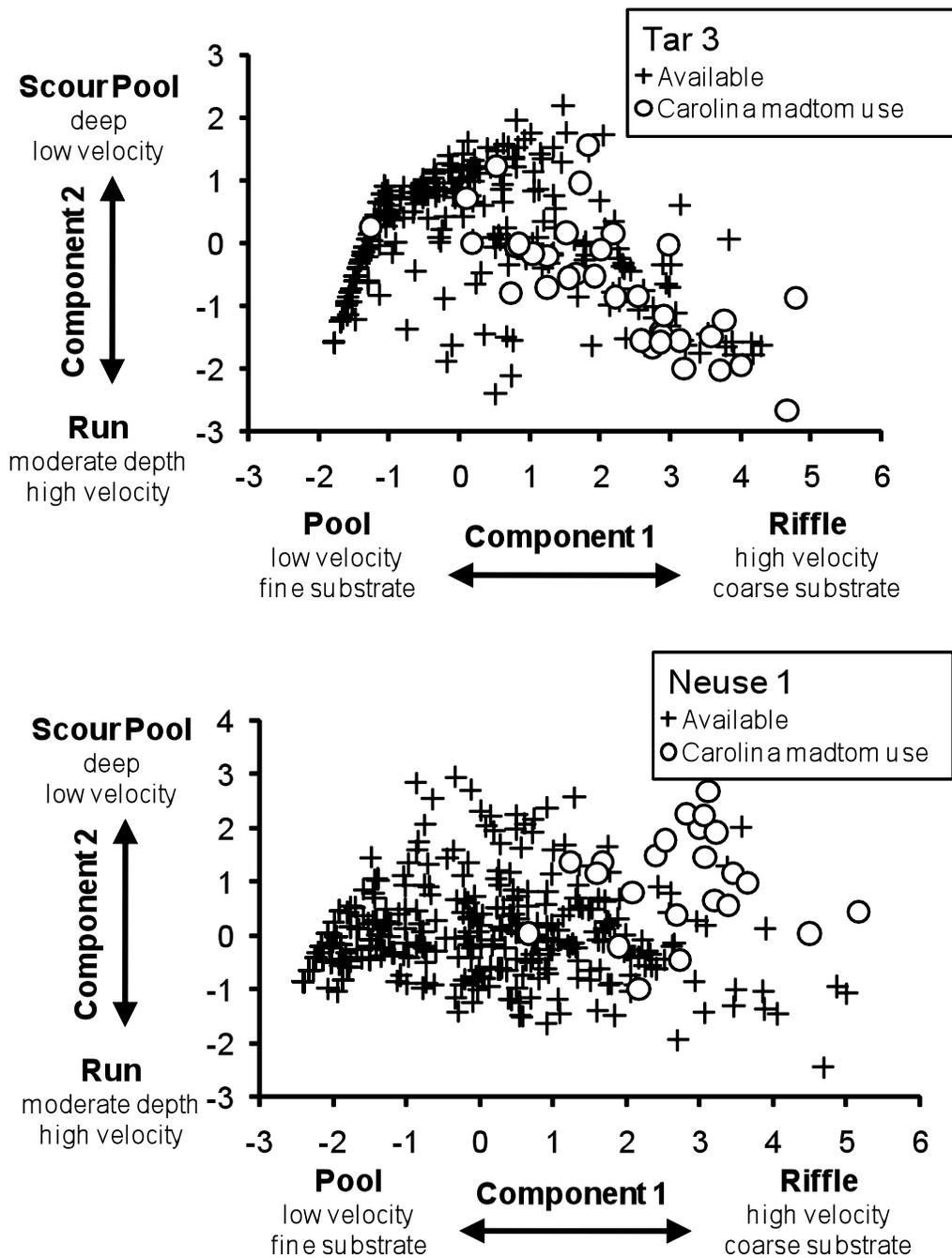


Figure 8. Plots of Carolina madtom microhabitat use and available habitat principal component scores for the Tar 3 and Neuse 1 study reaches. Component loadings appear in Table 7, and statistical comparisons appear in Table 8.

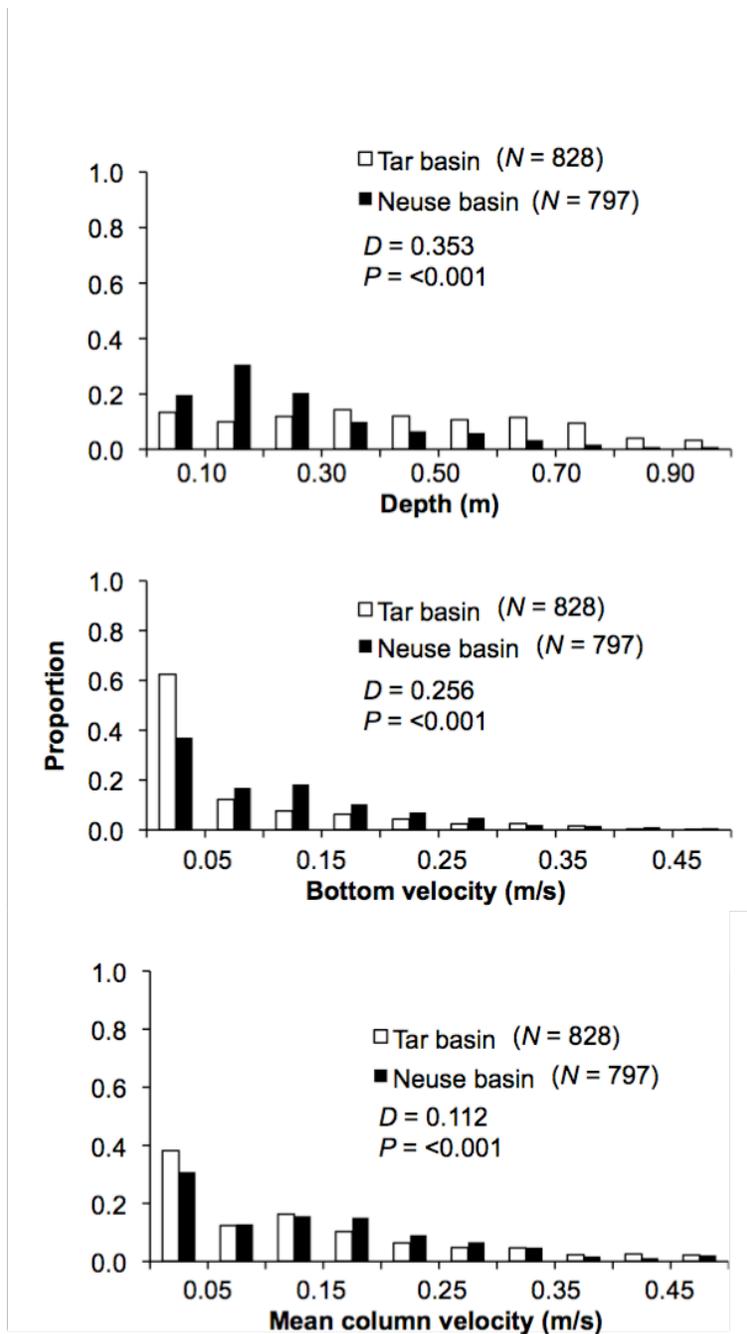


Figure 9. Frequency distributions of depth (top), bottom velocity (middle), and mean column velocity (bottom) for Carolina madtom microhabitat availability in the Tar and Neuse river basins. Use and availability were compared using a two-sample K-S test. Optimally suitable substrate habitat ranges were 0.10–0.19 m for depth, 0.10–0.24 m/s for bottom velocity, and 0.20–0.29 m/s for mean column velocity.

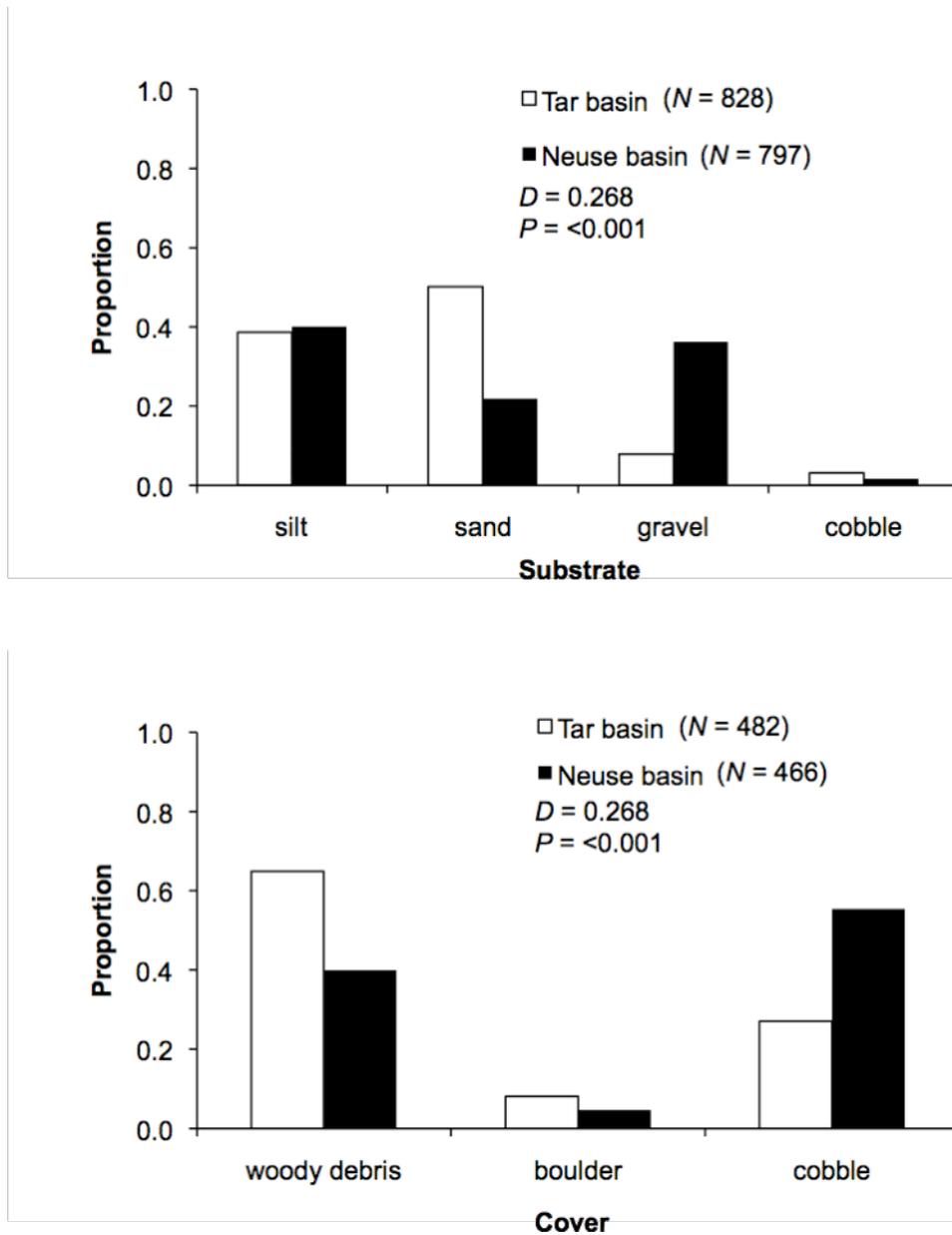


Figure 10. Frequency distributions of substrate (above) and cover (below) for Carolina madtom microhabitat availability in the Tar and Neuse river basins (2007). Use and availability were compared using a K-S two-sample test for substrate and a G-test for cover. Optimally suitable habitat categories were gravel for substrate and woody debris, boulder and cobble for cover.

**Chapter II – Cover Preference of the Carolina Madtom,
an Imperiled Endemic Stream Fish**

Abstract

Habitat destruction is a major threat to North American freshwater fauna, a problem exacerbated by lack of critical ecological information for many nongame species. Instream physical cover is a primary component of habitat that can influence species throughout their ontogeny. In a controlled, laboratory setting, we investigated cover preference of the Carolina madtom, *Noturus furiosus*, an imperiled, endemic southeastern U.S. stream fish. Fish were tested individually and given 24 hours to make a selection from four cover options, including rock, leaf pack, mussel shell, and an artificial cover unit. Among 30 trials, Carolina madtom preferred the artificial cover unit, selecting it 63% of the time. Rock was selected 23% of the time and leaf pack 13%. Mussel shells were not selected during any trial. Given the species' State Threatened status, declining numbers and restricted distribution, our results have implications for conservation and restoration of this native and endemic catfish.

Introduction

Viable habitat is essential to stream fish populations and the quality of lotic systems. Degradation and loss of stream habitat has been widespread and well documented in recent decades, particularly in the southeastern United States where fish diversity is high (Angermeier 1995; Warren et al. 2000; Jelks et al. 2008). This habitat loss is directly related to species declines (Schlosser 1982; Ricciardi and Rasmussen 1999), yet many systems lack appropriate management plans. Even when streams are managed for suitable habitat, the effectiveness of management strategies is often limited by poorly understood habitat requirements of nongame species (Hubert and Rahel 1989). Further, nongame species can be adversely effected by management plans developed for game species, which often have fundamentally different habitat requirements (Aadland 1993). As a result, habitat remains a primary focus of research on stream species, especially nongame species that are threatened or endangered (Pajak and Neves 1987; Wildhaber et al. 2000).

Stream habitat is generally described as a place where fish can find the physical and chemical features required for life (Orth and White 1999), though it can be highly parameterized. One particularly important aspect of habitat is physical instream cover, which is generally defined as any object or formation that offers concealment or visual isolation (Orth and White 1999). Cover is an especially critical habitat component to many benthic species, serving a number of purposes throughout a fish's ontogeny, such as a location for spawning and egg rearing, and shelter from predators (Vogele and Rainwater 1975; Wills et al. 2004). Protection of offspring and increased recruitment

have also been linked to cover (Hoff 1991; Hunt et al. 2002). For example, Tabor and Wurtsbaugh (1991) found that juvenile rainbow trout mortality decreased by 34% when suitable cover was available. Later in life, cover may continue to provide sites for predator avoidance (e.g., Savino and Stein 1982; Gotceitas and Colgan 1989).

Species conservation is perhaps the most important reason to study the habitat and cover associations of nongame species, specifically because habitat destruction has been considered the primary threat to imperiled species (Wilcove et al. 1998). This is particularly true for benthic and endemic guilds, which are among the most intensely impacted species in degraded systems (Angermeier 1995; Piller et al. 2004). In particular, the Madtoms *Noturus* spp. have been documented as significantly imperiled (Piller et al. 2004), likely because nearly all members of this group are both benthic and endemic. Loss of viable habitat has been cited as the leading impediment to madtom conservation (Robison and Harp 1985; Etnier and Starnes 1991). Empirical attempts at quantifying cover requirements for madtoms are rare and additional studies are needed for the development of robust conservation and management plans. One strategy for species conservation involves cover restoration, by either re-establishing natural cover or introducing a known and suitable artificial cover. Studies on the effectiveness of restoring habitats through artificial cover have produced equivocal results (Heggenes and Traaen 1988; Glova 1999), though evidence for successful introduction of artificial cover exists for a number of commercially and recreationally valuable species (Butler and Hawthorne 1968; Moring and Nicholson 1994; Eklöv and Greenberg 1998).

The Carolina madtom, *Noturus furiosus*, is an endemic, cover associated fish species found in only two river basins entirely within the state of North Carolina, USA. In 2008, the conservation status of the species was listed as State Threatened (Le Grand et al. 2008). Recent work has shown that rock cobble and leaf packs are the most suitable instream covers (Chapter 1), though Burr et al. (1989) noted anecdotal observations of mussel shell use. In addition, Carolina madtom use of artificial cover, such as 355-ml aluminum cans, has also been reported (Burr et al. 1989). Midway (Chapter 1) found that artificial cover significantly increased the abundance of Carolina madtom in paired stream reaches when compared to adjacent control reaches with no artificial cover.

The goal of this study was to quantify cover preference of the Carolina madtom based on choice experiments involving one artificial and three naturally occurring cover types, in a controlled, laboratory setting. While conclusions from microhabitat field research may be limited by habitat availability and lack of microhabitat choices, controlled laboratory studies may reveal clear microhabitat preferences with strong inference. In previous work, Carolina madtom clearly demonstrated use of artificial cover (Chapter 1); however, it is not clear how this use might be impacted by availability (or loss) of natural habitat. Herein, we examine cover use of the Carolina madtom in a controlled setting, and quantify use of the artificial cover unit relative to natural cover types. Any tendency to use the artificial cover would be highly relevant given the species' recent decline, and could be useful for stream managers in developing conservation plans.

Methods

Field Collection

Carolina madtom were collected from populations in Swift and Little Fishing creeks (Tar River Basin) near Red Oak, North Carolina during June and July 2008. Individuals were collected through a combination of visual snorkel inspection of natural habitat as well as from artificial cover units deployed earlier at field sites. Because of its State Threatened status, it was necessary to limit experimentation to a small number of individuals. Upon collection, fish were placed into 19-L buckets with aerated water at ambient stream temperatures (23 - 25°C) for no more than 90 minutes. Captured fish were transferred and acclimated to a laboratory at the Historic Yates Mill County Park in Raleigh, North Carolina.

Setup and Holding

Fish were housed in 19- or 38-L aquarium units. Each fish was assigned to a holding tank outfitted with one of two types of cover, either a PVC pipe with one open end or two (15 x 5 cm) tiles glued together at a right angle. These cover types were unlike any of the experimental cover types. The bottom of the holding tanks (and the experimental system) were covered in approximately 2.5 cm of crushed river rock that closely resembled the most suitable substrate size for Carolina madtom in natural systems (Midway 2008). Water for all tanks was supplied from a local well, and 20% water changes were performed weekly. Temperature and light conditions were identical among tanks; temperature remained constant at 18-19°C and lighting was timed to mimic local diurnal patterns (14 hours of sunlight and 10 hours of darkness).

Laboratory Experiments

Cover preference experiments were conducted in a 530-L recirculating Living Stream (Frigid Units, Toledo, Ohio). The living stream tank provided an 133 x 44 cm experimental area physically separated from a chiller unit at one end and a filter at the opposite end (Figure 1). The experimental area was divided into an acclimation zone (1/3 of the experimental space) with a removable divider, and an experimental zone where the cover types were placed and made available (Figure 1). Four cover types were deployed in the experimental zone and included rock, leaf pack, mussel shell, and artificial cover unit. The rock and mussel shell were collected from a stream within the species' native drainage. A leaf pack was composed of native tree leaves and held together with a thin mesh net. Our artificial cover unit (Figure 2) consisted of two flowerpot saucers, one 100 mm and the other 150 mm in diameter. The smaller, top saucer had a 25-mm opening on one side and three small vent holes on the other. This saucer was then inverted and glued on a larger flowerpot saucer, also inverted. Rocks were glued to the bottom of the entire unit to provide weight. Cover configurations (i.e., the specific location of rock, leaf pack, mussel shell, and artificial cover unit) within the experimental tank were randomly generated for each trial, and individual fish never received the same configuration twice. Pilot trials of different experimental times (1, 12 and 24 hours) indicated that 24 hours was the best experimental time frame to maximize the likelihood of detecting a stable cover selection and minimize intrusive observation. Each trial was conducted as follows: an individual fish was transferred to the acclimation zone for 30 min. At the end of the acclimation period, the divider was temporarily lifted

and the fish was moved into the test area, either on its own or with gentle prodding. At this time, the divider was re-deployed and the observer left the room and returned 24 hours later to record which cover (or if no cover) was selected. Final observations and recordings were made without any changes (e.g., light, temperature, noise) introduced to the room and by an observer using a viewing pane that alleviated any need to touch the experimental system or hover over it before recording. A total of 30 trials were conducted using 12 fish, each completing between one and six trials.

Analyses

Because the Carolina madtom is a protected species, we only collected a small number of animals. To maximize the amount of information collected from these individuals, most experimental individuals were used in multiple cover-choice trials. For statistical analyses, we used a non-parametric bootstrap to account for the repeated use of some individuals. We generated bootstrap data sets by first sampling individual fish with replacement from the set of experimental fish, and then mimicking a cover-choice trial by choosing a cover type based the actual cover choices of each fish. Each bootstrap fish was used for a variable number of cover-choice trials to match the structure of the actual data. We then calculated the proportion of times that the artificial cover type was chosen in each of 100,000 bootstrap data sets. These bootstrap proportions were used to calculate a percentile-based 95% confidence interval for madtom preference for the artificial cover, and to generate a p-value to test the null hypothesis that cover choice was random (i.e., artificial cover was chosen 25% of the time). Significant *P*-values (<0.05) indicate non-random or preferential use of the artificial cover unit.

Prior to analyzing the results for cover preference, we also tested for an effect of holding tank cover (PVC vs. tile) and experimental cover position (i.e., configuration of cover types in the experimental system). Both associations were tested with a standard chi-square goodness-of-fit test. Significant results would indicate a relationship between experimental cover choice and holding tank cover or preference of a position within the test area, regardless of cover type.

Results

Carolina madtom used in the experiment averaged 80 mm total length and ranged from 62 to 97 mm. A chi-square test on experimental cover position did not detect a trend ($X^2=1.733$, $P=0.6295$), suggesting that fish did not favor any location within the Living Stream. Likewise, chi-square analysis did not reveal a correlation between holding tank cover type and ultimate experimental cover choice ($X^2=0.2694$, $P=0.8740$), demonstrating that cover exposure during non-experimental periods did not influence experimental cover preference.

Carolina madtom overwhelmingly selected the artificial cover unit, choosing this cover in 19 of 30 trials (63%). Rock was selected seven times (23%) and leaf pack four (13%), whereas the mussel shell was never used. In multiple trials with individual fish, cover selections often varied, suggesting the high rate of artificial cover use was not influenced by a few, highly selective fish (Figure 3). The bootstrap analysis generated 100,000 data sets, where each data set included 30 simulations (i.e., trials) representing the size of the original data set. We used the results of the simulated data sets to calculate

a 95% confidence interval of 37%-87% for use of the artificial cover unit. Though a fairly large range, this bootstrap result effectively rejected ($P=0.0025$) the null hypothesis that use of the artificial cover type was 25%, the expected use given the actual ratio (1:3) of the artificial cover unit to natural cover types.

Discussion

Carolina madtom clearly preferred the artificial cover unit when presented a choice of natural cover types in our experiment. This result is perhaps surprising, given that an artificial (unfamiliar) cover type might be expected to be less desirable than known, instream covers. On the other hand, our results are consistent with field observations of artificial cover use by Carolina madtom (Chapter 1) as well as their observed tendency to use artificial structure or human refuse (Burr et al. 1989). Our findings, combined with documented use of the same artificial cover unit in natural systems (Chapter 1), provide useful information relevant to development of habitat conservation plans for the Carolina madtom. In streams where cover is found to be limiting, introduction of these cover units could provide a suite of potential benefits such as predator avoidance and increased spawning habitat. However, because cover is only one component of habitat, it should not be assumed that high levels of occupancy would result from a random instream distribution of artificial cover units. Instream cover placement, which has been most successful in areas with suitable microhabitat (Chapter 1, Table 10), would need to inform artificial cover use and management.

Though we believe this artificial cover unit could be a valuable component of Carolina madtom conservation and restoration strategies, we recognize a number of limitations in this investigation. While we selected a suitable, coarse gravel substrate for our laboratory experiments, other microhabitat parameters such as water depth, velocity and chemistry influence instream cover selection (Fausch 1984; Baltz et al. 1987; Chapter 1). Madtoms have been observed enlarging or otherwise modifying cover structure by oral movement of pebbles, demonstrating fine-scale nest qualities that are widely overlooked (Cochran 1996). Structural complexities we didn't account for, such as dimensions, complexity, and orientation of cover, suggest that even within a cover type, there are desirable characteristics that may require additional consideration to ultimately determine effectiveness of the cover (Johnson 1993; Monzyk et al. 1997). Variation in habitat cover use has been demonstrated to change in the presence of predators (Power and Matthews 1983; Gilliam and Fraser 1987; Johnson 1988), providing evidence that within a lifetime and under different biotic environments, fish may use multiple cover types. It is also advisable to understand seasonal cover use for a managed species, and this information remains incomplete for the Carolina madtom. Cover introduction to swift moving water over gravel beds may be appropriate for spring and summer, however, instream overwintering habitat requirements may differ substantially.

Wilcove et al. (1998) identified habitat loss as the primary threat to imperiled fauna in the United States, and while the Carolina madtom may be declining due to habitat loss, declines in other assemblages may be influencing Carolina madtom

behavior. For example, we recorded no use of mussel shell cover in the lab, but previous work listed mussel shells as a primary natural cover (Burr et al. 1989). This observation could be correlated to declining mussel populations (North Carolina Wildlife Resources Conservation, unpublished data; Neves et al. 1997; Vaughn and Taylor 1999) and this faunal change may have shifted Carolina madtom cover use from mussel shells to rock and human refuse based on availability.

Habitat degradation is also the single greatest threat to madtom conservation (Robison and Harp 1985; Etnier and Starnes 1991). Of the 14 future research needs for madtom species, Burr and Stoeckel (1999) included three relating to physical habitat: nest construction, critical habitat, and nest fidelity. This study directly informs the critical habitat component, and has implications for nesting issues, as evidence suggests that these fish use cover extensively during spawning (Burr et al. 1989; see Chapter 1). Effective madtom conservation also includes accounting for not only a top-down species-approach, but also a bottom-up process-approach, whereby the stream processes that create viable habitat will be conserved (Rosenfeld 2003). Though conservation is in place for a number of madtom species (Burr and Stoeckel 1999; Wildhaber et al. 2000), more widespread and increased protection may be required to ensure survival of imperiled populations. Due to the declining range and lack of protection, the Carolina madtom aligns well with those species in which more work is required to guide improved management.

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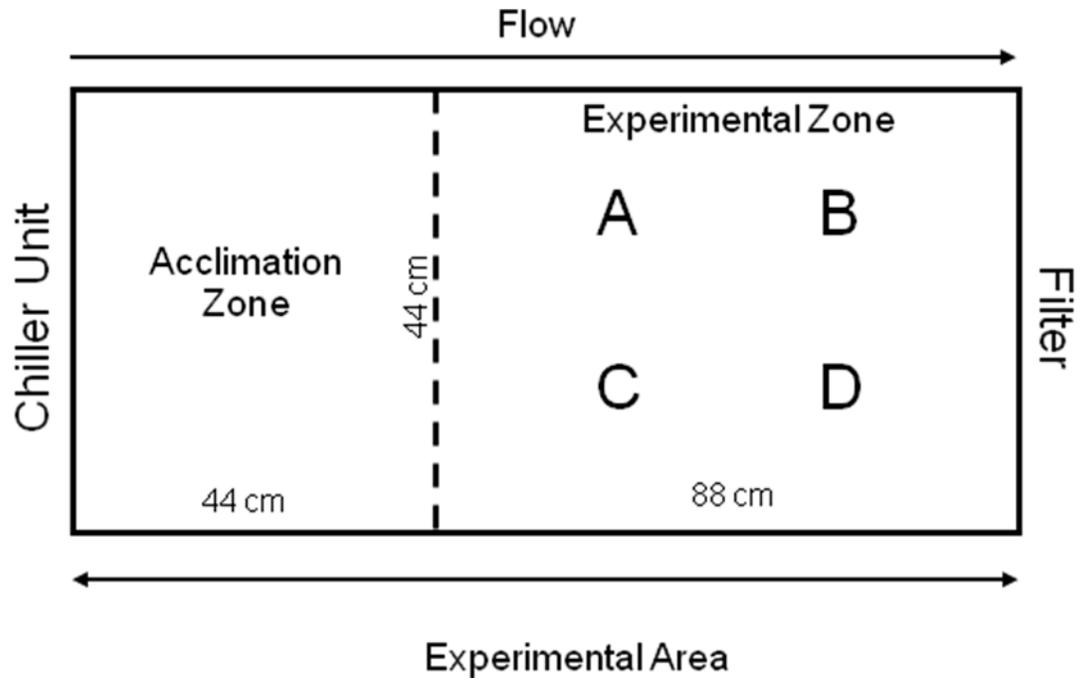


Figure 1. Diagram of the Living Stream experimental area, including the acclimation zone and configuration of randomly selected cover types (A, B, C, D) within the experimental zone.



Figure 2. The artificial cover unit employed in cover choice experiments.

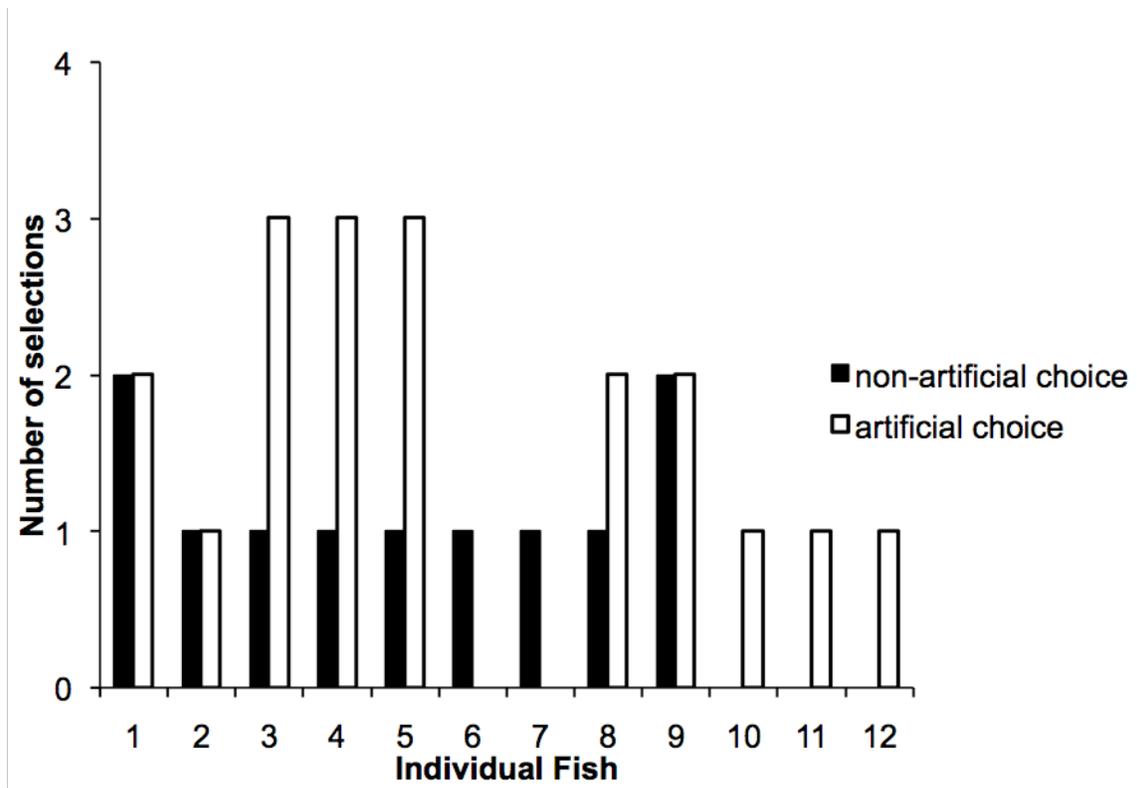


Figure 3. A comparison of selection of non-artificial (i.e., natural) and artificial cover types by individual fish.