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[Article]

Constructed Microhabitat Bundles for Sampling Fishes and Crayfishes in Coastal Plain Streams

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Abstract.—We investigated fish and crayfish use of standardized, constructed microhabitats (bundles) in three northern Mississippi streams. Cypress Creek and the Little Tallahatchie Canal were channelized and incised and had little woody cover; Puskus Creek was unchannelized and unincised and had abundant woody cover. We constructed three types of bundles (cane, leaf, and string) and deployed replicates of each in winter and spring. Occupancy of bundles by fish and crayfish was high and included 32 fish species representing eight families. Fish abundance did not differ among bundle types or between channel positions (bank or midchannel), but abundance of crayfish showed mixed responses to bundle type and position. Fish and crayfish use of bundles was higher in the channelized streams (89% occupied) than in the unchannelized stream (49% occupied). Furthermore, after a winter storm, fish use increased in the channelized streams but not in the unchannelized stream. Bundles yielded abundance estimates with modest to poor precision (40–73% for fish; 37–125% for crayfish); about 110–140 bundles would be necessary to consistently achieve precision of 30%. Bundles were effective for sampling a subset of fish assemblages (e.g., darters *Etheostoma* spp. and *Percina* spp. and madtoms *Noturus* spp.), but other fish species were conspicuously underrepresented or absent in our samples relative to sampling by electrofishing and seine (e.g., open-water species and large individuals). Nevertheless, microhabitat bundles can be effective for sampling small fish and crayfish that associate with woody cover and that are difficult to sample with conventional methods.

Wood in streams defines flow patterns and channel dimensions and provides food and shelter for a wide array of aquatic organisms (Benke et al. 1985; Dolloff and Warren 2003; Jowett et al. 2008). Large woody material has received the most attention from researchers (Gregory et al. 2003), but small woody material (e.g., twigs, stems, and leaves; Smock et al. 1989; Henderson and Walker 1990; Jowett et al. 2008) and fine roots of living riparian trees or aquatic plants (Wood and Sites 2002) also contribute to structure and diversity of stream habitats. Woody material appears to be especially important in Atlantic and Gulf coastal plain streams of North America, where rocks can be rare and sand is often the dominant substrate (Drury and Kelso 2000; Warren et al. 2002; Benke and Wallace 2003). In these habitats, woody material represents the major source of fish cover, an important source of crayfish food (e.g., Huryn and Wallace 1987; Whitledge and Rabeni 1997), and the primary substrate for macroinvertebrate production (Benke and Wallace 2003). Therefore, woody material can be a strong determinant of fish and macroinvertebrate assemblage structure (e.g., Angermeier and Karr 1984; Meffe and Sheldon 1988; Schofield et al. 2001; Shields et al. 2006).

Experimental studies and quantitative descriptions of woody microhabitats and warmwater fish or crayfish use are few (e.g., Angermeier and Karr 1984; Monzyk et al. 1997; Crook and Robertson 1999; Fletcher et al. 2004; Wright and Flecker 2004; Hrodey and Sutton 2008; Jowett et al. 2008), in part because of the difficulty of sampling instream woody material. In marine and freshwater invertebrate ecology, standardized artificial substrates are used widely to study faunal use of substrates that are difficult to sample in situ (Cairns 1982; Sheldon 1984). Artificial substrates also are used to sample marine fishes in applications ranging from censuses (Silberschneider et al. 2001;

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TABLE 1.—Location and characteristics of study sites used to evaluate fish and crayfish use of constructed microhabitat units (bundles) in coastal plain streams of northern Mississippi. Mean wetted width and water depth for Cypress Creek are from Adams et al. (2004; eight site visits across seasons); wetted width and depth for other streams are from Warren et al. (2002, and unpublished; summer measures only). Mean (±SD) depth and current at bundles are presented with sample size (in parentheses). Planar watershed areas were determined from a geographic information system.

Stream (latitude, longitude)	Mean wetted width (m)	Mean water depth (cm)	Mean depth of bundles (cm)	Mean current at bundles (m/s)	Watershed area (km^2)
Tallahatchie Canal (34°31′45.6″N, 89°21′58.7″W)	24	23	24.9 ± 11.79 (71)	0.21 ± 0.114 (71)	1,802
Cypress Creek (34°26′32.0″N, 89°17′25.0″W)	7	22	14.0 ± 7.58 (80)	0.20 ± 0.129 (80)	74
Puskus Creek (34°26′41.0″N, 89°20′14.8″W)	6	21	21.4 ± 10.40 (49)	0.20 ± 0.183 (49)	49

Upton and Booth 2003) to complex experiments (Hixon and Beets 1993). In freshwater fish ecology, artificial substrates have been used to evaluate movement corridors (rocky overhead cover) for benthic fishes, to model fish community assembly in artificial rocky microhabitats, and to provide spawning surfaces and cover for life history studies and enhancement of fish populations (Piller and Burr 1999; Hunt et al. 2002; Knaepkens et al. 2004; Arrington et al. 2005; Roberts and Angermeier 2007). To our knowledge, no studies have used standardized artificial substrates to evaluate microhabitat use of small woody cover by stream fishes or crayfishes.

We investigated fish and crayfish use of three types of standardized, constructed microhabitats in three northern Mississippi streams with varying disturbance histories. We focused on two measures of microhabitat usage: (1) fish and crayfish abundance and (2) fish assemblage structure. To examine abundance, we compared occupancy patterns of fish and crayfish in microhabitats and explored potential size or abundance interactions between fish and crayfish in the microhabitats. We also evaluated the effect of exposure time, season, microhabitat type, and position on abundances of fish and crayfish. To examine fish assemblage structure, we compared species richness and distinctiveness of assemblages. Finally, we evaluated the utility of the microhabitats as instream samplers by (1) comparing the precision of our abundance estimates for fish and crayfish relative to different sample sizes, (2) examining the relationship between sample size and the cumulative number of fish species captured, and (3) comparing our results with fish species richness estimates obtained by conventional sampling methods.

Methods

Study area.—We placed microhabitats into three streams (Table 1) in the Little Tallahatchie River drainage (upper Yazoo River basin) of northern Mississippi. Since European settlement, upland erosion, channelization for flood control, and subsequent stream incision and headcutting have profoundly altered most streams in this region (Schumm et al. 1984), thereby producing strong effects on fish habitat and fish assemblages (Shields et al. 1994; Warren et al. 2002; Adams et al. 2004). Modified streams have high banks, little woody material, shallow water, and shifting sand substrate. Hydrographs are flashy, being exacerbated by stream incision, channelization, and deforestation (Shields and Cooper 1994; Doyle and Shields 1998). Fish assemblages are diverse but highly variable among seasons and streams (Adams et al. 2004). In our study streams, Cypress Creek is incised and channelized, and the Little Tallahatchie Canal (henceforth, Tallahatchie Canal) is a constructed linear bypass carrying most of the flow of the original meandering channel of the Little Tallahatchie River. Study sites in both of these streams were characterized by a near-absence of woody material. Puskus Creek has a largely natural channel within our study reach, but this reach is bounded by a small impoundment upstream and a channelized section downstream. Within our study reach, Puskus Creek was characterized by an abundance of instream small and large woody material as well as other cover, such as overhanging vegetation and undercut banks.

Microhabitat construction.—We constructed three types of microhabitat units, hereafter referred to as bundles (Figure 1). We made cane bundles (60 cm long

FIGURE 1.—Cane (left), string (middle), and leaf (right) microhabitat units (bundles) deployed in three northern Mississippi streams (1-m rule at bottom).

× 45 cm in distal diameter) from freshly cut branching tops of Asian bamboo *Phyllostachys* spp. fastened together with a plastic cable tie about 15 cm from the proximal base of the branches. We made leaf bundles by enclosing newly fallen oak (*Quercus* spp.) leaves in nylon gill netting (7.5-cm bar mesh) to form spheres about 60 cm in diameter. We made string bundles by forming mop heads from 50 strands of 40-cm-long, degradable synthetic baling twine; string bundles were designed to mimic submerged, living roots of riparian plants. We secured bundles in the stream by attaching them with a 60-cm string to an iron rebar stake driven into the stream bottom. In this arrangement, bundles were in contact with the stream bottom and moved freely in the current.

Experimental design.—We were interested in examining effects of bundle type, channel position, exposure time, and season on use of the habitat units by fish and crayfish. In each stream, we delineated a 180-m study reach and divided the reach into sixty 3.0-m intervals. On 27–28 November 2000, we placed 60 bundles (20 of each type) in each of the three streams. We randomly assigned a single bundle type to each 3.0-m interval and randomly assigned the bundle position as either near the bank (bank) or in midchannel (middle); in each stream, each bundle type was represented by 20 bundles with 10 in each position (3 bundle types × 2 positions × 10 bundles/position). In this arrangement, bundles were at least three linear stream meters apart, a distance that minimized the likelihood of interaction between bundles. We planned to recover 30 bundles (3 bundle types × 2 positions × 5 bundles/position) from each stream on 11 December 2000 after a 14-d exposure (hereafter, 14-d winter exposure) and to recover the remaining 30 bundles on 10 January 2001 after a 44-d exposure (hereafter, 44-d winter exposure). We randomly preassigned 30 bundles for recovery on the first sample date, but because of bundle loss we recovered fewer than 30 bundles on both dates. We initiated a second experiment on 14 June 2001 in Cypress Creek and the Tallahatchie Canal but did not deploy bundles in Puskus Creek on that date due to time constraints. We deployed 30 cane, 10 leaf, and 10 string bundles in Cypress Creek and 10 of each type in the Tallahatchie Canal and randomly assigned the position of each bundle. We retrieved all bundles from both streams on 28 June 2001 after a 14-d exposure (hereafter, 14-d summer exposure).

Recovery of bundles.—To recover and sample a bundle, we carefully enclosed it with a seine (3 × 2 m; 3.2-mm mesh), quickly detached the bundle, and carried it ashore in the seine. We shook fish and crayfish from cane and string bundles into the seine; for the leaf bundles, we cut and removed the gill netting and examined the contents for fish and crayfish. We identified, measured (standard length; nearest mm), and released all fish except for a few that were retained for laboratory examination. We measured (total length; carapace and abdomen) and released all crayfish but did not identify them below the family level (Cambaridae). Prior to recovering a bundle, we visually estimated the portion that was exposed to air or buried in the substrate (nearest 10%) to quantify how much of the bundle was actually capable of serving as habitat. After removing the bundle, we measured depth and flow velocity (at 0.6 × depth) at the bundle position with a Marsh-McBirney Model 201 flowmeter. To avoid disturbing the bundles, we recovered them in an upstream direction.

Data analysis.—We used mean fish and crayfish abundances and 95% bootstrap confidence limits (CLs; 10^4 resamples; Manly 1997) to estimate differences in abundance in relation to main effects (stream, exposure time, season, bundle type, and bundle position). Approximate significance tests involved inspection for overlap of 95% bootstrap CLs. We used bootstrap CLs instead of analysis of variance (ANOVA) for estimation of differences among means because (1) the abundance data were nonnormal (strongly right skewed) and variances were unequal among streams (as determined by F_{max} tests; Sokal and Rohlf 1995), and neither of these properties was remedied by logarithmic or square-root transformation; (2) sample sizes among effects were highly unequal (because of bundle loss); and (3) the data contained extreme values. These factors, particularly in combination, seriously reduce reliability of ANOVA (i.e., estimates of main effects, variances, F-tests, P-values; Quinn and Keough 2002).

We used multiresponse permutation procedures (MRPPs; Bray–Curtis distance; McCune and Mefford 1999) to test the hypothesis that there were no fish assemblage differences among bundle types within each stream. The MRPP is a nonparametric randomization analog of parametric procedures like discriminant analysis, but it has the advantage of not requiring distributional assumptions (Mielke and Berry 2001). We used the chance-corrected within-group agreement statistic (A) to evaluate effect size. This statistic is independent of sample size and describes within-group homogeneity.

We estimated relative precision of mean abundances for exposure time and season in each stream as a percentage of one-half the bootstrap confidence interval (CI) divided by the mean (i.e., 100 × {CI/[2 × mean]}), such that high percentages indicate decreased precision and low percentages indicate increased precision. To assess the relationship between sample size and precision for abundance estimates, we calculated the 95% bootstrap CL (10^4 iterations each) and relative precision for a series of increasing sample sizes drawn from our data set using standard resampling techniques (Simon 1998). First, for each stream (all bundle types combined), we generated precision estimates around total abundance (all fishes combined) for sample sizes of 25–200 bundles (increments of 25) to construct relative precision curves. Second, for each stream we constructed relative precision curves (25–350 bundles; increments of 25) for crayfish and two fish habitat guilds: benthic fishes (all darter species combined) and water column species (all minnow species). We then plotted the mean precision across streams for each group relative to sample size.

We compared fish species richness in bundles with site species richness estimated from conventional standardized fish sampling that was conducted previously in our study streams (combination of electrofishing and seining; Warren et al. 2002; Adams et al. 2004, and unpublished). First, we compared species richness in bundle samples with richness estimates obtained from conventional sampling with similar effort (~1–3 samples). For Cypress Creek, we tallied cumulative richness from three fish samples collected during seasons in which bundles were sampled (winter and summer). For the Tallahatchie Canal and Puskus Creek, we used species richness estimated from single fish samples taken in summer 1999 because we had no samples from other times of the year. Second, we compared richness in bundle samples with estimates of total site richness based on repeated conventional sampling in Cypress Creek (eight samples over an 18-month period; Adams et al. 2004) and Puskus Creek (three samples taken during summer in 1999, 2000, and 2002; Warren et al. 2002, and unpublished). We did not have multiple samples from the Tallahatchie Canal site. To evaluate the number of bundles needed to completely sample the fish assemblage at each site, we generated species accumulation curves for each stream by consecutively selecting each bundle and adding the number of new species contributed by that bundle to the total number from previous bundles. For each site, we randomly shuffled bundle order five times to yield five species accumulation values for each bundle sample size. We used the means of the accumulation values to construct the curves.

Results

Recovery of Bundles

Fluctuating flow and shifting substrates resulted in loss or compromise of many bundles in all three streams. Loss of bundles (bundle not recovered; all bundle types combined) was 8% in Puskus Creek, 16% in Tallahatchie Canal, and 19% in Cypress Creek. Bundles that were completely buried or completely emersed contributed further to losses (8% in Puskus Creek; 13% in Tallahatchie Canal; 6% in Cypress Creek). Highest losses were associated with two winter storm events that affected all three streams and occurred after the 14-d exposure period but before the end of the 44-d exposure period. Discharge in the Tallahatchie Canal peaked at 42 and 190 m^3/s on 14 and 16 December, respectively; prior to these storms, flows during the study period ranged from 1 to 5 m^3/s (USGS 2008). Across streams, the total percentages of bundles that were lost, completely buried, or completely emersed were similar among bundle types: 12–19% for cane bundles, 20–33% for leaf bundles, and 15–40% for string bundles. Recovered bundles that were over 50% but less than 100% buried or emersed ranged from 6% to 12% among streams and from 0% to 12% among bundle types.

TABLE 2.—Percent occupancy of constructed microhabitats (bundles) by fish and crayfish in three northern Mississippi streams, 2000–2001 (N = total number of bundles).

Stream	Fish only	Crayfish only	Fish and crayfish	Unoccupied	N
Tallahatchie Canal	39.4	8.5	40.8	11.3	71
Cypress Creek	16.0	19.8	53.1	11.1	81
Puskus Creek	22.4	14.3	12.2	51.0	49

Overall Patterns of Bundle Use

Use of bundles by fish and crayfish was high. In Cypress Creek and the Tallahatchie Canal, 89% of bundles were occupied by fish, crayfish, or both (Table 2). Use of bundles was lower in Puskus Creek, where only 49% of bundles were occupied. Across all streams and bundle types, we captured 32 fish species representing eight families: Cyprinidae, Centrarchidae, Percidae, Ictaluridae, Fundulidae, Aphredoderidae, Catostomidae, and Poeciliidae (Table 3). Six species contributed 68% of total individuals captured: the blacktail shiner, brighteye darter, bluntface shiner, blackspotted topminnow, ribbon shiner, and dusky darter (Table 3). Fish captured in bundles either represented small-bodied species or were small individuals of larger species (e.g., channel catfish, spotted bass, and blacktail redhorse); maximum fish size was 14 cm. Crayfish size ranged from 1 to 10 cm, but crayfish smaller than 2.0 cm were rare (4 individuals). Size of fish and crayfish captured in bundles was similar among streams (Figure 2).

Co-occurrence of fish and crayfish in bundles showed little consistent pattern with regard to body

TABLE 3.—Fish species (percentage of the total number of sampled fish) captured in constructed microhabitats (cane, leaf, and string bundles combined) that were placed in three northern Mississippi streams, 2000–2001.

Species	Tallahatchie Canal	Cypress Creek	Puskus Creek	Overall
Blacktail shiner *Cyprinella venusta*	39.28	4.40	3.13	26.79
Brighteye darter *Etheostoma lynceum*	14.99	4.40	12.50	11.65
Bluntface shiner *C. camura*	7.24	9.89	34.38	8.82
Blackspotted topminnow *Fundulus olivaceus*	0.78	19.78	9.38	6.99
Ribbon shiner *Lythrurus fumeus*	5.94	9.34	0.00	6.66
Dusky darter *Percina sciera*	8.01	1.10	21.88	6.66
Brown madtom *Noturus phaeus*	1.03	10.99	0.00	3.99
Bullhead minnow *Pimephales vigilax*	6.20	0.00	0.00	3.99
Western mosquitofish *Gambusia affinis*	3.10	5.49	0.00	3.66
Yazoo shiner *Notropis rafinesquei*	0.26	10.99	0.00	3.49
Bluegill *Lepomis macrochirus*	0.00	4.95	9.38	2.00
Longear sunfish *Lepomis megalotis*	2.07	2.20	0.00	2.00
Yellow bullhead *Ameiurus natalis*	2.07	1.65	0.00	1.83
Harlequin darter *E. histrio*	2.84	0.00	0.00	1.83
Redspot darter *E. artesiae*	0.00	3.85	6.25	1.50
Green sunfish *Lepomis cyanellus*	0.52	3.30	0.00	1.33
Brindled madtom *Noturus miurus*	0.00	3.30	3.13	1.16
Mimic shiner *Notropis volucellus*	1.03	1.10	0.00	1.00
Orangespotted sunfish *Lepomis humilis*	1.29	0.00	0.00	0.83
Emerald shiner *Notropis atherinoides*	0.52	1.10	0.00	0.67
Slough darter *E. gracile*	0.52	0.00	0.00	0.33
Cypress darter *E. proeliare*	0.52	0.00	0.00	0.33
Blackstripe topminnow *F. notatus*	0.52	0.00	0.00	0.33
Pirate perch *Aphredoderus sayanus*	0.26	0.00	0.00	0.17
Mississippi silvery minnow *Hybognathus nuchalis*	0.26	0.00	0.00	0.17
Channel catfish *Ictalurus punctatus*	0.26	0.00	0.00	0.17
Warmouth *Lepomis gulosus*	0.00	0.55	0.00	0.17
Redfin shiner *Lythrurus umbratilis*	0.26	0.00	3.13	0.17
Blacktail redhorse *Moxostoma poecilurum*	0.26	0.00	0.00	0.17
Spotted bass *Micropterus punctulatus*	0.00	0.55	0.00	0.17
Largemouth bass *Micropterus salmoides*	0.00	0.55	0.00	0.17
Bluntnose minnow *Pimephales notatus*	0.00	0.55	0.00	0.17
Total number of fish	387	182	32	601

size or abundance. In all three streams, 95% CIs around estimates of mean fish length and mean number of fish in bundles overlapped widely between bundles with fish only and bundles with both fish and crayfish (Figure 2). Similarly, mean crayfish lengths and numbers of crayfish were similar between bundles with crayfish only and bundles with both crayfish and fish. In bundles with both fish and crayfish, fish abundance and crayfish abundance were negatively correlated in the Tallahatchie Canal (randomized Pearson's product-moment correlation coefficient $r = -0.301$, $P < 0.013$; 10^4 iterations) but were not correlated in Cypress Creek ($r = -0.038$, $P < 0.49$); small sample sizes in Puskus Creek precluded correlation analysis. Mean fish length and mean crayfish length in bundles inhabited by both organisms were weakly and positively correlated in Cypress Creek ($r = 0.23$, $P < 0.05$) but were not correlated in the Tallahatchie Canal ($r = 0.23$, $P < 0.10$).

Exposure Time and Season

Fish abundance in bundles was significantly higher after the 44-d exposure than after the 14-d exposure in Cypress Creek and the Tallahatchie Canal, but abundance did not differ with exposure time in Puskus Creek (Table 4). For both exposure times, fish abundance was similar between Cypress Creek and the Tallahatchie Canal but significantly lower in Puskus Creek. Fish abundance did not differ between 14-d winter and summer exposures in Cypress Creek. In the Tallahatchie Canal, mean fish abundance after 14 d was higher in summer than in winter, and 95% CIs around these two estimates overlapped minimally. Fish abundance after 14 d in summer was higher in the Tallahatchie Canal than in Cypress Creek.

Crayfish abundance in bundles decreased with increasing exposure time in the Tallahatchie Canal but did not differ with exposure time in Cypress Creek or Puskus Creek (Table 4). Crayfish abundance after 14 d was similar between Cypress Creek and the Tallahatchie Canal but significantly lower in Puskus Creek; crayfish abundance after 44 d did not differ among streams. Crayfish abundance did not differ between winter and summer 14-d exposures in either Cypress Creek or the Tallahatchie Canal.

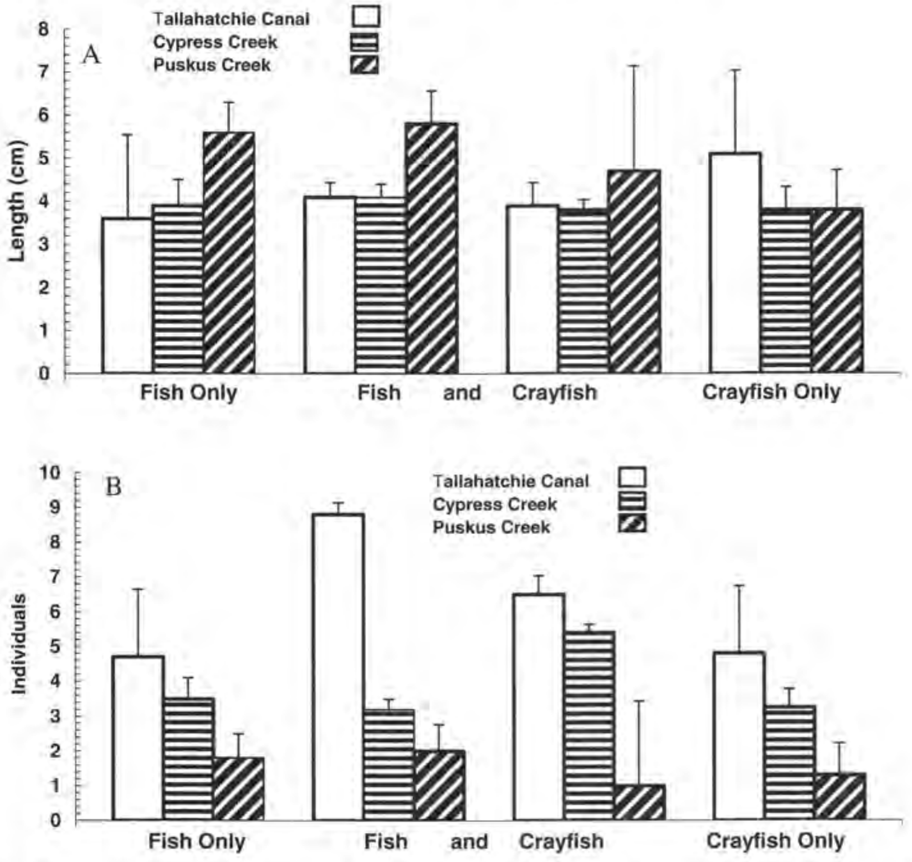

FIGURE 2.—Mean (with 95% confidence interval) (**A**) lengths of fish and crayfish occurring together or separately and (**B**) numbers of fish and crayfish occurring together or separately in constructed microhabitats (bundles) within three northern Mississippi streams, 2000–2001.

Bundle Type and Position

Fish abundance was not associated with bundle type or position, but crayfish abundance showed mixed relationships with these variables. The 95% CIs around fish abundance overlapped for all three bundle types and both bundle positions within each stream (Table 4). For each bundle type, mean fish abundance was highest in the Tallahatchie Canal and lowest in Puskus Creek. Crayfish abundance was lowest in string bundles in the Tallahatchie Canal and Cypress Creek. Crayfish abundance was higher in leaf bundles than cane bundles in Cypress Creek, but these bundle types did not differ in the Tallahatchie Canal. Crayfish abundance did not differ among bundle types in Puskus Creek. Crayfish abundance was high and similar in cane and leaf bundles in the Tallahatchie Canal and Cypress Creek relative to the low values in Puskus Creek (Table 4). Crayfish abundance in string bundles did not differ among streams. Across all bundle types, crayfish were more abundant near the bank in the Tallahatchie Canal, but crayfish abundance did not differ relative to bundle position in Cypress Creek or Puskus Creek.

A large proportion of fish species across all bundles were represented in each bundle type. Across streams, cane bundles contained 63–95% of the fish species observed in all bundle types, leaf bundles contained 38–76%, and string bundles contained 40–63%. Among streams, minimal and maximal values for richness in bundle types varied. Species richness of bundles ranged from 10 (string) to 19 (leaf) in the Tallahatchie Canal, 8 (leaf) to 20 (cane) in Cypress Creek, and 3 (leaf) to 5 (cane and string) in Puskus Creek.

Distinctiveness of fish assemblages among bundle types was detected only in Puskus Creek, where within-bundle-type homogeneity relative to all bundle types was greater than that expected by chance (MRPP:

TABLE 4.—Mean abundance (with 95% confidence interval [CI] in parentheses) of fish and crayfish in constructed microhabitats (bundles) placed in three northern Mississippi streams, 2000–2001 (NA = not applicable). For each exposure period, sample size (number of bundles) and relative precision (%) are given below mean and 95% CI. For each bundle type and bundle position, sample size is given below mean and 95% CI.

Effect	Fish			Crayfish		
	Tallahatchie Canal	Cypress Creek	Puskus Creek	Tallahatchie Canal	Cypress Creek	Puskus Creek
Exposure time and season						
14 d, winter	2.1 (1.23–3.04) 30, 43%	1.7 (0.96–2.54) 26, 47%	0.6 (0.28–0.93) 29, 56%	4.3 (2.27–6.90) 30, 52%	4.8 (1.96–8.96) 26, 72%	0.3 (0.17–0.55) 29, 55%
44 d, winter	12.2 (5.60–21.87) 15, 67%	7.7 (2.86–13.71) 7, 70%	0.8 (0.25–1.35) 20, 73%	0.6 (0.2–1.2) 15, 83%	1.42 (0.14–3.71) 7, 125%	0.3 (0.05–0.50) 20, 90%
14 d, summer	5.4 (3.00–8.62) 26, 52%	1.75 (1.13–2.52) 48, 40%	NA	2.8 (0.96–5.19) 26, 73%	3.1 (2.06–4.33) 48, 37%	NA
Bundle type						
Cane	8.1 (4.16–14.3) 25	2.6 (1.63–3.74) 43	1.0 (0.41–1.65) 17	2.3 (0.76–4.36) 25	2.6 (1.95–3.39) 43	0.3 (0.11–0.53) 17
Leaf	5.5 (2.75–9.08) 24	1.4 (0.65–2.3) 20	0.3 (0–0.60) 15	6.4 (3.58–9.29) 24	7.6 (3.40–12.95) 20	0.3 (0–0.60) 15
String	2.3 (0.81–4.41) 24	1.4 (0.71–4.88) 17	0.6 (0.18–1.18) 17	0.3 (0.09–0.50) 22	1.1 (0.39–2.17) 18	0.4 (0.11–0.65) 17
Bundle position						
Bank	5.4 (2.77–9.25) 44	2.9 (1.91–4.18) 45	0.6 (0.20–1.08) 25	4.5 (2.66–6.61) 44	4.4 (2.29–7.07) 45	0.3 (0.12–0.52) 25
Midchannel	5.6 (3.11–8.70) 27	1.4 (0.61–2.39) 36	0.7 (0.33–1.13) 24	0.7 (0.30–1.11) 27	2.4 (1.75–3.22) 36	0.3 (0.13–0.58) 24

$A = 0.099$, $P < 0.028$). Values of A suggest that the primary contrast in assemblages was between cane and string bundles. When leaf bundles were excluded from the analysis, cane and string assemblages showed an effect size nearly as large as that observed with all bundle types included ($A = 0.088$, $P < 0.023$). In contrast, when string bundles were excluded, the effect size was close to zero and was nonsignificant ($A = -0.014$, $P < 0.44$). In Puskus Creek, brighteye darters occurred only in string bundles, redspot darters occurred only in cane bundles, and bluegills and blackspotted topminnow occurred in cane and leaf bundles but not string bundles; however, these patterns are based on few (2–4) individuals. No differences in fish assemblages among bundle types were detected in the Tallahatchie Canal ($A = -0.005$, $P < 0.70$) or Cypress Creek ($A = -0.000081$, $P < 0.45$).

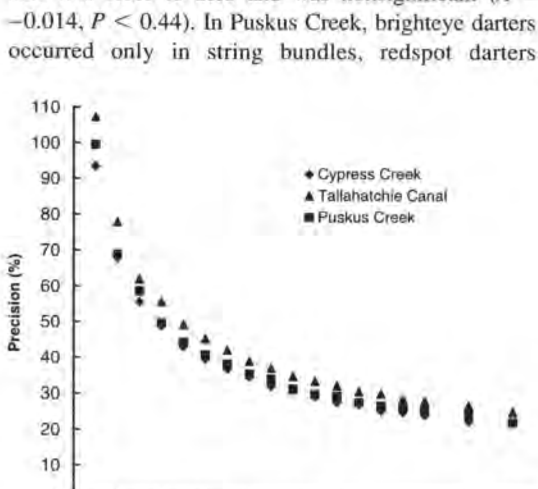

FIGURE 3.—Relative precision (%) of mean abundance across fish species for each stream plotted in relation to the number of constructed microhabitats (bundles) placed in three northern Mississippi streams during 2000–2001.

Relative Precision of Bundle Abundance Estimates

Precision of estimates for fish and crayfish abundance was low in all streams and for all bundle types, indicating that large numbers of bundles are needed for substantial improvement in precision. Relative precision of abundance estimates among the three sample dates ranged from 40% to 73% for fish and from 37% to 125% for crayfish (Table 4). Mean relative precision across samples was similar among streams but was slightly greater (i.e., abundance estimates were less precise) for crayfish (69–78%) than for fish (52–65%). For fish, the relationship of sample size to relative precision was similar for all three streams; sample sizes of 110–140 bundles were required to achieve a relative precision of 30% around mean abundance estimates (Figure 3). Across streams, the number of samples necessary to achieve 30% relative precision was lowest for crayfish (150 bundles), intermediate for darters (210), and highest for minnows (300; Figure 4).

Bundles and Conventional Fish Samples

Bundles sampled a large proportion of the fish assemblage detected by conventional sampling in the

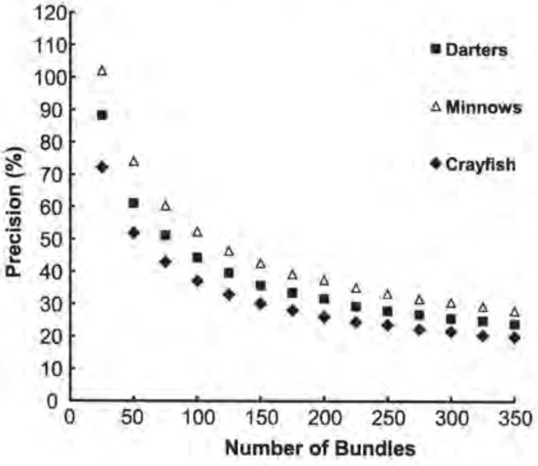

FIGURE 4.—Relative precision (%) of mean abundance across three taxonomic groups (darters = benthic fish group; minnows = water column fish group; and crayfishes) plotted in relation to the number of constructed microhabitats (bundles) placed in three northern Mississippi streams during 2000–2001.

Tallahatchie Canal and Cypress Creek but sampled richness poorly in Puskus Creek. Relative to richness estimates from one to three conventional samples, bundle samples yielded 104% of fish species (25 of 24) for the Tallahatchie Canal, 78% of fish species (21 of 27) for Cypress Creek, and 38% of fish species (9 of 24) for Puskus Creek. Bundles yielded 49% of the total known fish fauna of the Cypress Creek site (43 species) and 27% of the total known fish fauna of the Puskus Creek site (33 species).

The potential effectiveness of bundles for estimating total fish species richness differed among streams (Figure 5). The species accumulation curve for the Tallahatchie Canal and Cypress Creek were steeper than the curve for Puskus Creek within the range of our sample sizes. Therefore, additional samples would be expected to yield a substantial increase in species in the Tallahatchie Canal and Cypress Creek. In Puskus Creek, the rate of increase for species accumulation began to decrease markedly with increasing sample size, suggesting that additional samples would only minimally increase the estimated total richness. Estimates of total fish richness (standardized at 400 bundles) were 38 species for the Tallahatchie Canal, 29 species for Cypress Creek, and 12 species for Puskus Creek. These estimates represent 67% (Cypress Creek) and 36% (Puskus Creek) of the total richness estimated by conventional sampling (total richness estimate was unavailable for the Tallahatchie Canal).

Relative to conventional samples, bundles selected for some species but against others. The emerald shiner, an open-water minnow, was one of the most abundant fishes in conventional samples at all three sites (Table 5) but was rare or absent in bundle samples (Table 3). Similarly, the Mississippi silvery minnow in Cypress Creek and the mimic shiner and gizzard shad in the Tallahatchie Canal were among the five most abundant species in conventional samples but were rare in bundle samples within these streams. The blackspotted topminnow was one of the five most abundant species in bundles from Cypress and Puskus creeks but not in conventional samples. Benthic fishes (brighteye darter, brown madtom, and dusky darter) were well represented in bundle samples from all streams but were not among the five most abundant species in conventional samples from Cypress Creek or the Tallahatchie Canal. Benthic species were well represented in Puskus Creek in both bundle and conventional samples. Other common species were well

TABLE 5.—List of the five most abundant fish species (rank order; relative abundance percentage in parentheses) in constructed microhabitat (bundle) samples (all bundle types and sample periods combined) and in conventional samples (i.e., collected by electrofishing and seining) from three northern Mississippi streams.

Stream	Bundles	Conventional samples
Tallahatchie Canal	Blacktail shiner (39%)	Emerald shiner (31%)
	Brighteye darter (15%)	Blacktail shiner (16%)
	Dusky darter (8%)	Bluegill (12%)
	Bluntface shiner (7%)	Mimic shiner (11%)
	Bullhead minnow (6%)	Gizzard shad *Dorosoma cepedianum* (6%)
Cypress Creek	Blackspotted topminnow (20%)	Emerald shiner (39%)
	Brown madtom (11%)	Yazoo shiner (21%)
	Yazoo shiner (11%)	Western mosquitofish (14%)
	Bluntface shiner (10%)	Bluntface shiner (9%)
	Ribbon shiner (9%)	Mississippi silvery minnow (3%)
Puskus Creek	Bluntface shiner (34%)	Bluntface shiner (27%)
	Dusky darter (22%)	Emerald shiner (20%)
	Brighteye darter (13%)	Brighteye darter (12%)
	Bluegill (9%)	Dusky darter (8%)
	Blackspotted topminnow (9%)	Blacktail shiner (8%)

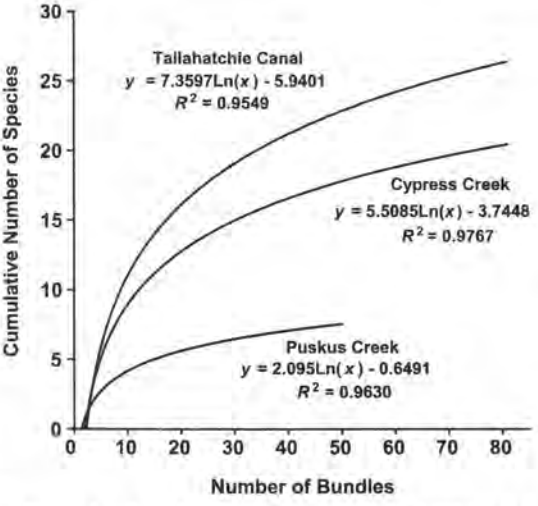

FIGURE 5.—Cumulative number of sampled fish species as a function of the number of constructed microhabitats (bundles) placed in three northern Mississippi streams during 2000–2001.

represented in both bundle and conventional samples from two or more streams (e.g., blacktail shiner, bluntface shiner, and Yazoo shiner).

Discussion

Microhabitat bundles attracted crayfish and a diversity of fishes and can provide a useful method for sampling small fish and crayfish that occur predominantly in woody habitats and are difficult to sample with conventional methods. We found little evidence that different bundle types attracted different fish species. Even though we found few strong differences among bundle types, we recommend cane bundles over the other two types that we evaluated. Leaf bundles were effective but were laborious to sample because of the necessity of sorting through large volumes of leaves to find fish and crayfish. String bundles were easy to sample but attracted fewer crayfish overall than the other two bundle types. Even though CIs around fish abundance overlapped among bundle types, string bundles consistently had lower mean fish abundance than cane bundles. Cane bundles tended to sample higher percentages of total fish species encountered in bundles than either leaf or string bundles. By focusing on a single, easily sampled bundle type and approximately doubling the number of bundles that we used, reasonably precise fish and crayfish abundance estimates can be obtained for monitoring or other applications.

Despite their usefulness in some applications, microhabitat bundles have strong sources of bias. Because of their small size, bundles provide habitat for only small fish and crayfish. We captured small individuals of several large-bodied species, but large individual crayfish, predatory fish (e.g., gars *Lepisosteus* spp., basses *Micropterus* spp., and flathead catfish *Pylodictus olivaris*), and nonpredatory fish (e.g., suckers *Moxostoma* spp. and common carp *Cyprinus carpio*) either were not attracted to bundles or easily avoided capture during daytime sampling of bundles. However, because of low water clarity, we were not able to quantify or qualify capture avoidance. Bundles also show strong bias against fish species that are not typically associated with cover (e.g., gizzard shad and open-water minnow species). These sources of bias are reflected in our species accumulation curves for two study streams, which predicted much lower total richness than that revealed by conventional sampling. Therefore, bundles may efficiently sample a subset of the fish assemblage even with relatively small sample sizes but are unlikely to provide accurate estimates of total site richness even with greatly increased effort. Because of macrohabitat partitioning among species, we suspect that similar bias will probably occur in the sampling of crayfish assemblages (e.g., DiStefano et al. 2003).

We expected to see interactions between fish and crayfish sizes or abundances within bundles due to predator avoidance by crayfish or displacement and predation of small fish by crayfish. Fish predators selectively prey on small- to medium-sized crayfish, and the presence of large fish predators increases cover use by crayfish of vulnerable sizes (Garvey et al. 1994; Adams 2007). Crayfish can evict small benthic fish from cover, thus increasing the fish's predation risk; if large enough, crayfish can also act as predators of small fish (Rahel and Stein 1988; Light 2005). From this, we predicted that (1) crayfish abundance would be lower in bundles with fish than in bundles without fish, (2) fish size in bundles with both fish and crayfish would be smaller than fish size in bundles with fish only, and (3) crayfish size in bundles with fish and crayfish would be smaller than crayfish size in bundles with crayfish only. In one stream, fish and crayfish abundances were negatively related, but we did not find any other relationships that would be indicative of predator–prey or behavioral interactions between fish and crayfish. We believe that the nearly equal size of fish and crayfish in the bundles limited predation of fish on crayfish or displacement of small fish by crayfish (e.g., Rahel and Stein 1988; Englund and Krupa 2000; Adams 2007; Bishop et al. 2008). Two other microhabitat-scale studies also found no evidence of interaction between abundances of co-occurring fish and crayfish (Bishop et al. 2008; Jowett et al. 2008).

The lack of interaction between fish and crayfish abundance or size is probably another result of sample bias associated with bundles. Because no large predatory fish species were captured in bundles, the potential for exclusion of crayfish by predators was low. Obversely, the bundles may have provided cover of adequate complexity to render predatory or behavioral interactions between fish and crayfish nonexistent or too weak to detect in size or abundance patterns (Stein 1977; Savino and Stein 1982; Dibble and Harrel 1997).

Bundle effectiveness appeared to vary strongly with habitat conditions, especially the availability of natural woody cover. The Puskus Creek site had a relatively natural channel with an abundance of natural woody cover, but the two disturbed sites were nearly devoid of woody debris or other cover. In Puskus Creek, the percentage of bundles occupied by fish and crayfish was nearly half that of the disturbed sites, the abundance of fish and crayfish in bundles was consistently lower than abundances at the disturbed sites, and bundles attracted fewer fish species and a smaller percentage of the known fish fauna for the site. Furthermore, the species accumulation curve for bundles in Puskus Creek showed evidence of saturation even at low sample sizes and predicted a total richness that was nearly one-third of the richness predicted for the disturbed streams, even though richness estimated by conventional sampling was similar among all three streams.

We hypothesize that fish and crayfish sought out and used bundles more in the streams where natural woody cover was rare than in the stream where such cover was abundant. Fish and crayfish use woody cover in response to several factors, including increased predation risk in open-water habitats (Power 1984; Schlosser 1988a,b; Everett and Ruiz 1993; Englund and Krupa 2000; Adams 2007), increased availability of food resources produced on woody substrates (Benke et al. 1985; Benke and Wallace 2003), and the presence of fast current or other metabolically demanding conditions (Maude and Williams 1983; Harvey 1987; Ross et al. 1992; Nakata et al. 2003). The observed increase in fish abundance in bundles between the 14- and 44-d winter exposures for Cypress Creek and the Tallahatchie Canal could be explained by a simple diffusion effect over time (e.g., Sheldon and Meffe 1995; Lonzarich et al. 1998; Arrington et al. 2005) as more individuals discovered and utilized bundles or by the time needed for colonization of the bundles by invertebrates that would attract foraging fish. In Louisiana coastal plain streams, abundance of macroinvertebrates on experimental tree branch units did not peak until at least 3 weeks after placement in streams in the fall (Drury and Kelso 2000). However, fish use of bundles did not increase over time in Puskus Creek, suggesting that in streams where natural cover was limiting (Cypress Creek and Tallahatchie Canal), fish actively sought out bundles to obtain refuge from extreme weather conditions.

Between our 14- and 44-d sampling periods in winter, water temperatures dropped quickly from about 12°C to 4°C and the study streams flooded. In addition to increased fish abundance in Cypress Creek and the Tallahatchie Canal, we observed other anomalous patterns of fish occurrence that were associated with extreme weather conditions and that were not seen on other sample dates. During the 44-d winter exposure, many fish found in bundles in the Tallahatchie Canal (water temperature = 2°C) were lethargic, and all western mosquitofish were dead. In both streams, single bundles yielded high numbers of minnows ($\leq$69 individuals; mostly blacktail shiners, bluntface shiners, and Yazoo shiners), which were not abundant in bundles after the 14-d winter or summer exposure. This anomalous abundance of minnows in bundles could be explained by seasonal movements of these fish, but blacktail shiners, bluntface shiners, and Yazoo shiners are common elements of the fauna at these sites year-round (Warren et al. 2002; Adams et al. 2004). In Puskus Creek, we observed neither an increase in fish abundance nor any anomalous pattern of species occurrence in bundles after this extreme weather event. We suspect that in streams where cover was limiting (Cypress Creek and Tallahatchie Canal), fish congregated in the bundles in response to low temperature (e.g., Cunjak and Power 1986; Ross et al. 1992; Cunjak 1996), whereas this did not occur in Puskus Creek because abundant natural woody cover existed elsewhere in the stream.

Our evaluation of the use of constructed microhabitats by crayfish and fish revealed several positive attributes and emphasizes the potential of these microhabitats as monitoring and research tools. Although we determined that some bundle materials were less suitable, cane bundles were relatively easy to construct, place, and sample. A four-person team was able to sample three streams (90 total bundles) within 1 d, including travel time. With a feasible increase in sample size, precision would be sufficient for detecting at least modest changes in abundance. Because a bundle attracts fish and crayfish from surrounding areas, it could potentially influence the numbers and species that use surrounding bundles, resulting in a lack of independence of samples. Although our study was not designed to measure independence among bundle samples, we suggest that spacing effects be considered in future studies. Bundles sampled a high diversity of fishes, especially cryptic species (e.g., madtoms and darters). Bundles could be useful in monitoring species that use woody habitats but that are difficult to sample by conventional methods. We envision several applications of bundles as an experimental research tool, such as in studying colonization dynamics (e.g., Sheldon 1984; Sheldon and Meffe 1995), interspecific habitat associations (e.g., Banks and DiStefano 2002), habitat use in the vertical dimension, diel behavioral patterns (e.g., Greenberg 1991; Arrington and Winemiller 2003), and species site fidelity and turnover. Bundles also could be useful tools for understanding fish and habitat relationships in damaged streams, particularly disturbed, wood-deficient streams in the coastal plain. Even in highly degraded streams, fish species richness can remain high (Adams et al. 2004). Bundles, therefore, provide an opportunity to evaluate the functional responses of fishes to channelization and incisement (e.g., relative use of cover) in addition to the structural responses that are more easily determined by conventional sampling.

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