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Captive Propagation and Early Life History of the Yazoo Darter (*Etheostoma raneyi*)

Crystal L. Ruble¹, Ken A. Sterling^{2,*}, and Melvin L. Warren Jr.²

Abstract - Captive propagation of fishes to achieve a variety of management goals is common and is increasingly included in recovery plans for imperiled species. Here, we present a protocol for the captive propagation of *Etheostoma raneyi* (Yazoo Darter) and summarize early life-history information. From 9 males and 17 females, we collected >1000 larvae, of which >83% survived to the juvenile stage. Water temperature during spawning was 15.6–21 °C and larval production peaked at 17.2–20.6 °C. Spawning abruptly ceased when daily high water temperatures exceeded 21 °C for 3 consecutive days. Newly hatched larvae were able to swim vigorously, were pelagic, and were about 4.4–4.5 mm total length (TL). Compared to other darter species, the Yazoo Darter is among the easiest to propagate, and the protocol presented should be suitable to meet most management goals. The protocol also provides a sound basis for the development of species-specific captive-propagation techniques for ≥17 closely related and imperiled snubnose darters.

Introduction

Development and modification of captive propagation protocols for imperiled freshwater fishes is now a widespread management action and is increasingly included in recovery plans (ADCNR 2015, Fisch et al. 2013, Guy and Allen 2018, Hutson et al. 2012, Mercy et al. 2015, Raghavan et al. 2016). In the southeastern United States, ≥25% of freshwater fish species are imperiled (Warren et al. 2000), and the percentage is apparently increasing (Jelks et al. 2008). Captive propagation techniques for imperiled southeastern fishes have been developed and published for various taxa including minnows (Rakes et al. 1999), madtom catfishes (Shute et al. 2005), and darters (Rakes et al. 1999; Ruble et al. 2014, 2016). Development of captive-rearing methods based on previous experience and through trial and error yields an appreciable amount of life-history information for the target species and often for closely related fishes as well (Rakes and Shute 2005). The data from these efforts are especially valuable because the life history of embryonic and larval forms of most fishes is rudimentary or completely unknown.

Etheostoma raneyi Suttkus (Yazoo Darter) is a range-limited, benthic stream fish distributed among tributaries of the Little Tallahatchie and Yocona River drainages, upper Yazoo River basin, north-central Mississippi (Johnston and Haag 1996, Sterling et al. 2013, Suttkus et al. 1994). Yazoo Darters are most commonly collected in less-disturbed, small perennial streams and are more abundant

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in streams with substantial ground water input near springs (Suttkus et al. 1994). The species is categorized as vulnerable by the American Fisheries Society (Jelks et al. 2008) and the Southeastern Fishes Council (Warren et al. 2000), globally imperiled by the Nature Conservancy (NatureServe 2013), sensitive by the USDA Forest Service (USDA Forest Service 2013), and a Tier 1 species of greatest conservation need by the Mississippi State Wildlife Action Plan (Mississippi Museum of Natural Science 2015).

A life history of the Yazoo Darter (Johnston and Haag 1996) shows remarkable consistency with life-history information from other closely related snubnose darters (clade *Adonia*, sensu Near et al. [2011]), especially in reproductive traits. Spawning behavior is described for 14 other closely related snubnose darters, (9 field and 5 aquaria studies) and is apparently identical among species. All attach eggs to appropriate substrate, usually coarse gravel, rubble, or cobble in upland streams (Hubbell 2014; Johnston and Shute 1997; Johnston et al. 1999; Keevin et al. 1989; O'Neil, 1981; Page et al. 1982; Porterfield 1997, 1998; Storey et al. 2006) and, presumably, wood in lowland streams lacking rocky substrates (Carney and Burr 1989; Johnston and Haag 1996; K.A. Sterling, pers. observ.). Spawning commences in early spring when daily high water temperatures are consistently above ~10–11°C, and continues for 2–3 months until daily high water temperatures are consistently above about 21 °C (Anderson 2009, Carney and Burr 1989, Hubbell 2014, Porterfield 1998, Rakes and Shute 2005, Storey et al. 2006, Suttkus et al. 1994). Snubnose darters are fractional spawners, producing clutches of eggs throughout the spawning season (Barton and Powers 2010, Carney and Burr 1989, Hicks 1990, Johnston and Haag 1996, Khudamrongsawat and Kuhajda 2007, Khudamrongsawat et al. 2005, O'Neil 1981, Stallsmith and Bedingfield 2015). Data from 8 closely related snubnose darters indicate that newly hatched larvae generally vary in total length (TL) from 3.5 to 5 mm and can swim upon hatching (Carney and Burr 1989, Rakes and Shute 2005, Simon and Wallus 2006). Such consistency lends confidence that new reproductive data obtained from one species are likely similar for other closely related snubnose darters (Khudamrongsawat and Kuhajda 2007, Rakes and Shute 2005). Here, we present the results of a captive propagation protocol for the Yazoo Darter developed by Conservation Fisheries, Inc. (CFI; Knoxville, TN), summarize early life-history information, and provide a supplemental description of larval and juvenile development.

Methods

On 16 March 2017, we used a seine to capture 26 Yazoo Darters (9 males, 17 females) from Hurricane Creek, Lafayette County, MS. We measured water temperature, which was 9.65 °C, when we commenced sampling at about 0900 and observed that males and females were in spawning condition (brightly colored males, gravid females). Because the winter of 2017 was unusually mild, Yazoo Darters likely had been spawning for several weeks at the time of capture. We transported Yazoo Darters from Hurricane Creek to CFI in covered, water-filled, plastic coolers with bubblers and *Sparganium* sp. (burr-reed).

We housed Yazoo Darters at CFI from 17 March to 17 July 2017, when we conformed to the Guidelines for the Use of Fishes in Research published by the American Fisheries Society and the American Society of Ichthyologists and Herpetologists (AFS 2014) and euthanized all remaining Yazoo Darters and preserved them in 5% buffered formalin.

Recirculating system and feeding

Upon arrival at CFI, we divided males and females into 3 breeding groups, each housed separately in 75-L aquaria with an air sponge filter for added biological stability (Fig. 1). The 3 aquaria were part of a larger recirculating system consisting of twenty-one 75-L aquaria and a 378-L sump. Breeding groups 1 and 2 each consisted of 3 males and 6 females, and breeding group 3 consisted of 3 males and 5 females. Each aquarium had sand substrate and a variety of cover types including *Ceratophyllum* (hornwort) and *Taxiphyllum* (java moss), PVC pipe, natural rocks, and slate tile. Water temperatures and photoperiod followed seasonal changes. We controlled photoperiod using an astronomic timer, and manipulated water temperatures using air exchange with the outside environment and air conditioning. We introduced Yazoo Darters into the system with water temperatures varying from 11.6 to 12.8 °C and gradually increased temperatures through the spring. The goal was to create a temperature ramp roughly corresponding to headwater spring-run temperatures (Fig. 2). We fed Yazoo Darters live food daily including the water fleas *Daphnia pulex* Leydig and *Ceriodaphnia dubia* Richard, *Enchytraeus buchholzi* Vejdovský (Grindal Worm), *Lumbriculus variegatus* Müller (Blackworm), and *Gammarus* sp. (amphipod). As fish became acclimated to feeding routines, we also offered prepared foods (e.g., frozen chironomid larvae and *Artemia* sp. [brine shrimp]).

The 75-L aquaria holding each breeding group drained into a passive larval collection tub with a light at the overflow to attract larvae in case the larvae exhibited strong, positive phototactic behavior (Fig. 1). Tubs were white plastic, 30 cm in diameter, 20 cm deep, and about 19 L in volume. The tubs had a 15-cm central standpipe with a 7.7-mm-diameter PVC reducer drain (see Supplemental Figure 1, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>). We glued a 400- μ mesh screen to the top of the reducer to allow water to pass through but keep larvae in the collection area. We manually transferred larvae from all the collection tubs (all breeding groups) twice a day to a 53.3 cm x 66.0 cm black, plastic, oval rearing tub with a volume of about 56.8 L. The rearing tub had a 12.7-cm vertical central standpipe with a 10.2-cm-diameter reducer with a 400- μ mesh screen attached. The rearing tub received water from the recirculating system through a restricted 6.35-mm Loc-Line[®] valve with a 3.2-mm nozzle opening (see Supplemental Figure 2, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>). The restricted flow insured proper water turnover without flushing food too quickly out of the rearing tub; it also created a consistent current

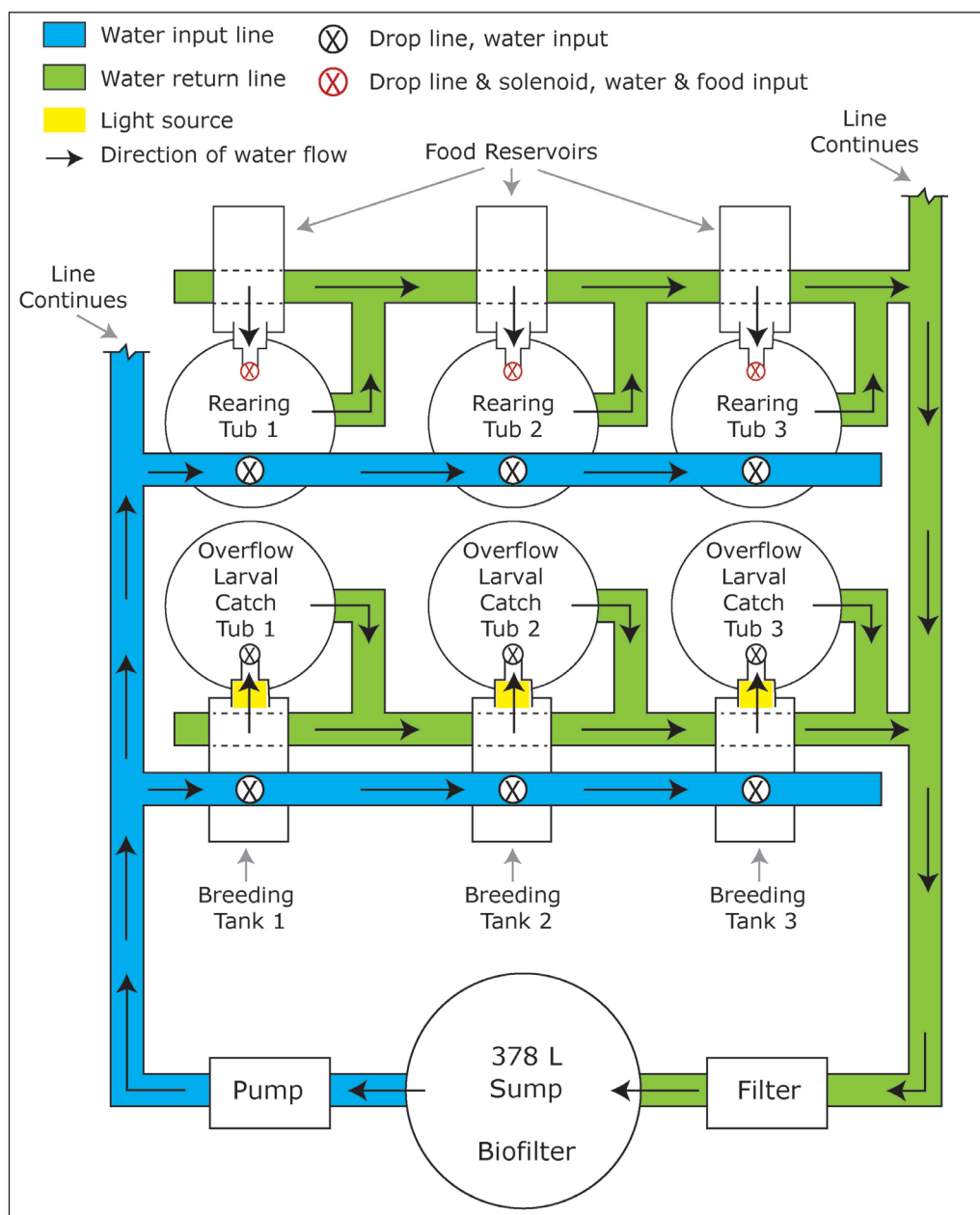


Figure 1. Simplified schematic drawing of the captive propagation recirculating aquaria system used to breed Yazoo Darters. Adults were housed in breeding tanks where spawning took place and hatched larvae (attracted by the light source) were passively moved through the aquarium overflow into the larval catch tubs (see Supplemental Figure 1). Larvae were manually moved to rearing tubs (see Supplemental Figure 2) daily. Food was input into rearing tubs from the food reservoirs through a solenoid to control the amount and timing of food input; see text for details. (Supplemental figures available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>).

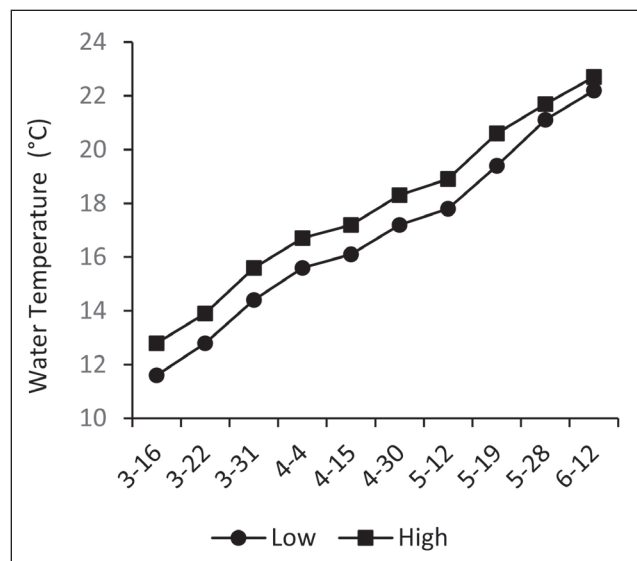
often needed for proper feeding and swimming behavior of the larvae. We transferred larvae to a rearing tub until the number of larvae in the tub was >200 or the age of the oldest larvae was ≥ 7 days old. At that point, we released newly collected larvae into a new rearing tub of the same specifications.

Feeding small larvae requires a constant distribution of food. We used a 7.5–11.4-L container with a spigot as a food reservoir for each rearing tub. We attached a solenoid to the spigot, and a timer opened the solenoid for 10 seconds every 2 minutes during daylight hours to provide a constant source of food. We filled feeding reservoirs twice daily with brine shrimp larvae, marine *Brachionus* sp. (rotifer), *C. dubia* neonates, and Instant Algae[®]. As larvae transformed to benthic juveniles, we removed them from the rearing tub, split them into groups of 30–40, and placed them in 75-L aquaria (also part of the larger recirculating system) to track further development for each breeding group. We fed juveniles brine shrimp nauplii, adult *C. dubia*, and Grindal Worms. As juveniles developed, we offered chopped frozen chironomid larvae.

Life-history data

We recorded high water temperatures daily and low temperatures haphazardly during the study. We recorded the number of swim-up larvae transferred to the rearing tubs daily and the number of juveniles transferred to holding aquaria. We estimated survival from larval to juvenile stages not including larvae preserved through time for the developmental series (described later). We qualitatively recorded larval and juvenile behavior. Because we tracked cohorts of larvae through time, we could estimate rate of growth and age in days post-hatch for the transition from yolk-sac larvae to post yolk-sac larvae and from larvae to juvenile as well as the range in TL (mm) at each transition. We recorded spawning behavior and substrates used for egg attachment.

Figure 2. High and low water temperatures within the recirculating system that housed Yazoo Darters during the spawning period (16 March–14 June 2017) of the study; dates when water temperatures were recorded are on the x-axis.



We held 11 embryos from breeding group 1 and 61 from group 2 (members of breeding group 3 died, see results) in flow-through trays (30 cm x 15 cm x 10 cm) with a 400- μ mesh screen (trays were connected to the larger recirculating system) to estimate hatching times in relation to temperature. We recorded water temperatures daily until all embryos hatched or died. We classified embryos collected from breeding groups as dead or viable and recorded mortality through time. We measured mean diameter of newly spawned, water-hardened eggs across the longest and shortest axes.

Developmental series

We collected a series of embryos representing all stages of development on 24 April 2017 from Breeding Group 1. We photographed embryos and preserved them in buffered 5% formalin for examination. We collected Yazoo Darter larvae and juveniles as opportunity offered to form a time series of development from newly hatched yolk-sac larvae to benthic juveniles ≤ 60 days old. We preserved larvae in 5% buffered formalin and used them for a supplemental description of development through time.

We used descriptive terminology following Simon and Wallus (2006). We employ the terms “pelagic” to refer to larvae that spend most of their time up in the water column where they primarily feed, and “benthic” to refer to juveniles that spend most of their time on the substrate where they primarily feed.

Material examined

All specimens examined are archived with the Mississippi Museum of Natural Science, Jackson, MS: MMNS 73630-73666.

Results

Passive collection of larvae in overflow tubs commenced on 9 April 2017 and ceased on 14 June 2017. For unknown reasons, 2 females from breeding group 2 and all individuals in breeding group 3 (26 April and 9 May, respectively) died. We treated affected aquaria with kanamycin sulfate (an anti-biotic) on 11 May for 10 days of treatment. We found hydra on 9 June 2017 in 1 rearing tub, which almost certainly lowered survivorship (only 39 of 60 larvae survived for transfer to juvenile aquaria). We introduced salt (NaCl) to achieve an initial concentration of 0.7 ppt and increased salt over 7 days to 2.21 ppt to control hydra, but by then production of larvae had ceased.

Water temperatures varied from 11.6 to 22.7 °C during the study (Fig. 2). Spawning occurred from 26 March through 9 June (75 days). Water temperature during spawning was 15.6–21 °C. The number of larvae entering the collection tubs peaked when daily high water temperatures reached 17.2–20.6 °C, and larval production at 19.4 °C was almost double that at 17.2 °C. However, the number of larvae collected daily abruptly decreased from the peak when daily high water temperatures were >20.6 °C (Fig. 3). Based on estimated rates of egg development, spawning ceased when daily high water temperatures had been ~ 21 °C for

the previous 3 days. At that time, male coloration had diminished significantly, and they were no longer following females. Females were clearly not as gravid as judged by abdominal distension, but their ovipositors were still distended. A total of 1034 larvae survived to swim-up for collection and transfer to rearing tubs. Of these yolk-sac larvae, 861 (83.3%) survived to the juvenile stage. The number of larvae produced per female was 26.2 (17 females) from 28 March to 17 May (after mortality of adults ceased) and 53.5 (11 females) from 18 May to 14 June. These were the number of larvae that survived for transfer to the rearing tubs.

Spawning behavior was consistent with a previous description for Yazoo Darters in captivity (Johnston and Haag 1996). Eggs were attached singly, usually in vegetation or yarn mops, but as the eggs developed they lost adhesion and were easily dislodged into the substrate. Rock, slate, and filter sponges were examined for eggs, but they were rarely found in these substrates.

A total of 23 embryos, 11 alive and 12 dead or unfertilized, were collected from breeding group 1 on 7 April to estimate hatch times (flow-through trays). Embryo development varied from high morula (~1–2 days old) to eyed and almost hatching. All embryos hatched by 17 April, 10 days after collection. Estimated time to hatch at 15.6–17.2 °C was 11–13 days. A total of 72 embryos, 61 alive and 11 dead or unfertilized, were collected from breeding group 2 on 18 April. Development varied from early somite (3–5 days old) to eyed (7–10 days old). All embryos hatched by 28 April, 10 days after collection. Estimated time to hatch at 16.1–17.2 °C was

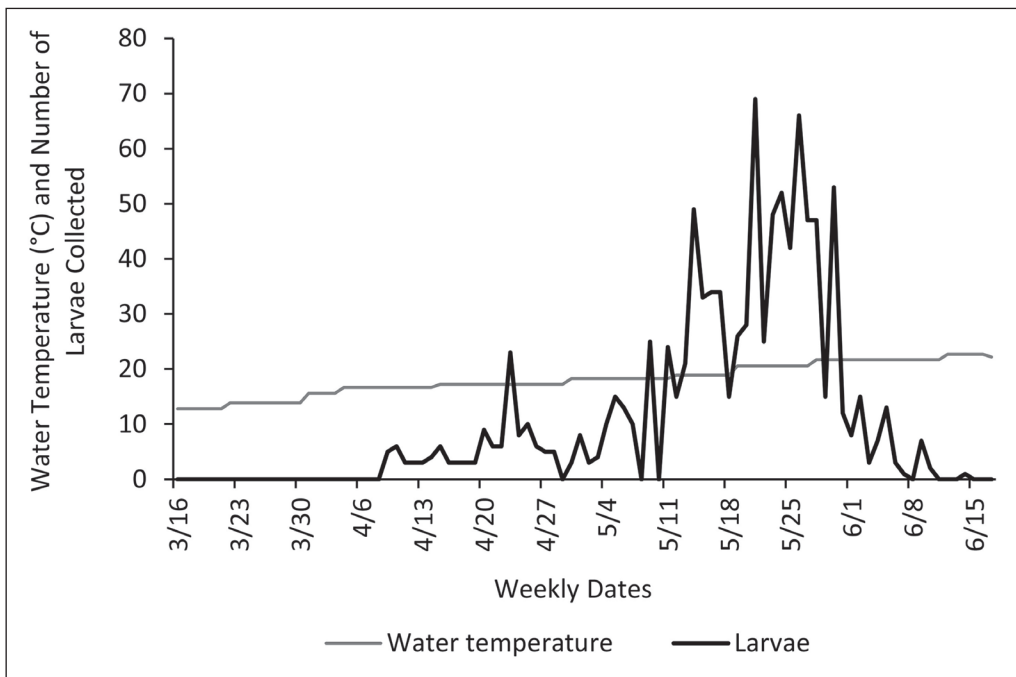


Figure 3. Changes in daily high water temperatures and the number of Yazoo Darter swim-up larvae that were collected daily for transfer to the rearing tubs during the spawning period (16 March–15 June 2017).

13–15 days (see Supplemental Figure 3, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>).

Larvae swam robustly upon hatching, but usually spent ≤ 24 hours resting on the substrate before swimming up into the water column to begin feeding. Newly hatched larvae were 4.4–4.5 mm TL (Fig. 4; Supplemental Figure 4 and text, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>) and stationed themselves in areas with the least current. Swim-up larvae were ~ 5.2 – 5.4 mm TL. A reduced yolk-sac was still present, but the digestive system was working, and there was evidence of feeding (Supplemental Figure 4 and text, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>). After becoming active, swim-up larvae were pelagic, positively phototactic, and selected for progressively deeper water and faster currents as they developed. Yolk-sac larvae transitioned to post yolk-sac larvae at about 5.5 mm TL and 2–3 days old. When post yolk-sac larvae transitioned to benthic juveniles, they positioned themselves directly under the inflow of water in the highest flow. At 11–13 mm TL and 20–23 days old, larvae began to settle to the substrate but still swam up into the water column to feed. At 13–15 mm TL and 23–27 days old, larvae permanently

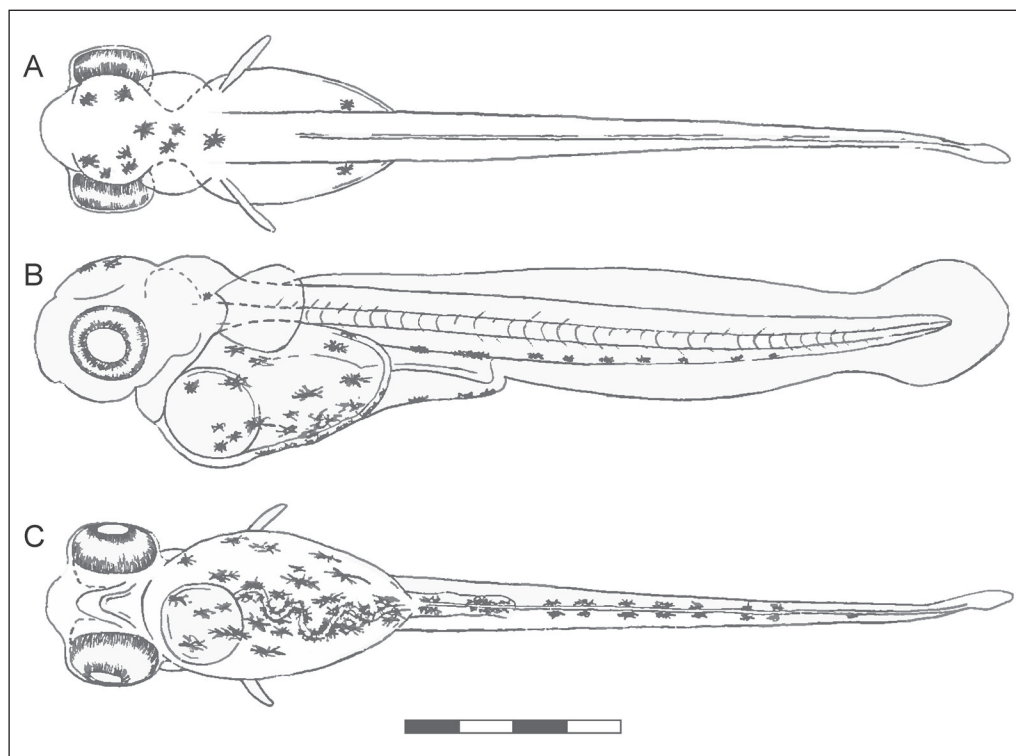


Figure 4. Drawings of (a) dorsal, (b) lateral, and (c) ventral views of a newly hatched Yazoo Darter yolk-sac larvae, about 4.5 mm TL; scale bar = 1 mm; illustration by K.A. Sterling.

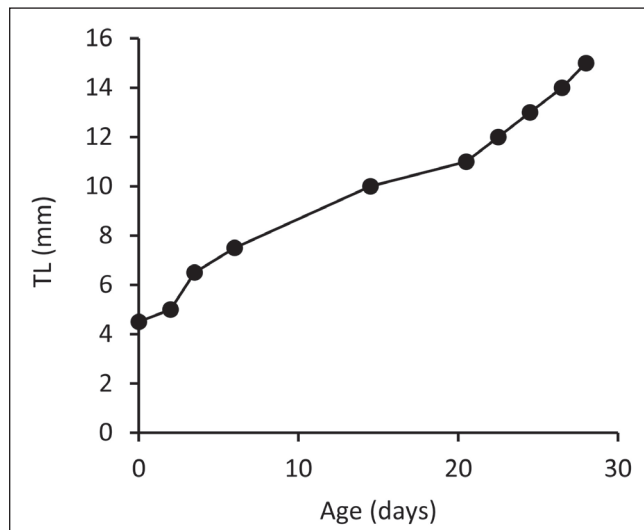
transitioned to benthic juveniles (Supplemental Figure 5 and text, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>). Larvae grew rapidly for the first week. Growth was slower over the next 10–11 days, then became more rapid as the larvae transitioned to benthic juveniles (Fig. 5). By ~15 mm TL, the gut is almost entirely within the body cavity and the overall form of juveniles closely resembles that of the adult. (Supplemental Figure 6 and text, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s18-4-S2525-Sterling-s1>, and for BioOne subscribers, at <https://dx.doi.org/10.1656/S2525.s1>).

Discussion

Life history

Our results are consistent with the documented effects of water temperature on timing and duration of fractional spawning snubnose darters (see Introduction). Because we collected adult Yazoo Darters after spawning had commenced in Hurricane Creek, our study was unable to document the effects of water temperature on the initiation of spawning. However, our results show that the daily number of swim-up larvae that survived for collection peaked as daily high water temperatures approached 20 °C (Fig. 3). This finding is consistent with results for another species of snubnose darter, *Etheostoma rafinesquei* Burr and Page (Kentucky Darter) (Weddle and Burr 1991), as well as research indicating the survival of fertilized eggs and larvae of *Etheostoma lepidum* Baird and Girard (Greenthroat Darter) and *E. spectabile* Aggasiz (Orangethroat Darter) peaks at about 21–22 °C (Hubbs 1961, Hubbs et al. 1969). Spawning in our study ceased when water temperatures consistently exceeded ~21 °C, which is generally congruent with other snubnose darters (Anderson 2009, Carney and Burr 1989, Hubbell 2014, Porterfield 1998, Rakes and Shute 2005, Storey et al. 2006, Suttikus et al. 1994) and similar to darters in general (Hubbs 1985). The abrupt transition from peak production of larvae

Figure 5. Rate of growth for Yazoo Darters from hatching (age 0 days) to about 28 days post-hatching; larvae and juveniles were measured haphazardly and the x-axis shows estimated median age; the y-axis shows the total length (TL) category for larval and juvenile Yazoo Darters.



to a cessation of spawning triggered by warming water temperatures suggests that in streams with greater inputs of ground water, greater canopy cover, and without upstream surface-release impoundments (e.g., epilimnetic releases), the spawning season for closely related snubnose darters may be more protracted than in streams in which water temperatures rise faster during the spring (Hubbs 1985). Production of captive Yazoo Darter larvae could likely be increased by slowing the rate of temperature increase over time or preventing water temperatures from exceeding $\sim 19^{\circ}\text{C}$, but it is uncertain how protracted spawning would be under those conditions (Hubbs 1985, Hubbs and Strawn 1957).

The number of swim-up larvae collected each day abruptly decreased when water temperatures reached $\sim 20\text{--}21^{\circ}\text{C}$ (Fig. 3), but spawning did not cease until the daily high temperature exceeded $\sim 21^{\circ}\text{C}$ for 3 successive days. Spawning decreased for Kentucky Darters held within in-stream cages when water temperatures approached an apparent heat threshold of about 21.5°C but then increased following a return to cooler water temperatures (Weddle and Burr 1991). It is unknown how many days of exceeding the heat threshold would result in complete cessation of spawning for the year. Termination of spawning early in the season, perhaps as a consequence of climate change, would have negative effects on fitness, particularly for a short-lived fish (≤ 3 years; Johnston and Haag 1996) such as the Yazoo Darter, and may have direct impacts on persistence of populations, especially those inhabiting small, isolated tributaries with warm, headwater impoundments (i.e., epilimnetic releases) and reduced canopy cover.

As discussed earlier, production of Yazoo Darter larvae peaked at water temperatures of $\sim 19\text{--}20^{\circ}\text{C}$ (usually occurring by about the beginning of May for Yazoo Darters in streams), which roughly coincides with peak survival of fertilized eggs and larvae in Greenthroat and Orangethroat Darters (Hubbs 1961, Hubbs et al. 1969). However, it is unknown if the peak in larval production was due to increased survival of embryos and newly hatched larvae or an increase in the number of fertilized eggs. One study of the Kentucky Darter showed that the number of eggs spawned and the frequency of spawning increased during the spawning season and peaked shortly before temperatures reached 20°C (Weddle and Burr 1991). However, results from another closely related snubnose darter, *Etheostoma bellator* Suttkus and Bailey (Warrior Darter), indicated that batch fecundity decreases during the spawning season (Khudamrongsawat and Kuhajda 2007), which is consistent with gonadosomatic index (GSI) data from other snubnose darters, which generally peak in the early spring (Barton and Powers 2010, Carney and Burr 1989, Hicks 1990, Johnston and Haag 1996, Khudamrongsawat and Kuhajda 2007, Khudamrongsawat et al. 2005, O'Neil 1981). Though results appear contradictory, they may not be. Even if batch fecundity or GSI values are higher early in the spring, reproductive output may peak later in the spawn if the frequency of spawning, fertilization rates, or survival of embryos increases through time. More data is needed to fully understand fecundity and spawning rates.

Our methods do not allow for a reliable estimate of annual or batch fecundity in Yazoo Darters; however, our estimate of 79–80 swim-up larvae/female over the

course of the study relative to an estimate of batch fecundity (*sensu* Heins and Baker 1993) of 80 ripening ova/female in Yazoo Darters (Johnston and Haag 1996) provides a benchmark for future research on fecundity and survival in captive and natural settings. Other estimates of mean batch fecundity (min–max = 50–109) and 1 estimate of seasonal fecundity (500–1000 ova spawned) for snubnose darters suggest that ova, embryo, and newly hatched larval survival in a captive setting are low (Hartup 2005, Khudamrongsawat and Kuhajda 2007, Khudamrongsawat et al. 2005, Weddle and Burr 1991). Possible non-mutually exclusive factors that may have contributed to suppressed production of Yazoo Darters are cannibalization, hydra infestation, disease, and low fertilization rate. Collection of adults earlier in the year for quarantine purposes and acclimation may have reduced chances of adult mortality, which may have boosted production of larvae.

Information on habitat use by larval snubnose darters is relatively scant, but accounts consistently describe post yolk-sac larval habitat as slack-water areas, usually behind structure, immediately downstream of spawning areas (Simon and Wallus 2006). This finding indicates that larvae do not drift post-hatch for a prolonged period of time during normal flows and actively select for slack-water areas, which is consistent with our observations of swimming ability in Yazoo Darters during this study and also an earlier attempt to hatch Yazoo Darters when we observed a larva that hatched and immediately swam, holding its position against a relatively strong current from a bubbler (K.A. Sterling, unpubl. data). Aquarium observations on swimming ability in other snubnose darters indicate that upon hatching all are pelagic, active swimmers (Simon and Wallus 2006).

The number of larvae swept downstream during spring spates is potentially large, but a relatively fast transition to the benthic juvenile stage may limit the numbers swept downstream. If so, this would have obvious consequences on dispersal and connectivity among demes (Turner 2001, Turner and Trexler 1998). We observed that Yazoo Darter larvae began to use benthic habitat at about 11 mm TL, though they still swam up into the water column to feed, and became predominantly benthic at about ≥ 13 mm TL at ~ 3 –4 weeks after hatch, which is generally consistent with other closely related darters. Eight species of snubnose darter larvae become benthic juveniles at ~ 7 –14 mm TL, though most (5 of 8) transition at ~ 13 –14 mm TL (Simon and Wallus 2006). However, genetic evidence from Yazoo Darters indicates greater population structure at small spatial scales than would seem likely given a 3–4 week period as pelagic larvae (Sterling et al. 2012). Other life-history attributes (e.g., relatively small body and poor swimming ability) that are related to gene flow among darters, as well as abiotic factors such as watershed configuration, may be more influential in structuring demes than larval drift (Fluker et al. 2014, Johnston and Haag 1996, Kral 2010, Turner 2001, Turner and Trexler 1998).

Spawning behavior has been described for 14 closely related snubnose darters, of which 9 were field studies and the remainder aquarium observations. No differences in spawning behavior were noted between field and aquarium observations except that in aquaria, eggs were often deposited on walls, pumps, or filters and

occasionally on gravel (Carney and Burr 1989, Hubbell 2014, Johnston and Haag 1996, Johnston and Shute 1997, Johnston et al. 1999, Porterfield 1998, Storey et al. 2006). Yazoo Darter spawning behavior in our aquaria was consistent with these published descriptions. Eggs were mostly deposited in plant material but were often found scattered on the sand substrate, likely after the eggs had lost adhesion over time and were dislodged from their sites of attachment. Coastal Plain streams inhabited by Yazoo Darters almost entirely lack coarse rock for use as spawning substrate (Sterling and Warren 2017, Warren et al. 2002). Carney and Burr (1989) speculated that 2 other Coastal Plain snubnose darters, *Etheostoma cervus* Powers and Mayden (Chickasaw Darter) and *E. pyrrhogaster* Bailey and Etnier (Firebelly Darter), deposited eggs on wood, a conclusion supported by a life-history study of the Yazoo Darter using aquarium data (Johnston and Haag 1996) as well as unpublished data (K.A. Sterling and M.L. Warren Jr.) from a pilot field project on Yazoo Darters. In our study, wood was not offered as a spawning substrate (to avoid possible complications such as the introduction of unwanted aquatic organisms), and Yazoo Darters relatively infrequently used the rock that was offered. Though an aquarium setting undoubtedly alters behavior to some degree, the frequent use of aquatic vegetation may reflect spawning substrate preferences in natural settings.

Development and captive propagation

Relative to our experience with other darters (Rakes et al. 1999; Ruble et al. 2014, 2016), the Yazoo Darter is easy to propagate in captivity, develops quickly to the benthic juvenile form, and is voracious (C.L. Ruble, pers. observ.). Several modifications to our captive-rearing protocol may increase production of Yazoo Darters. For example, if breeding stock were captured before early January, spawning could possibly be initiated earlier in the season and might be extended by manipulating water temperature. Even so, survival from newly hatched larvae to benthic juveniles was much higher than expected, and our results demonstrate that production of Yazoo Darters in large numbers for conservation management efforts is possible. The protocol and associated life-history data also provides a reliable starting point for the development of species-specific captive propagation techniques for ≥ 17 closely related, imperiled snubnose darters, should the need arise.

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