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Captive Propagation, Reproductive Biology, and Early Life History of the Diamond Darter (Crystallaria cincotta)

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ABSTRACT.—Reproductive biology and early life history data are critical for the conservation and management of rare fishes. During 2008–2012 a captive propagation study was conducted on the Diamond Darter, *Crystallaria cincotta*, a rare species with a single extant population in the lower Elk River, West Virginia. Water temperatures during spawning ranged from 11.1–23.3 C. Females and males spawned with quick vibrations, burying eggs in fine sand in relatively swift clean depositional areas. Egg size was 1.8–1.9 mm, and embryos developed within 7 to 11 d. Diamond Darters were 6.7–7.2 mm total length (TL) at hatch. Larvae ranged from 9.0–11.0 mm TL following a 5–10 d period of yolk sac absorption. Larvae had relatively large mouth gapes and teeth and were provided brine shrimp *Artemia sp., Ceriodaphnia dubia* neonates, marine *Brachionus* rotifers, and powdered foods (50–400 μm) but did not appear to feed in captivity, except for one observation of larval cannibalization. Larvae survived for a maximum of 10 d. To increase larval survival and reduce the possibility of cannibalism, other alternative food sources are needed during captive propagation.

INTRODUCTION

The Diamond Darter, *Crystallaria cincotta,* is a rare and recently described fish of the Ohio River drainage (Welsh and Wood, 2008). Currently, only a single population is known; it inhabits the lower 37 km of the Elk River (an Ohio River tributary), Kanawha County, West Virginia (Welsh *et al.*, 2013). Based on museum specimens, this species previously occurred over a wider range within the Ohio River drainage, but populations are considered extirpated from Kentucky, Ohio, and Tennessee (Welsh *et al.*, 2009). In Nov. 2009, the Diamond Darter was included as a candidate for listing as endangered or threatened (USFWS, 2009). Recently, USFWS (2013) published a final rule in the Federal Register listing the Diamond Darter as endangered under the U.S. Endangered Species Act.

Data on reproduction and early life history of the Diamond Darter are needed for conservation efforts and can be obtained through studies of captive breeding and rearing (Rakes *et al.*, 1999). The reproduction of darters is partly controlled by the synchronization of temperature and photoperiod (Hubbs, 1985; Bonner *et al.*, 1998), and captive propagation studies allow for an increased understanding of how these variables influence reproduction. In addition to environmental controls on reproduction, data on egg size and development and egg viability and survival are critical to understanding reproductive success. Also, information on the development, growth rate, and survival rate of larvae is needed for documenting the early life history of the Diamond Darter.

Reproductive biology is known, in part, for many darter species (Page, 1983; James and Maughan, 1989; Fisher, 1990; Simon *et al.*, 1992; Brandt *et al.*, 1993; Mattingly *et al.*, 2003;

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Simon and Wallus, 2006). However, no previous studies have examined the reproductive biology and early life history of the Diamond Darter. Simon and Wallus (2006) reported the Crystal Darter, a close relative of the Diamond Darter, is an egg burier that spawns from Feb. through Apr. They also noted breeding started typically at temperatures of 12–13 C. Given genetic relatedness, life history traits of the Diamond Darter may be similar to those of the Crystal Darter. However, field and laboratory studies are needed to fully understand the reproductive biology of the Diamond Darter.

Through captive propagation, we examined reproduction and early life history of the Diamond Darter. Our objectives were to document important parameters of reproduction and characteristics of early life history including water temperature range during spawning; egg size and development; egg viability; and the development, growth, and survival of larvae. Objectives were also to document specific methods for breeding as well as specific methods for care of eggs and rearing of larval darters.

METHODS

A total of 17 Diamond Darters were collected from Elk River during four sampling trips: 12 Aug. 2008 (n = 1, female), 8 Oct. 2008 (n = 2, juveniles), 23 Sep. 2009 (n = 2, female and male), and 13–14 Sep. 2011 (n = 12, 8 females and 4 males). Diamond Darters were successfully transported to Conservation Fisheries, Inc. (CFI), Knoxville, Tennessee and acclimated to aquarium conditions in the laboratory. No disease or excessive stress was noted with the Diamond Darters during this time of transition. During winter months we manipulated water temperature and photoperiod which conditioned Diamond Darters for the upcoming spring spawning period. Photoperiod was controlled with artificial lights (twin 40W natural light fluorescent bulbs) and an astronomic timer (Intermatic Next Generation Year Long Double Circuit Electronic Timer). The astronomic timer mimicked natural lighting conditions by slowly decreasing, then increasing day length on a schedule comparable to ambient seasonal changes. Water temperatures, manipulated by using ventilation of outdoor air, were reduced to a low of 2 C to winter-condition the fish. During summer water temperatures were manipulated with either ambient air circulation or air conditioning and maintained below 25 C using a programmable thermostat.

AQUARIUM DESIGN FOR ADULT DIAMOND DARTERS

For captive propagation in 2009 through 2011, Diamond Darters were placed in a 170 liters aquarium, which was part of a larger recirculation system of approximately 900 liters as described by Rakes *et al.* (1999). A mixture of sand and gravel (up to 25 mm diameter) was used as bottom substrate in the aquarium. Filtration included individual tank sponge filters, airstones, and system biotower filters. A circulation pump (Hydor® Koralia 2) maintained a flow rate of 2300 L/h. The recirculating aquaria system was treated with salt (maintained at ~2 ppt) to reduce fish stress and fight parasitic infections.

During Mar. 2012 the breeding set up was modified, in part, to accommodate the larger number of brood fish. Diamond Darters were moved to an 1140 liters opaque plastic oval vat (PRE 328; Behlen Country Poly Stock Tank). A centrifugal water pump, rated at 3500 L/h, generated substantial flow within the oval vat. A reverse-flow undergravel filter was installed within the substrate in ¼ of the bottom of the vat. The undergravel filter provided mechanical and biological filtration, and the reverse-flow system kept the substrate cleaner and less compacted while providing oxygenated water to interstitial spaces. The filter consisted of a grid of perforated PVC pipes (1.3 cm diameter, Schedule 40) placed on the bottom of the vat and covered in gravel and sand. Two underwater video cameras

(VideoSecu® 1/3'' Color CCD Bullet Camera f3.6, enclosed in PVC housing) recorded individual interactions, including territorial behavior of male, and male/female interactions during spawning events.

Aquarium Design for Larvae

For the initial breeding tank design, separate tubs were used for the capture and rearing of larvae. For capturing pelagic darter larvae, an overflow at the back of the breeding tank drained into an oval black plastic tub of approximately 50 liters (~50 × 70 cm with ~15 cm depth). A rearing tub with a slower turnover rate was also set up for swimming larvae. This tub had a 70 cm diameter, a 30 cm depth, and approximately a 100 liters capacity. Water from the recirculation system was piped to the rearing tub, entering as a swift fine stream through a PVC end cap with a 4 mm hole, creating circular flow. The reduced overall turnover rate increased food retention within the rearing tub. Vertical center standpipes, which were topped with 250–500 μm screens, drained both capture and rearing tubs. Airstones at the base of standpipes prevented larval escape or impingement on the screens. Also, in some cases, viable eggs and yolk-sac larvae were placed in a rearing tray (30 × 18 cm) nested in a 76 liters aquarium with a flow through screen in the same system as the adults.

Collection and rearing tubs were modified in 2011 and 2012, respectively. The collection tub was replaced with a shortened 50 liters white plastic trash can, equipped with a central overflow standpipe and a fine mesh (420 μm) screen. The screen prevented larval escape and an air wand around the stand pipe reduced larval drift into the screen. The white color of the collection tub allowed for easier detection of larvae. Larvae were transferred with a baster to a black oval rearing tub (~50 × 70 cm, ~15 cm depth). A dark rearing tub was necessary because phototropic pelagic darter larvae typically do not feed properly in glass or light colored containers. Pelagic larvae see and capture food items more efficiently in dark containers with overhead lighting. The oval shape of the tub helped to maintain a directional flow pattern of the water. To precisely control flow and water exchange within the rearing tub, the PVC end cap with 4 mm hole was replaced with a LOC-LINE® system with in-line valve and 1.6 mm round nozzle tip. This allowed more control over water exchange, which reduced larval impingement on the overflow screen and increased food density within the tub. In order to increase flow without increasing water exchange, a lift tube was placed on the opposite side of the LOC-LINE® inflow. The lift tube consisted of 10 and 5 cm long PVC tubes (6.4-mm diameter) joined with a 45° elbow. An airstone inside the lift tube created a gentle current through the rearing tub. The force of the current was regulated by the amount of air going through the lift tube. The collection tub was monitored daily for larvae from Apr. through Jul. An overhead shop light positioned near the water surface was intended to attract larvae to the breeding tank overflow, thereby promoting passive collection of larvae in the tub. Substrates were also searched and vacuumed periodically with an aquarium cleaning siphon to recover deposited eggs. In 2012 gravel-siphoning events were minimized in an effort to reduce disturbance and thus, to increase larval survival.

Adult and Larval Food

Adult Diamond Darters were fed live blackworms, live *Daphnia,* frozen bloodworms (chironomids), and frozen adult brine shrimp (*Artemia*). Food quantities were determined by water temperature, fish activity levels, and the willingness of fish to feed. For larvae, an automated feeder system was constructed. This feeder system (a reservoir, timer, and solenoid) dispensed food to the rearing tub. The feeding reservoir was an 11.4 liters opaque

plastic tub with a solenoid-controlled bottom spout. The feeding reservoir was filled with water from the system, then with a portion of *Brachionus* rotifers, Nanno 3600™ *Nannochloropsis* sp. (Instant Algae®, Reed Mariculture Inc.), newly hatched brine shrimp nauplii, and *Ceriodaphnia dubia* neonates. The feeder system dispensed food for 8–10 s. every 2 min. Initially, food was provided to larvae during daytime, but in 2012 the automated feeder was modified to also provide food at night, given a possibility that larvae were nocturnal foragers. To supplement automated feeding, several powders were lightly dusted on top of the rearing tub several times daily. The powders were all in equal parts from a premixed batch: A.P.R. (Artificial Plankton – Rotifer, O.S.I.), Larval AP 100 (<100 μm and 100–150 μm, Zeigler Bros., Inc.), and Spirulina (Salt Creek, Inc.). During the latter part of the 2012 spawning season, larvae of Sawfin Shiner (*Notropis sp.*) and Redline Darter (*Etheostoma rufilineatum*) were added to rearing tubs, because Diamond Darter larvae were suspected to be obligatory larvae eaters. Also, given the possibility that larval feeding might improve in green water conditions, Instant Algae® was added directly to larval rearing tubs in 2012.

Data Collection

For each year the duration and water temperatures of the spawning period were recorded. The spawning period was defined by the first and last appearance of eggs. Prior to spawning, behaviors of females and males were documented, including sand burying, male territoriality, and courtship. Short movie clips of some spawning events were recorded to document reproductive behaviors. The total numbers of fertile and infertile eggs were counted and the number of eggs per female calculated. Gravel siphoned egg and larvae counts were recorded separately. If larvae were observed without previously seeing the eggs, then those individuals were included in the egg count. Diameters were measured for subsamples of eggs and embryos given that handling of the eggs and larvae could result in losses. Lengths were measured via photos taken by a Canon Rebel® SLR camera with a Canon microscope lens mount that was fitted on a Nikon dissecting scope. A section of clear ruler was used to take measurements for subsamples of eggs, yolk-sac larvae, and developing larvae. Myomeres were also counted using these photos.

Results

Diamond Darter behavior during fall/winter and reproduction during spring was examined during three time periods (fall 2009–spring 2010, fall 2010–spring 2011, and fall 2011–spring 2012). Spawning during an earlier time period (fall 2008–spring 2009) was unsuccessful because the spawning group consisted of an adult female (~97 mm total length, TL) and two smaller (55–65 mm TL) individuals, which were sexually-immature or female. Spawning group sizes (individual lengths not measured) for the other three time periods consisted of two females and three males, two females and three males, and nine females and five males, respectively.

During fall/winter Diamond Darters were often buried in the sand during the day and active at night. As temperatures decreased below 15 C, Diamond Darter activity decreased and individuals were often buried in the sand. Low temperatures of about 2 C were obtained during the wintering conditioning period. Diamond Darters time buried declined as water temperatures increased above 15 C; once water temperatures exceeded 21 C, darters were consistently above the sand substrate during day and night. Adult females became noticeably gravid as early as Mar., and eggs or larvae were observed from Apr. to mid-Aug.

Table 1.—Parameters associated with captive spawning of the Diamond Darter during three spawning seasons

Spawning parameter	Spawning season		
	2010	2011	2012
Sex ratio (Female:Male)	2F:3M	2F:3M	9F:5M
Spawning duration	31 Mar.–21 May	1–29 Apr.	13 Mar.–1 Jul.
Spawning days	53	28	111
Water temperature (C), mean ± SE	19.1 ± 0.21	17.7 ± 0.28	19.5 ± 0.15
Water temperature (C), range	14.9–21.2	14.7–20.1	15.9–23.3
Ratio of eggs/female	178/2	15/2	179/9

In 2010–2011, males persistently solicited females in the smaller breeding tank, but unreceptive females simply ignored males. In contrast, male territoriality was observed in the larger vat during the 2012 spawning season, where a dominant territorial male chased both males and unreceptive females. Females influenced the site deposition of eggs during 2010–2011, but a dominant territorial male solicited females during 2012 and chased away other individuals, except for receptive females. Receptive females would swim into the territory and accept the male's vibration solicitation for spawning. During courtship males repeatedly swam directly in front of the female. Sometimes the male would position perpendicularly in front of the female and wag his tail, while at other times just quiver, then circle around and swim above her, almost resting on her back. The male would also approach beside the female and vibrate. Females would either swim away, or vibrate with the male, descending into the substrate. During this process the posterior 1/3–1/2 of the female's body descended into the sand and the male would be positioned along her side, tilted almost at a 45° angle with tail down and head in an upward position. Vibrations and burial into the sand usually coincided with a wide gape of the female's mouth which appeared to be associated with opercular/buccal flaring. Total spawn time was 7–8 s. (CFI, 2010a, b).

Spawning period duration, egg production, and temperature at spawning cessation differed among the three study periods, but water temperature at spawning initiation was similar among periods. Spawning periods lasted for 53 d (31 Mar.–21 May 2010), 29 d (1–29 Apr. 2011), and 111 d (13 Mar.–1 Jul. 2012, Table 1). Water temperatures during the three periods (mean ± se, C) averaged 19.1 ± 0.21 (range 14.9 to 21.2 C), 17.7 ± 0.28 (range 14.7–20.1 C), and 19.5 ± 0.15 (range 15.9–23.3 C), respectively (Table 1, Fig 1). For the spawning periods of 2010–2012, the ratios of eggs/female were 178 eggs per 2 females, 15 eggs per 2 females, and 179 eggs per 9 females, respectively (Table 1). On several occasions adult Diamond Darters were observed eating eggs, but the condition of these eggs (fertile vs. infertile) was unknown. Well-developed fertile eggs measured 1.9–2.0 mm (Figs. 2A, B). Each embryo had a clear chorion and a lightly tinted yellow to nearly clear yolk sac with a moderate amount of melanophores present. Oil droplets on embryos were large with a dark yellow coloration.

The estimated hatch time of eggs was 7–9 d. Based on observed numbers of eggs and larvae, hatch rates were 15.8% (164 eggs, 26 larvae) and 33.3% (15 eggs, 5 larvae) for the 2010 and 2011 spawning periods, respectively. A total of 179 eggs were observed during the 2012 spawning period, but hatch rate was not estimated because gravel-siphoning of eggs was minimized in an effort to increase egg survival. During all spawning periods, many eggs were infertile or displayed bacterial or fungal infection. Also, fecundity decreased during

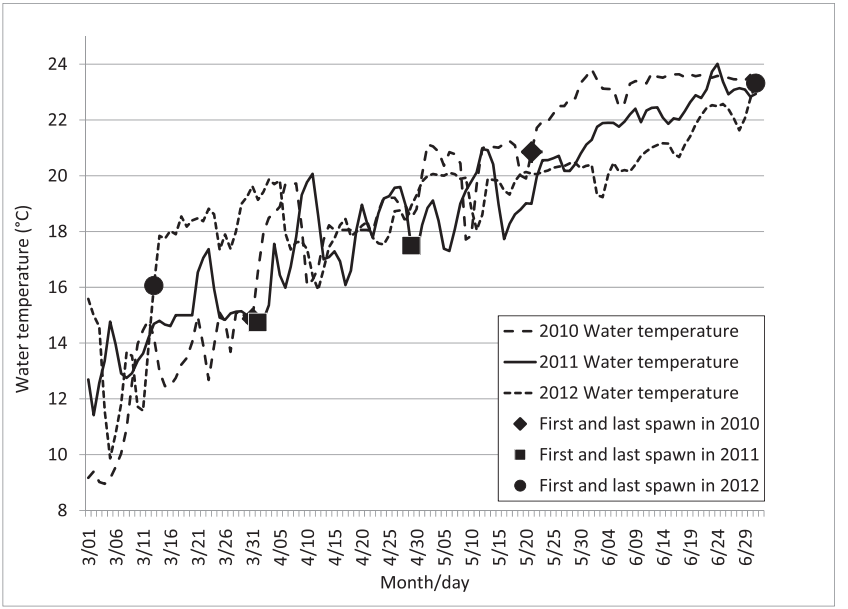

FIG. 1.—Aquarium water temperatures for spring 2010, 2011, and 2012 including dates of the first and last spawning events of captive Diamond Darters

the spawning season. Females continued to deposit eggs as the spawning season progressed, but fewer of these eggs were fertile relative to those from earlier in the spawning period. Even when fertile eggs were recovered later in the season, many succumbed to fungal or bacterial infections. Efforts to reduce the organic load were implemented, including discontinued use of rotifers and instant algae, given that larvae were not consuming these food items. Poly-filter (PolyBioMarine Inc.®) pads were added on top of the biotower for removal of excessive organic compounds that might fuel harmful bacteria in the system. Neither alteration seemed to help in increasing fertility or survivorship of eggs. Reverse flow filtration appeared to increase egg survival in 2012, but the magnitude of increase was not measured because we relied on passive collection of the larvae instead of gravel siphoning of eggs in an effort to reduce disturbance.

Larvae were 6.7–7.2 mm TL with heavy yolk sacs (Figs. 2C, D). Larvae had an initial swim-up period of several minutes to possibly an hour, but then settled to the bottom for another day before maintaining a steady position in the water column. These early swim-up larvae did not maintain a particular position in the water column and were positively phototaxic. They would occasionally swim-up when disturbed. Yolk-sac larvae began swimming consistently in 1–2 d, and changed from being positively to negatively phototaxic (*i.e.*, photophobic). Larvae also paced the side of the rearing tub, but this behavior was reduced in 2012 after adding Instant Algae®. With green water conditions, larvae were often positioned in the water column away from the side of the rearing tub.

Pigmentation of larvae after hatch consisted of melanophores concentrated on the ventral surface of the yolk sac, as well as posterior to the anus both on ventral and dorsal areas along the myomeres. Protolarvae had 22–23 post anal myomeres and 23–24 preanal

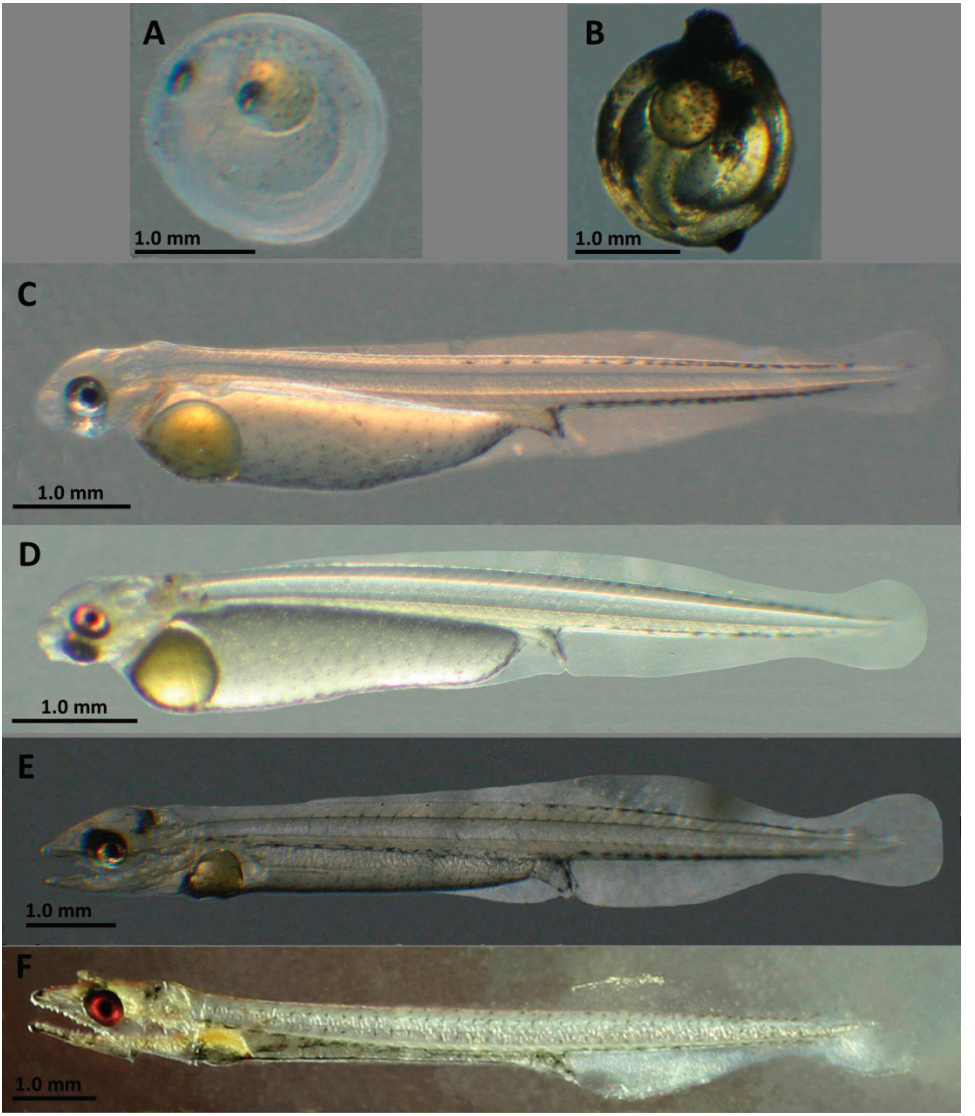

Fig. 2.—Photographs of eggs and larvae of the Diamond Darter: (A) eyed egg, (B) well-developed egg, (C) 1 d old larva with heavy yolk sac, (D) 2 d old larva, (E) 5 d old larvae, (F) 10 d old larva with large gape size and large teeth

myomeres. Larvae estimated to be 5 d post hatch were 9.7–10.0 mm TL, had a large yolk sac, and displayed pigment along myomere edges (Fig. 2E). Head and mouth morphology were developed; the snout was elongated and gape size was almost 1 mm (Fig. 2E). A 10 d old larva, the oldest larva observed during our study, measured 10.8 mm TL and had a gape length of over 1 mm, with a total lower jaw length of 1.4 mm, large teeth, and little to no remaining yolk-sac (Figs. 2F, 3).

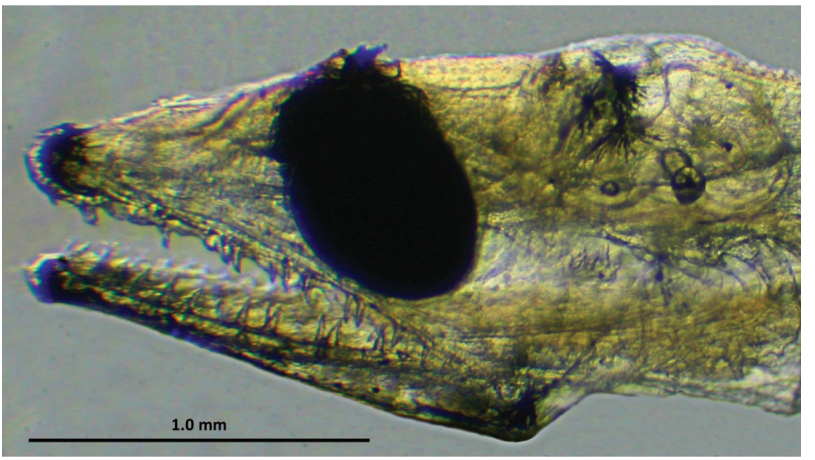

FIG. 3.—Large gape size and teeth of a 10 d old Diamond Darter larva photographed during a captive propagation study

Larvae did not survive *(i.e.*, 100% mortality) and did not appear to eat the provided food items. Diamond Darter larvae darted frequently and purposefully in and out of flow in the rearing tub, which may indicate flow avoidance (or preference) or prey-seeking behavior. However, larvae did not eat *Artemia sp.* or *Ceriodaphnia dubia.* Larvae of other darter species typically have an orange or dark gut after feeding on these two food items, but Diamond Darter larvae always had clear guts. The large teeth of 10 d old larvae suggest the possibility for predation on other smaller fish larvae. In 2012 a larva was observed cannibalizing a smaller larva. During 2012 larvae of Sawfin Shiners and Redline Darters (5–7 mm TL) were provided as forage, but Diamond Darter larvae did not feed on this alternative food source.

DISCUSSION

This captive-propagation research represents the only study of reproduction and early life history of the Diamond Darter. Spawning behavior, including male territoriality, was observed and documented. Spawning periods of Diamond Darters and associated water temperatures differed among study periods. Eggs and larvae were successfully propagated, although production differed among study periods. Larvae did not survive beyond 10 d. The large gape width and large teeth of larvae were important findings, which suggest that larvae may be obligatory piscivores. Larval mortality appeared to result primarily from starvation. Diamond Darter larvae did not feed on invertebrates, but we observed one case of a larva cannibalizing a younger larva.

Territorial aggression in male Diamond Darters occurred only during the breeding season. Male aggression is common in darters (Page, 1983). Aggressive and territorial behaviors of Diamond Darter males occurred in the larger breeding tank but were not exhibited in the smaller breeding tank. Possibly, the larger tank provided necessary space for territorial nesting behavior, which in turn, promoted aggressive behavior. If a male is guarding a territory, then it is more likely that there will be aggressive postures and pecks in order to assure that only receptive females enter into his preferred spawning habitat.

Differences in spawning period duration among study periods may have several explanations. First, the longer spawning period of 2012 (111 d) was associated with higher

temperatures during early spring and lower temperature during late spring relative to those of 2010 (53 d) and 2011 (28 d). Water temperatures at the start and end of the 2012 spawning period (15.9–23.3 C), however, were higher than those of 2010 (14.9–21.2 C) and 2011 (14.7–20.1 C). Individual variation and ages within the larger breeding group of 2012 may explain differences in spawning period duration. Also, younger females were possibly maturing into condition at later dates, lengthening the overall observed spawning time.

Differences in egg and larvae production among study periods may have resulted from condition of adult breeders, methods of egg collection, an increase in the size of the breeding tank, and associated increase in male territoriality, or egg and larvae cannibalism. Two females produced 89 eggs each during the 53 d spawning period of 2010, but spawning success was reduced the following year where only one of these females produced eggs (n = 15). Reduced spawning success possibly resulted from advanced age or poor condition of females. Adults were slightly emaciated during winter months prior to spring 2011 even though food quantities provided were similar to the previous more productive year. Spawning success was also relatively low in 2012, where nine females produced a total of 179 eggs. The lower egg count may reflect the reduced frequency of vacuuming to recover infertile eggs as compared to 2010, but it might also be explained by the intense territoriality of the dominant male. Possibly, only a few of the females were contributing to the total egg counts. Also, on several occasions during our study, adult Diamond Darters were observed eating eggs. We do not know if these were infertile eggs or eggs that were uncovered during spawning events. It also was not clear if the adult darters were rooting for the eggs. Cannibalism of eggs likely reduced egg counts and likely reduced production of Diamond Darters during captive propagation. The swim up period of early larvae also is a critical period when larvae could be cannibalized by the adult breeders. It is possible that a large proportion of larvae in the larger vat were cannibalized just after swim up and before being passively collected in the overflow.

Several interesting findings concerning Diamond Darter larvae involved yolk-sac persistence, swimming behavior, and reaction to light. Larvae had a very heavy yolk sac and many were collected via gravel siphoning, suggesting that they were not yet swimming. Also, larvae tended to swim up early following hatch then settled down for another day before maintaining a steady position in the water column. This may be a dispersal strategy — avoiding high predation areas immediately after hatching and settling into safer benthic habitat before swimming up to feed. Early hatching Diamond Darter larvae moved towards light for a few days after swim up dispersal but later became photophobic and were observed pacing along the side of the rearing tub. After addition of green water in 2012, larvae were often positioned in the water column near the water inflow and away from the side of the rearing tub. This might have been a visual, or chemical-induced, change in orientation, and possibly the limited sight range in green water improved focus on prey items.

Other interesting results were the discoveries of large gape size and large teeth of Diamond Darter larvae (Fig. 3). Large teeth are not present in adult Diamond Darters; hence, teeth are either embedded within tissue or lost during ontogeny. Ontogenetic tooth loss occurs in other fishes, including members of elopiformes and anguilliformes (Fahay, 1983), acipenseriformes (Nelson, 2006), and siliformes (Huysseune and Sire, 1997; Kakizawa and Meenakarn, 2003). To our knowledge larval teeth have been reported for only a few species of darters. Simon and Wallace (2006) noted small teeth in *Etheostoma blennioides pholidotum* and *Percina caprodes*. Larval teeth were also noted in *Etheostoma variatum*, *Etheostoma cf., zonale* ''Jade Darter'', *Percina macrolepida*, *Percina rex*, and *Percina austoperca* (T. P. Simon, pers. comm.). These larval teeth, however, are not as large as those

documented on the Diamond Darter. Simon and Wallace (2006) did not report the presence of teeth in larval Crystal Darters. Further study of larval teeth in the Diamond Darter is warranted to contribute to an area of interest in evolutionary biology (Stock, 2001; Davit-Béal *et al.*, 2009; Huysseune *et al.*, 2009).

Survival of Diamond Darter larvae was limited by starvation and cannibalism and possibly by nutrition and condition of the adult breeders. Larvae did not eat *Artemia sp.* or *Ceriodaphnia dubia*, although these food items, which were successfully consumed by larvae of other darter species at CFI, were within size limits of the large-gaped Diamond Darter larvae. The large teeth of 10 d old larvae suggest the possibility for predation on other smaller fish larvae. Given that a larva was observed cannibalizing a smaller larva, it is possible that older larvae commonly cannibalized newly hatched larvae, which may explain why only older larvae were found in the rearing tub after several days. However, Diamond Darter larvae did not feed on larvae of Sawfin Shiners and Redline Darters. Condition of the adult breeders may have contributed to larval mortality. Adult Diamond Darters were fed frozen bloodworms and brine shrimp during spring but were only provided with live blackworms during early acclimation and winter temperatures in order to reduce uneaten food waste and maintain water quality. Because food storage for reproduction occurs in the fall, a more diverse diet during this time may influence early oocyte development.

If Diamond Darter larvae are cannibalistic and obligate larval piscivores, then this presents considerable difficulty for future captive rearing efforts. Providing Diamond Darter larvae with larval fish prey will require propagating a second species that breeds at the same time to insure a steady supply of smaller larvae. This may also preclude housing Diamond Darter larvae together that are more than a few days apart in age. To reduce the risk of cannibalism, a larger number of rearing tubs may be needed for successful propagation of larvae. Age classes of larvae may require segregation every few days to prevent larval cannibalism.

Captive propagation of Diamond Darters was hindered by low survival rates of eggs and early larvae. Water temperature, dissolved oxygen, siltation, and general water quality parameters typically affect egg survival. Eggs succumb to secondary infections when weakened by these stressors or when development is negatively affected by poor physiological condition of the parent. Microbial populations are typically high in low oxygen, silted, or organic substrates, leading to increased likelihood of bacterial and fungal infections. Survival of larvae is influenced by the type, variety, and size of food items. Excessive food accumulation can encourage bacterial growth that then infect sensitive early life stages. Disease did not appear to affect survival of Diamond Darter larvae, but egg survival was low due to bacterial and/or fungal infection(s).

Low dissolved oxygen levels in aquarium substrates likely impact captive propagation of egg-burying darters. In natural habitats egg-burying darters often spawn in clean sand and gravel substrates with high flow and interstitial water movement. Inadequate water flow or compaction of interstitial spaces in aquarium substrates may result in anoxic conditions, causing eggs to die either directly or through secondary infections. Low oxygen levels in the substrate could also lead to poor egg development and poor larval condition. In addition to an oxygen issue, a lack of interstitial flow could also influence sperm mobility and fertilization. Higher temperatures were associated with reduced egg survivorship — no eggs developed over 20 C even when spawning activity continued. This could result directly from decreasing dissolved oxygen in the substrate as temperatures increase. As a possible solution, we designed and implemented an undergravel reverse-flow system in 2012 for supplying fresh, oxygenated water within the substrate. The reverse-flow system improved larval swim up and will be used in future efforts.

In conclusion this study successfully produced eggs and larvae of the Diamond Darter. Several important parameters of reproductive biology and early life history were documented including hatch time, egg size and development rate, and the early development and growth rate of larvae. Also, specific methods for breeding, as well as specific methods for care of eggs and rearing of larval darters were developed, problem areas identified, and potential solutions devised. Possibly, improved fall and winter diets will increase egg production by female Diamond Darters and increased interstitial water movement will enhance egg survivorship during in situ incubation. It is suspected that poor larval survivorship resulted from a lack of appropriate diet items. The large teeth and gape size suggests that Diamond Darter larvae are predators of larger prey, and future efforts should examine alternative prey items for larvae. Also, segregation of Diamond Darter larvae may increase survival. Information from this study aids protocol development for captive propagation of Diamond Darters and other darter species and provides ecological information for conservation and management efforts.

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