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REPRODUCTION IN THE SPOTTAIL DARTER IN INDIANA: USE OF ARTIFICIAL NEST SITES

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ABSTRACT: The spottail darter (*Etheostoma squamiceps*), an Indiana special concern species, is found in a few small streams in the extreme southwestern portion of the State. This species uses rocks and other solid debris as egg deposition sites, and previous surveys have indicated that the small populations and patchy distribution in Indiana may be due to a lack of suitable nest sites. This hypothesis was addressed by adding 108 half-cylinder sections of ceramic tile to the experimental halves of six stream sections containing spottail darters. Tiles and natural nest sites were checked biweekly for spottail darter nests during the two month breeding season. Of the 386 *E. squamiceps* nests located during the study, 122 (31.6%) were found under tiles. Experimental areas contained significantly more nests per census than did reference areas. Tile use correlated negatively with rock availability, indicating that rocks were preferred nest sites. The average nest contained 368 eggs, and rock nests and tile nests did not differ significantly in the number of eggs per nest. Densities of spottail darters were higher than expected from previous surveys but did not correlate with the numbers of nests located. This observation, combined with the fact that the 142,000 spottail darter eggs counted during this study represented approximately 13 times the predicted reproductive output of the 167 darters captured during the post study census, suggests that darters migrate during the breeding season.

INTRODUCTION

The spottail darter (*Etheostoma squamiceps*; Perciformes, Percidae), like other members of the subgenus *Catonotus*, is an egg clusterer (Page, 1985), with males defending rocks and other solid benthic debris under which females attach their eggs. Females do not participate in nest site defense or egg maintenance. Spottail darters range from northern Tennessee through western Kentucky, with the farthest northern populations in southern Illinois and southwestern Indiana (Braasch and Mayden, 1985). The first Indiana record of *E. squamiceps* was by Jordan (1890), who found them only in Gresham's Creek and Black River in Posey County. Subsequent surveys of the area either failed to locate any spottail darters (Gerking, 1945; Kozel, *et al.*, 1980) or found relatively small, scattered populations. Page, *et al.* (1976) found only two spottail darters at 18 sites in Posey County and speculated that increasing siltation due to agricultural run-off and the lack of slab rocks used for breeding sites limited population sizes and distribution in southwestern Indiana. Grannan and Lodato (1986) found spottail darters at two sites in Posey County and four sites in the Bayou Creek drainage in adjacent Vanderburgh County, a range extension confirmed by Cervone, *et al.*(1989). The small populations and limited distribution earned the spottail darter a special concern status by the Indiana Department of Natural Resources (Indiana Discretionary Order W-12, revised August, 1984), although Whitaker and Gammon (1988) recommended the status be upgraded to endangered.

Spottail darter abundances in the tributaries of Bayou Creek do not seem to be strongly influenced by water chemistry (pH, dissolved oxygen, salinity, and conductivity; Bandoli and Leatherland, pers. obs.) despite the oil drilling activities and effluent discharges common in the drainage. Casual inspection of several sites with and without darters seemed to support the nest site limitation hypothesis of Page, *et al.* (1976), since areas with the largest populations generally had more rocks or other solid debris available as potential breeding sites. Previous studies with egg-clustering darters (Lindquist, *et al.*, 1984) and other benthic fishes (Downhower and Brown, 1977) that reproduce under solid benthic structures have found that artificial nest sites added to the streams were readily used. The goals of this study were (1) to quantify reproduction in *E. squamiceps* using natural nest sites in southwestern Indiana and (2) to evaluate the nest site limitation hypothesis through the addition of artificial nest sites to sections of two tributaries of Bayou Creek.

METHODS

Bayou Creek drains approximately 80 km^2 in Vanderburgh and Posey Counties in extreme southwestern Indiana. Six study areas (stations) were chosen on the basis of abundance of spottail darters following intensive seining in January, 1988. Three stations were located on Carpentier Creek, a first-order tributary of Bayou Creek draining suburban areas interspersed with bottomland hardwood woodlots. The remaining three stations were located on Sander's Creek, a first-order tributary of Bayou Creek which drains agricultural land. All stations were slow-flowing (2.7 m^3 to 6.5 m^3/min) and turbid with similar average widths (1.3 m to 1.8 m) and depths (0.1 m to 0.2 m). Station lengths ranged from 12 m to 41 m.

Spottail darters were censused in February 1988 using 4-mm-mesh minnow seines. Each station was subdivided into small sections that were seined (including dislodging of benthic debris) repeatedly until no more darters were captured. Darters were measured (standard length), held in stream water in plastic bags until the entire station had been censused, and returned to within 5 m of their capture sites. Age was estimated from standard lengths based on data from Page (1974). Spottail darters less than 4 cm long were considered yearlings, those between 4 and 6 cm long were 1 to 3 years old, and fishes over 6 cm long were classified as 3 years old or older. Following this census, each station was divided into 2 subsections of approximately equal lengths and densities of spottail darters. Subsections were randomly designated as reference or experimental at stations 1 to 4. At stations 5 and 6, one subsection at each station lacked rocks (potential nest sites) and was therefore designated as experimental.

Artificial nest sites (hereafter called tiles) were cut from 10 cm diameter ceramic field tile, with each tile a half-cylinder 15 cm long. Laboratory observations in 1987 confirmed that spottail darters would use tiles as hiding sites, and one naturally occurring *E. squamiceps* nest was found on the underside of a piece of broken field tile in April 1987. During the last week of February, tiles were individually marked and placed in the experimental halves of each station in sets of two, with one tile oriented with and one against the current. Sets were placed at 1 m to 2 m intervals and, where possible, pushed 1 cm to 2 cm into the substrate, leaving approximately 185 cm^2 of surface area on the concave underside of each tile available for egg attachment. Ten sets were placed at the four longest stations; eight sets and five sets were placed at the two shorter.

Censuses for darter nests began on 10 March 1988 and continued biweekly through 17 May 1988. Each census consisted of checking the undersurfaces of all tiles, rocks

Table 1. Spottail darter census data from 1988 and 1989. Numbers are darters caught in reference and experimental sections at each station. Numbers in parentheses are densities (darters per square meter of stream).

Census	Station 1	2	3	4	5	6
February, 1988						
Total	6 (0.31)	30 (0.43)	4 (0.06)	30 (0.80)	46 (0.93)	20 (0.48)
Reference	3 (0.31)	18 (0.48)	2 (0.07)	15 (0.68)	25 (0.88)	10 (0.43)
Experimental	3 (0.31)	12 (0.37)	2 (0.05)	15 (0.93)	21 (1.00)	10 (0.55)
May, 1988						
Total	2 (0.10)	46 (0.66)	27 (0.41)	19 (0.51)	59 (1.19)	14 (0.34)
Reference	0 (0.00)	29 (0.78)	10 (0.35)	6 (0.27)	34 (1.19)	9 (0.38)
Experimental	2 (0.21)	17 (0.53)	17 (0.45)	13 (0.85)	25 (1.19)	5 (0.27)
February-March, 1989						
Total	38 (1.98)	65 (0.88)	55 (0.87)	62 (1.66)	71 (1.34)	35 (0.84)
Reference	21 (2.19)	27 (0.68)	30 (1.10)	42 (1.90)	33 (1.09)	22 (0.94)
Experimental	17 (1.77)	38 (1.11)	25 (0.70)	20 (1.31)	38 (1.70)	13 (0.71)

(>10 cm in diameter), and other debris at each station for the presence of spottail darter eggs. In our study areas, only the bluntnose minnow (*Pimephales notatus*) deposits eggs in a manner similar to *E. squamiceps* (Trautman, 1981). Spottail darter eggs were easily distinguished from those of bluntnose minnows on the basis of color (orange versus gray) and size (average egg diameter for *E. squamiceps* is 1.9 mm versus 1.4 mm for *P. notatus* (Bandoli, unpubl. data)). Spottail darter eggs were counted directly or photographed and counted from projected slides. Nests were out of the water for 1 minute or less, and all rocks and tiles were returned to their original positions. The presence of spottail darters under tiles was also noted. An additional nest census was conducted on 23 May followed by a darter census using the same methods as in February. Tiles were moved during this census and replaced afterwards. Darters were again censused at each station in February and March 1989 to determine if nest site addition had a significant effect on subsequent population size or composition.

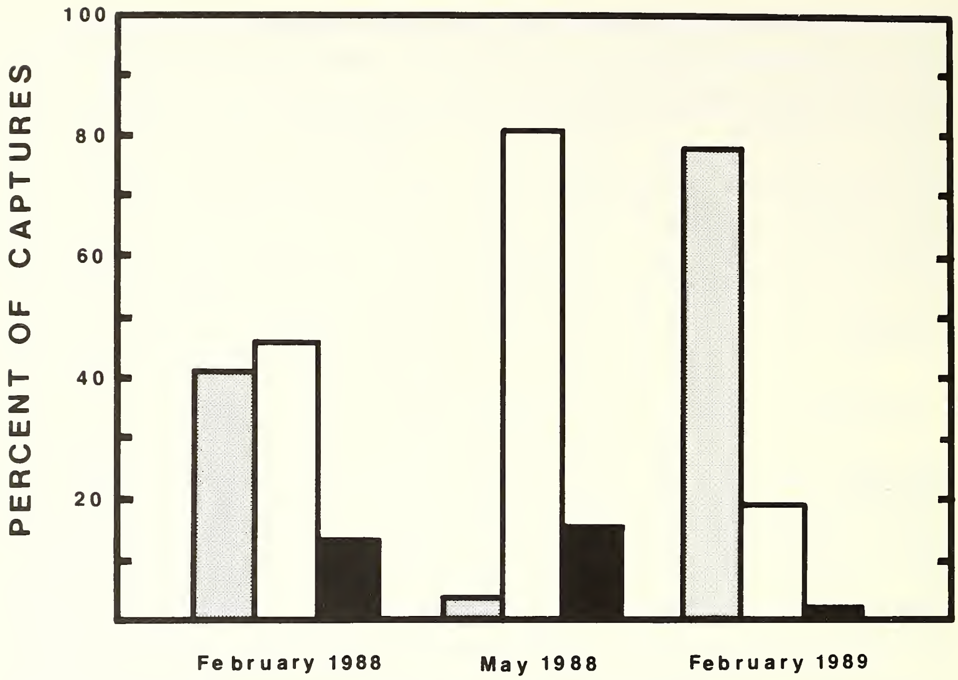

Figure 1. Percent frequencies of age classes in the 1988 and 1989 spottail darter censuses estimated from standard lengths (Page, 1974). Darters 4 cm or less (shaded bars) are in their first year; those between 4 cm and 6 cm (unshaded bars) are between 1 and 3 years; daters greater than 6 cm (black bars) are at least 3 years old.

RESULTS

Darter censuses. Rainfall was consistently low during the study, averaging 38% below normal levels. Streams maintained a relatively constant width and depth, and February width and depth measurements were therefore used for all 1988 density computations. These parameters were re-measured for the 1989 density estimates.

The February 1988 census yielded 136 individuals of *E. squamiceps*, with densities ranging from 0.06/m^2 to 0.93/m^2 (Table 1). The dominant age class was 1 to 3 years old, followed closely by the yearling cohort (Fig. 1). In the May 1988 census, 167 spottail darters were captured, an increase of 22.7% over the February census. The gain was not consistent, as three stations (1, 4, and 6) declined in total numbers of darters (Table 1). Overall, densities increased slightly from an average of 0.5/m^2 in February to 0.54/m^2, although the difference was not significant (Student's t-test, $t = 0.2$, $P < 0.9$). Age structure changed in the expected direction, as most yearlings passed 4 cm in standard length and entered the next age class. The February-March 1989 census yielded 326 spottail darters, a 139.7% increase over the February 1988 census. The increase was consistent across all stations, and the average density (1.26/m^2) was significantly higher than that observed during the previous February ($t = 3.22$, $P < 0.01$; Table 1). Virtually all of the increase in spottail darter population density was due to the yearling cohort, which increased from an average of 9.3/station to 42.2/station, a significant difference ($t = 4.24$, $P < 0.01$; Fig. 1).

Nest censuses. No nests were found during the first census (10 March); thereafter, spottail darter nests were found during each census (Fig. 2). A total of 386

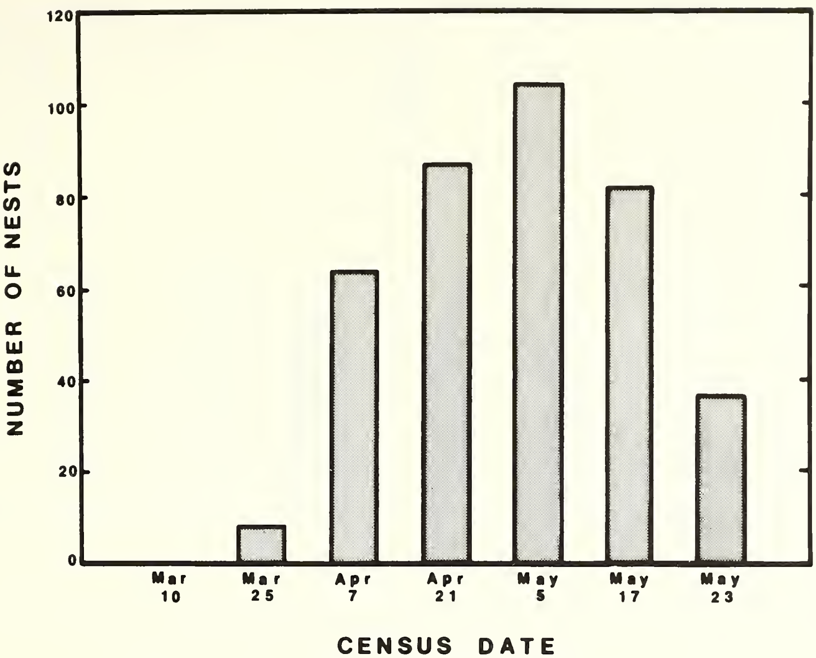

Figure 2. Spottail darter nests found in each census. Bars are totals across all stations.

E. squamiceps nests were located during the study, 122 (31.6%) of which were found under tiles. The majority of the 264 natural nests were found under rocks; however, bricks and other solid debris were also used on occasion, all of which will henceforth be included in "rock" nest and nest site designations.

Mean water temperature per census ranged from 10.2°C on 10 March to 18.6° C on 23 May, averaging 15.7° C. Extrapolating from Page's (1983) data on the effect of temperature on development in darters, the incubation period at this temperature was approximately 13 days. As this approaches the 14-day interval between successive nest censuses, it is possible that some nests were counted twice. To minimize this source of error, photographs of darter nests under the same tiles at successive censuses were compared, and nests which appeared to be the same were excluded. In cases where the nests appeared the same but new eggs had been added since the previous census, only the new eggs were counted. Rocks were not marked, however, which prevented comparisons of rock nests between consecutive censuses, thus allowing the possibility of overestimation of the number of nests under rocks. If this occurred with the same frequency as it did for tile nests (14.7%), then the rock nest total may have been closer to 225 and the total nest count 347. Some of this potential overestimation may have been balanced by rock nests missed due to periodic increases in turbidity, which occasionally prevented us from locating all rocks at a station. (For this reason, rock availabilities (below) are averages of the number of rocks checked per station rather than constants. Conversely, all tiles were located on each census.) Since neither effect is accurately quantifiable on a date- or station-specific basis, neither factor was used to adjust the data.

The use of tiles as nest sites varied between stations (see sample sizes on Fig. 3). None of the nests found at station 1 were under tiles, although on four occasions darters

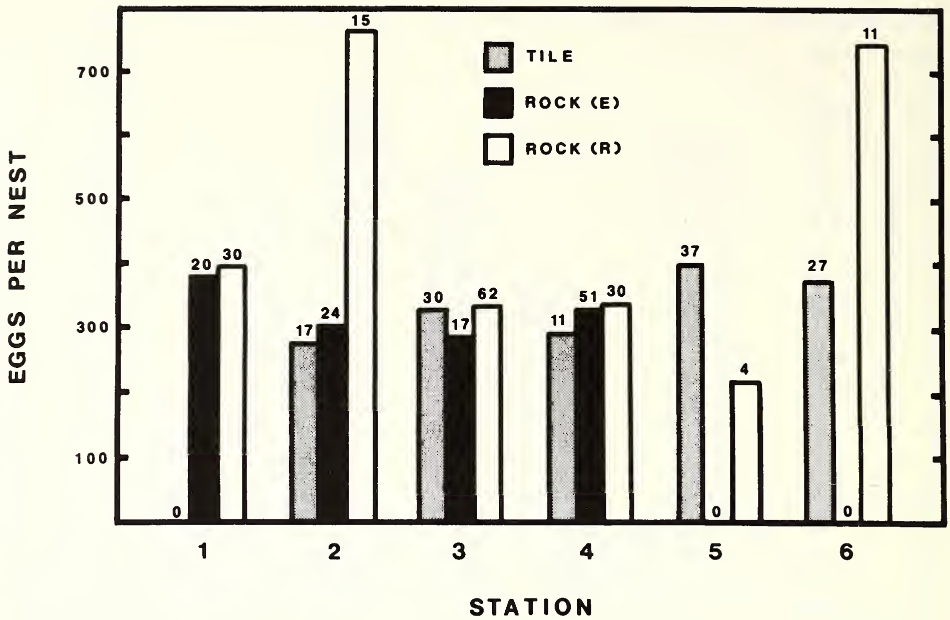

Figure 3. Average number of eggs per nest by type and station. Tile bars are averages of nests under tiles oriented both with and against the current. Rock nests are from reference (R) and experimental (E) areas of each station. Numbers above bars are sample sizes.

were found resting under the tiles. Tiles sheltered the majority of the nests at stations 5 and 6, both of which lacked rocks in the experimental sections. Tile orientation (with or against the current) did not affect utilization as nest sites (59 and 63 nests, respectively); these categories were therefore lumped in all subsequent analyses.

Experimental sections contained 234 nests (62.1% of the total), and outnumbered control section nests at four of the six stations. From 25 March through 23 May, the average experimental section contained 6.5 nests per census, which was significantly more than the 4.2 nests per census found in reference sections (Student's t-test, $t = 2.04$, $P < 0.04$).

The availability of rock nest sites averaged over all stations and censuses was not significantly different between reference (0.87 rocks/m^2) and experimental (0.74/m^2) sections ($t = 0.27$, $P < 0.9$; Table 2). The addition of tiles more than doubled the experimental nest site density to 1.68 sites/m^2, although the difference between average experimental and reference section nest site densities was still not significant ($t = 1.44$, $P < 0.2$). Average use of rock nest sites was similar between reference (0.21 nests/m^2; 22.6% of the sites in use per census) and experimental (0.19 sites/m^2; 20.2% site usage) sections. With the addition of tiles, experimental nest density increased to 0.33 nests/m^2 with a site usage of 23%. The difference between reference and experimental nest densities was not significant ($t = 1.09$, $P < 0.4$).

The experimental nest density per station averaged across all censuses exceeded that of the respective reference sections at four of the six stations (Table 2). The difference was significant at stations 2 ($t = 2.46$, $P < 0.05$) and 5 ($t = 2.75$, $P < 0.05$). Average nest density per station correlated strongly with nest site availability in both

Table 2. Summary of densities (per square meter of stream) of nest sites and spottail darter nests found at each station averaged over six censuses between 25 March and 23 May, 1988.

Parameter	Station					
	1	2	3	4	5	6
Reference sections						
Rock density	2.14	0.33	1.15	1.05	0.24	0.28
Nest density	0.52	0.07	0.36	0.23	0.02	0.08
Percent Use[1]	24.4	20.5	31.0	21.6	10.4	27.7
Experimental sections						
Rock density	2.06	0.27	0.28	1.80	0.00	0.00
Rock nest density	0.35	0.12	0.08	0.56	0.00	0.00
Percent use[1]	16.8	46.2	27.0	30.9	0.00	0.00
Tile density	1.04	0.62	0.53	1.05	0.95	1.10
Tile nest density	0.00	0.09	0.13	0.12	0.29	0.25
Percent use[1]	0.00	14.2	25.0	11.5	30.8	22.5
Total shelter density	3.10	0.89	0.81	3.24	0.95	1.10
Total nest density	0.35	0.21	0.21	0.68	0.29	0.25
Percent use[1]	11.2	23.8	25.7	23.8	30.8	22.5

[1] Percent use is determined as the average number of nests per census divided by the average number of nest sites in each category.

reference (product-moment correlation, $r = 0.98$, $P < 0.01$) and experimental ($r = 0.82$, $P < 0.05$) sections. A significant negative correlation existed between percent tile use and both experimental section rock availability ($r = -0.87$, $P < 0.05$) and overall rock availability per station ($r = -0.85$, $P < 0.05$).

Sixty-nine of the 106 tiles set were used as nest sites, 33 of which sheltered nests more than once during the two month breeding season. Of these, 18 (54.6%) were used as nest sites twice, 12 (36.4%) were used three times, and three (9.1%) had four nests. The average number of nests per tile over the entire breeding season varied from 0 (station 1) to 1.85 (station 5), averaging 1.04.

The average number of nests per rock, estimated by dividing the mean number of rocks available by the total number of nests under rocks, was 1.61, with those in experimental sections used slightly more than those in reference sections (1.99 and 1.35, respectively). On four occasions, a rock was found sheltering two spottail darter nests simultaneously, with the nests separated by substrate when the rock was in its original position. Nests per site (rocks and tiles combined) in experimental sections averaged 1.41, ranging from 1.01 at station 1 to 1.85 at station 5.

There were no significant correlations between the numbers or densities of darters in the 1988 and 1989 surveys and the numbers or densities of nests or nest sites in experimental, reference, or combined sections.

The average number of eggs per nest was 368.6 with a standard error of 16.1. The range was 4 to 2100, with a median number of 313. Tile nests contained an average of 352.6 eggs, while those under rocks averaged 376.2 eggs per nest. The number of eggs per nest did not differ significantly between stations (Kruskal-Wallace test, $chi^2 = 2.48$, $P < 0.8$) or between nest types (tile nests, rock nests in experimental sections, rock nests in reference sections; Kruskal-Wallace test, $chi^2 = 1.8$, $P < 0.4$; Fig. 3). The average experimental section nest contained 341.6 eggs, which was not significantly different from the 411.4 average in reference sections (Mann-Whitney U-test, $z = 1.22$, $P < 0.11$). There was, however, a change in average nest size during the study. For the period of 25 March through 21 April, the average nest contained 456.6 eggs, significantly more than the 304.4 average for the May censuses (Mann-Whitney U-test, $z = 4.82$, $P < 0.001$).

An average of 0.7 (4%) of the tiles at each station sheltered spottail darters without nests at each census period. One observation involved two darters under the same tile; the remaining 29 were solitary. Of the 122 nests found under tiles, 54 also contained spottail darters: 49 were solitary, four involved two darters, and one, three.

DISCUSSION

The primary objective of this study was to determine whether the spottail darter, a special concern species, is limited by a lack of nest sites in southwestern Indiana. The heavy use of the artificial nest sites (31.6% of all nests) clearly affirms this limitation, as each tile added to the drainage increased reproduction by an average of one new nest. Moreover, tile use increased where rock availability was lowest, being particularly evident at stations 5 and 6 which had the fewest natural nest sites. However, rocks appeared to be the preferred nest sites. The average rock sheltered more nests over the course of the breeding season than did the average tile, and the percent utilization of tiles was lower (although not significantly) than that for rocks. Additionally, many rocks were not suitable as nest sites due to position or lack of access to the undersurface, such that those rocks capable of supporting nests were used to a greater degree than the data indicate. Therefore, while the addition of tiles did significantly increase the number of *E. squamiceps* nests in experimental sections relative to reference sections, the darters appeared to prefer rocks as nest sites.

Both the mud darter (*Etheostoma asprigene*) and the slough darter (*E. gracile*) occur in Bayou Creek (Cervone, *et al.*, 1989), but both attach their eggs to vertical surfaces (Cummings, *et al.*, 1984; Page, 1983) and therefore do not compete with *E. squamiceps* for nest sites. The bluntnose minnow (*Pimephales notatus*) is a potential competitor for nest sites as it is common in the areas studied and also attaches eggs to the undersides of benthic debris (Trautman, 1981). Nests of *P. notatus* were encountered during this study under both rocks and tiles but were found only in mid and late May, well after the peak in spottail darter reproduction. It is therefore doubtful that the addition of nest sites released *E. squamiceps* from strong interspecific competition. It is more likely that nest site addition relaxed intraspecific competition, allowing either more male spottail darters to obtain nest sites or individual males to defend nest sites for a longer period.

The average number of eggs per nest (368.6) found in this study was smaller than the 449.2 average found by Page (1974), although the ranges were similar (8 to 1500, Page, 1974; 4 to 2100, this study). Rocks and tiles had similar average nest sizes despite the fact that tiles offered a relatively consistent surface area for egg deposition (185 cm^2) while rocks used as nest sites ranged from small half-bricks (95 cm^2) to large stones with over 1000 cm^2 of undersurface area, indicating that the surface provided by the tiles was adequate for most nests.

Some of the variation in nest size was due to censusing nests at different stages of formation. Many small nests may have contained only a partial clutch while others contained clutches from several females. Spottail darters practice a form of resource defense polygyny (*sensu* Emlen and Oring, 1977), with males defending nest sites visited by several females (Page, 1974). While the degree of polygyny and variation in male reproductive success is not known, some estimates are possible. The number of mature ova in a reproductive female ranges from 28 to 357, averaging 109.6 (Page, 1974). Assuming each female spawns once each year (supported by Page, 1983; but see Hubbs, 1985) and deposits all mature ova, all nests with 110 or more eggs (75.6% in the current study) may represent multiple clutches. The average nest (368 eggs) may therefore contain three clutches. The degree of polygyny may be even higher, if the same male defends a nest site long enough to hatch more than one set of multiple-clutch eggs. The addition of tiles may have relaxed the normal degree of polygyny by providing more nest sites, possibly allowing more males to breed simultaneously. This may partially explain the smaller average nest sizes in experimental areas relative to reference areas. Similarly, the decline in nest size in the latter half of the breeding season may be due to a decrease in the number of females contributing eggs to each nest or, alternatively, a decrease in the number of eggs laid per female. Since size and mature ova content are correlated (Page, 1974), it may also be that the larger (i.e., older) females are breeding earlier.

The large number of spottail darters found in this study is surprising considering the small populations found in previous efforts in southwestern Indiana. Page, *et al.* (1976) found only 2 *E. squamiceps* in extensive seining in Posey County. Grannan and Lodato (1986) found spottail darters at 6 of 46 localities in Posey and Vanderburgh Counties, with four of these sites yielding less than 10 darters each despite multiple seining efforts. Cervone, *et al.* (1989) collected spottail darters at 6 of 26 stations in these counties, but caught an average of only 3.7 darters per station at those stations yielding darters.

We captured an average of 25 darters per station over all stations and censuses in 1988. The difference may be due to two factors. First, variations in seining techniques could give different population estimates. The benthic habits of darters make them difficult to seine, and we found that single or a few seine passes through an area generally yielded only a few darters per pass, but multiple passes through the same area could accumulate relatively large numbers. Additionally, areas with moderate to large rocks are inherently difficult to seine efficiently for benthic organisms. Previous studies designed as surveys may therefore have underestimated the darter populations.

Second, there appears to be considerable temporal variation in darter abundance. One area found by Grannan and Lodato (1986) to contain 27 spottail darters yielded only one *E. squamiceps* in four surveys in 1987 and none in a single census in February 1988. Similarly, station 1 contained an average of 14 darters in February and May of 1987, but yielded an average of only 4 darters during the same period in 1988 despite more intensive seining during the latter year. Identical seining techniques and effort yielded 38 spottail darters at that station in March 1989. An area that yielded 15 darters in February 1987 contained only a single darter a year later despite seining a much larger area.

Page (1974) noted that young spottail darters disperse downstream but return to the breeding areas at the end of their first year. Otherwise, migration appears to be limited to movement between microhabitats (pools and riffles). Observations in this study, however, suggest that adults may migrate also. Assuming an average clutch size of 110, the over 142,000 eggs counted in this study would require nearly 1300 females. This is over 13 times the number of females captured in the May 1988 census assuming a 1:1.3 sex ratio (Page, 1974). Clearly, darters are moving through potential breeding areas during the reproductive season. For example, station 3 was seined intensively twice during February 1988, and on both occasions, four individuals of *E. squamiceps* were captured at the same sites within the station. Areas approximately 30 m long above and below station 3 were also seined and did not yield any darters. However, the 109 nests found at this station and the 27 darters captured in May 1988 attest to the local migratory capabilities of this species. Clearly, some long-term monitoring of population size and density of this species in southwestern Indiana is needed to distinguish between population fluctuation and local migration.

The lack of correlations between densities of darters at different censuses or between darter densities and nest densities is not surprising given the complications presented by seining difficulties and local migration as described above. Similarly, the increase in the yearling cohort at the six stations in 1989 may or may not represent the spottail darters born at those stations in 1988, and therefore no conclusions can be drawn regarding the direct effect of nest site additions on subsequent population size. The number of under 4-cm darters captured at each station in 1989 did not correlate with either total nests or tile nests in 1988. It is encouraging, however, that the only significant change in population structure between years was an increase in the cohort produced in 1988.

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