

Notes and Discussion Piece

Spawning Substrate Preferences of the Yazoo Darter (*Etheostoma raneyi*)

ABSTRACT.—The Yazoo Darter *Etheostoma raneyi* is a small imperiled snubnose darter endemic to Upper Gulf Coastal Plain streams in north-central Mississippi. Installation of rip-rap and wood is being considered in an effort to increase suitable habitat and spawning substrate for the species. In common with other snubnose darters, Yazoo Darters attach their eggs to appropriate spawning substrate. However, it is not known if Yazoo Darters, or other imperiled Coastal Plain snubnose darters, will use rock substrate for spawning given it is almost entirely absent from streams of occurrence. If Yazoo Darters show no preference between wood and rock (rip-rap) for spawning, management would have greater flexibility when designing stream habitat installations for imperiled Coastal Plain snubnose darters, because the installation of rip-rap is much less expensive and time consuming than installation of wood. We used a randomized block experiment in an outdoor mesocosm facility to test for spawning substrate preferences. We counted >7600 eggs and Yazoo Darters used both substrates but used wood more than twice as often as rip-rap. Though we cannot recommend habitat installations using only rip-rap, any installation using a mix of wood and rip-rap will enhance spawning habitat for the Yazoo Darter and at least four other imperiled Coastal Plain snubnose darters.

INTRODUCTION

The Yazoo Darter *Etheostoma raneyi*, is an imperiled, small (<65mm SL), benthic insectivore endemic to perennial headwater streams in the Little Tallahatchie River watershed (upper Yazoo River basin) of the Upper Gulf Coastal Plain of north-central Mississippi (Suttkus *et al.*, 1994; Johnston and Haag, 1996; Sterling *et al.*, 2013). The species is closely related to at least 27 other snubnose darters (clade *Adonia*, sensu Near *et al.*, 2011, hereafter snubnose darters), of which approximately 17 are imperiled (Jelks *et al.*, 2006; Ruble *et al.*, 2019), including at least five other imperiled forms inhabiting the Upper Gulf Coastal Plain of western Kentucky, Tennessee, and northern Mississippi. One of the primary causes of imperilment for Coastal Plain snubnose darters is habitat alteration and degradation across virtually every stream within the limited ranges of each species in the group. Streams are deeply incised as a result of widespread channelization and are wide and shallow with homogeneous habitat and unstable fine substrate. Spates sweep streams clear of structure and mature riparian forest is often lacking. Consequently, wood within streams is relatively scarce (Shields *et al.*, 1998, 2010; Warren *et al.*, 2002; Keck and Etnier, 2005; Powers and Warren, 2009). This factor may be limiting some populations of Coastal Plain snubnose darters given they are closely associated with complex, stable cover dominated by wood (Carney and Burr, 1989; Powers and Warren, 2009; Sterling and Warren, 2017).

Snubnose darters apparently share many life history attributes including identical spawning behavior (Johnston *et al.*, 1999; Storey *et al.*, 2006; Hubbell, 2014). Spawning commences when daily high water temperatures consistently reach about 10C (generally early March in northern Mississippi) and ceases when temperatures exceed about 21C for more than a few days (about mid-May) (Carney and Burr, 1989; Suttkus *et al.*, 1994; Ruble *et al.*, 2019), which is mostly consistent with patterns observed in other less closely related species of darters (Page, 1983; Hubbs, 1985). Batches of eggs are recruited throughout the spawning season and are spawned in clutches (Heins and Baker, 1993). Females attach eggs (about 1–1.5 mm diameter) singly to appropriate substrate, most often described as the vertical surfaces of coarse gravel, cobble, and rubble in upland rocky streams (Porterfield, 1998; Johnston *et al.*, 1999; Storey *et al.*, 2006; Hubbell, 2014). However, streams within the Upper Gulf Coastal Plain of western Kentucky and Tennessee and northern Mississippi lack coarse rock substrate and at least five imperiled snubnose darters in the region are likely reliant on wood as a spawning substrate (Carney and Burr, 1989; Johnston and Haag, 1996).

The Yazoo Darter is currently the focus of considerable conservation effort by numerous state and federal agencies. One management option to increase abundance of Yazoo Darters is the introduction of rock (rip-rap) and wood into streams to provide cover, refugia from high flows and drought, substrate for feeding and spawning, and greater variance in depth, water velocity, and substrate size. Although it is

apparent rip-rap and wood can each provide most of these services, it is uncertain if rip-rap would be used as a spawning substrate; this is a key consideration given the management goal of increasing abundance of Yazoo Darters. If rip-rap is used as a spawning substrate about as often as wood, managers would have much greater flexibility when designing and installing stream habitat for imperiled Coastal Plain snubnose darters. Here, we test the hypothesis Yazoo Darters show no preference for wood and rock as spawning substrates. This study represents the first experimental test of the spawning substrate preferences of a clade *Adonia* darter.

METHODS

MESOCOSMS AND MAINTENANCE OF FISH

A shaded outdoor flow-through mesocosm facility was used to house Yazoo Darters, each mesocosm consisting of an oval 1135 L aquaculture tub within a 29,148 L recirculating system (contact authors for details). Twelve mesocosms were used to collect data. Counterclockwise flow in each mesocosm was maintained at about 0.06 m/s using a 1500 L/h powerhead pump. Water temperatures were not manipulated.

Yazoo Darters were collected from Hurricane Creek (34.425, -89.496) on February 14, 20, and 27, 2019 and from Bay Springs Branch (34.428, -89.396) on February 26 and 27, 2019. The U.S. federal government shutdown (December 2018–January 2019) prevented collection of fishes earlier in the year and record high rains and flows prevented collection on a single day from a single stream as initially planned. Two males and six females (total 24 males and 72 females) were apportioned among mesocosms; no mortality occurred during the study. Though the mesocosms supported numerous and diverse invertebrate food resources, supplemental feeding using commercially available frozen chironomid larvae and brine shrimp was performed daily for a week after introduction of fish to the mesocosm and about three times a week thereafter.

TREATMENTS AND STUDY DESIGN

Four Sycamore (*Platanus occidentalis*) wood logs and four pieces of limestone rip-rap were installed in each mesocosm for spawning substrate. Each piece of substrate was mounted on a wooden dowel that was attached to a masonry base. The base was buried within the sand substrate, and the bottom of the wood or rock was about 7–8 cm above the substrate to provide access underneath the substrate (Fig. 1). Because our central question was whether or not Yazoo Darters would use rip-rap as often as wood, natural materials were used rather than manipulated and uniform substrates. Sycamore is a common riparian tree species with smooth bark that allowed us to easily find and count eggs. Tree trunks were sectioned into 30 cm lengths and diameters ranged from 52–115 mm. Rip-rap of various sizes but of approximately similar surface area as the sycamore logs was obtained from a stream supporting Yazoo Darters. Weight of rip-rap varied from 1.4–5.7 kg. We introduced our wood substrates into our mesocosm facility August 2018 to allow them to become waterlogged and accumulate biofilm. Rip-rap already with biofilm was introduced January 2019 directly from the stream from which they were collected.

A randomized complete block design (RCBD) was used in which each mesocosm was a block. Wood and rip-rap spawning substrates were randomly placed in two rows of four near one side of each mesocosm (Fig. 2). Random placement was determined by numbering each piece of substrate and using a stratified randomization procedure in which at least one piece of each type of substrate occurred in each row (Statistics.com LLC, 2009). A power analysis indicated about 12 blocks were needed to have <10% chance of committing a Type II error and to detect an effect size of about 25% ($\sigma = 5$).

DATA COLLECTION

Spawning substrates were removed one at a time from the mesocosm and examined for eggs. Each sample occurred over 2 d (about 6 h/d) beginning March 13, 2019, and counts were repeated every 4 d until April 3, 2019 for a total of five samples. Eggs were counted using reading glasses (3.0 diopter) and removed. At the end of the count, the substrate was wiped clean and rinsed in a bucket of mesocosm

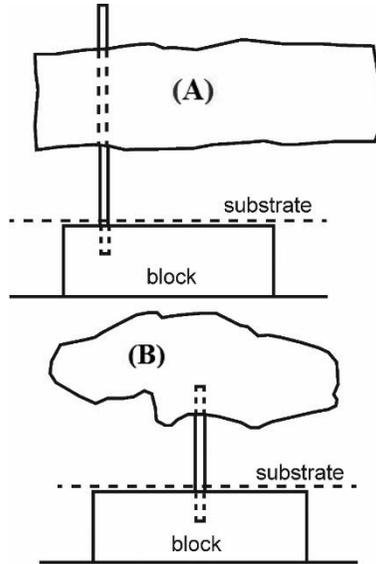


FIG 1.—Wood (sycamore log, **A**) and rip-rap (**B**) substrates used to estimate substrate preferences for attaching eggs; each piece of substrate is mounted on a masonry block using a wood dowel that was epoxied into the block. A stainless steel pin was inserted through the dowel to hold the wood log at the correct height above the block; because the dowel running through the waterlogged wood was offset, any tendency to float was prevented by binding against the dowel

water before being returned to the same position in the mesocosm from which it was removed. Egg locations on substrates were defined and recorded as follows: top = surface that would collect and retain all silt; sides = surfaces that would collect but not retain all silt; bottom = surface that would collect no silt. The vertical faces at each end of wood logs were not examined for eggs.

ANALYSIS

Our main question was whether or not Yazoo Darters show a substantial preference for either wood or rip-rap spawning substrate. Because more eggs on one substrate results in fewer eggs on another, a test of the null expectation of no preference is most suitable. We used the nonparametric Friedman’s χ^2 test of the summed egg counts for each substrate type across all samples within each mesocosm (randomized blocks) (Zolman, 1993; StatXact 8, 2007). Analyses were run using Cytel Studio version 8.0 (Cytel, Inc. Cambridge, MA). To estimate if substrate size had an effect on egg counts, we used regression analyses (Cytel Studio version 8.0) of egg counts (square root transformed) and size (Log_{10} diameter for wood and Log_{10} weight for rip-rap).

We calculated means and 95% Confidence Intervals (CIs) for egg counts on each substrate type across time and for total egg counts in each position (top, bottom, sides) for each substrate type. Because there was one top and bottom, but at least two sides for each piece of substrate, we divided the total egg count on sides in half.

RESULTS

We counted a total of 7642 eggs, 2111 on rip-rap and 5531 on wood. Yazoo Darters showed a decided preference for wood substrate, $\chi^2 = 12$, $df = 1$, $P < 0.0005$ (Table 1), and no changes in preference were apparent over time (Fig. 3). They also attached eggs to the bottom of both substrate types more often

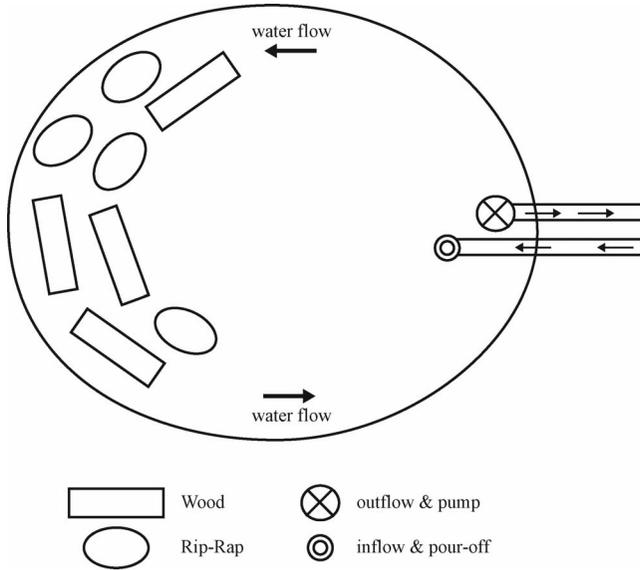


FIG. 2.—Diagram of one 1135 L mesocosm showing inflow and outflow of water and placement of spawning substrates (substrate was placed randomly, *see* text); a 1500 L/h powerhead pump was hung off the outflow and directed flow in the mesocosm

than to the sides or top (Fig. 4). Size of rip-rap ($R^2=0.08, P > 0.05$) or wood ($R^2=0.0002, P > 0.05$) was not associated with the number of eggs.

DISCUSSION

We were not surprised to find Yazoo Darters preferred the wood substrate, but we did not anticipate such a large effect size. Descriptions of spawning and substrate choice among snubnose darters indicate they will attach their eggs to all available substrates, but they usually appear to show a preference for

TABLE 1.—Data used for the Friedman’s (χ^2) test of substrate choice by Yazoo Darters showing egg count totals for each mesocosm (randomized block) for each substrate

Block	Rip-Rap	Wood
M1	213	284
M2	200	599
M3	294	635
M4	201	391
M5	117	467
M6	123	577
M7	116	413
M8	266	426
M9	187	551
M10	119	344
M11	176	531
M12	99	313

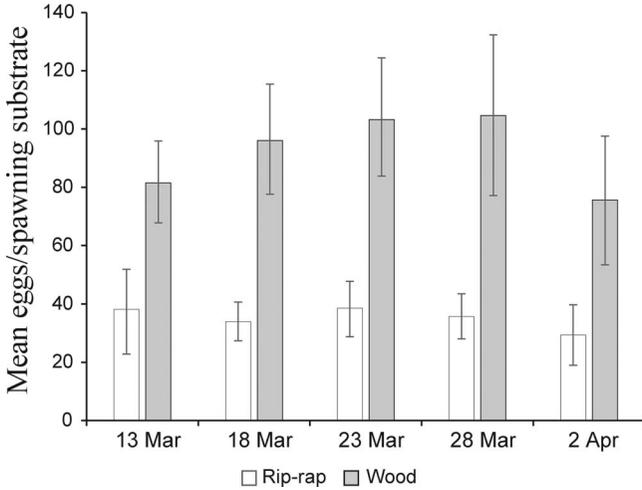


FIG. 3.—Mean egg counts (\pm 95% CIs) across mesocosms for each substrate type over time

certain substrate types (Porterfield, 1998; Johnston *et al.*, 1999; Storey *et al.*, 2006). Yazoo Darters appeared to show a preference for wood (and aquatic plants) over rock in an earlier limited observational study using aquaria (Johnston and Haag, 1996). As instream wood is the only widely available stable substrate within streams of occurrence, it seems logical Yazoo Darters would show a preference for wood spawning substrate. However, although rock is currently exceedingly rare within the range of the Yazoo Darter, this may not have always been the case. During repeated and frequent glacial cycles, streams likely alternated between higher gradient, coarse substrate habitat (glacial periods), and lower gradient, fine substrate habitat (interglacial periods) (Fisk, 1944; Rittenour *et al.*, 2007).

The avoidance of spawning on the top surfaces of wood or rip-rap substrates is likely a strategy to prevent eggs from being smothered in fines and organic matter and is consistent with published observations for other darter species (O’Neil, 1981; Page *et al.*, 1982; Porterfield, 1998; Storey *et al.*, 2006; Anderson, 2009). Likewise, in an instream pilot project using only sycamore wood logs, we found 44 eggs on the bottom of the logs, 19 on both sides, and no eggs on top (unpubl. data). Because we wiped substrates after counting eggs and before replacing them into the mesocosms, the top surfaces were regularly cleaned. Even so, a small accumulation of fines and the fast growth of periphyton created a top surface that was distinctly less clean than the sides and bottom surfaces of the substrates.

Though we did not measure the effects of rugosity on egg placement, it was clear a large majority of eggs were attached within crevices, knots, holes, and along the edges of tree bark. Nonetheless a few eggs were routinely found on all surfaces, including those that were smooth. This pattern of egg placement was commonly observed in other snubnose darters (O’Neil, 1981; Page *et al.*, 1982; Porterfield, 1998; Anderson, 2009) and is likely a bet-hedging strategy to avoid predation (Page, 1983).

The lack of any effect of size of the substrate on egg counts may be attributed to the relatively small variation in the size of substrates used. If we had used smaller substrates, we likely would have found some effect.

We were mildly disappointed Yazoo Darters showed a strong preference for wood substrate because the installation of wood substrate in streams is more expensive and time consuming than for installation of rip-rap (unpubl. data). We cannot recommend habitat enhancement installations using only rip-rap. However, Yazoo Darters did use rip-rap frequently and any installation using a mix of wood and rip-rap will enhance spawning habitat for the species and at least four other imperiled Coastal Plain snubnose darters.

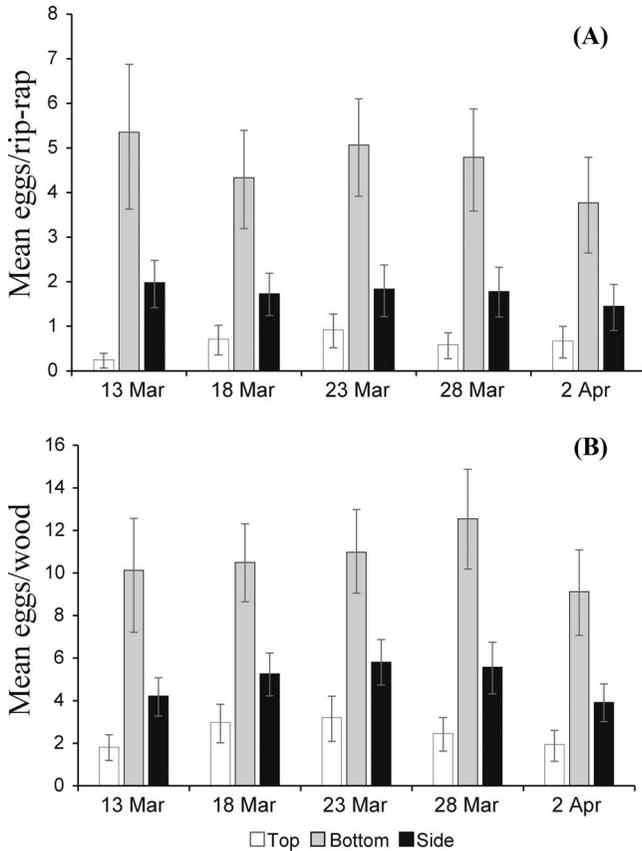


FIG. 4.—Mean number of eggs (\pm 95% CIs) attached to the top, bottom, and sides of rip-rap (A) and wood (B) over time

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