

Captive propagation, reproductive biology, and early life history of *Crystallaria cincotta* (Diamond Darter), *Etheostoma wapiti* (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter)

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Abstract

Captive propagation, reproductive biology, and early life history of *Crystallaria cincotta* (Diamond Darter), *Etheostoma wapiti* (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter)

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Reproductive biology and early life history data are important for understanding the ecology of fishes, and can be used for the management and conservation of rare species. During 2008–2012, a captive propagation study was conducted on *Crystallaria cincotta* (Diamond Darter), a rare species with a single extant population in the lower Elk River, WV. Also, captive propagation studies were conducted during 2008 on three species of darters of the subgenus *Nothonotus*: *Etheostoma wapiti* (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter). Water temperatures during Diamond Darter spawning ranged from 11.1–23.3°C. Females and males spawned with quick vibrations to bury eggs in fine sand in relatively swift clean depositional areas. Egg size was 1.8–1.9 mm, and embryos developed within 7–11 days. Diamond Darters were 6.7–7.2 mm TL at hatch. Larvae ranged from 9.0–11.0 mm TL following a 5–10 day period of yolk sac absorption. Larvae were provided *Artemia* nauplii, *Ceriodaphnia dubia* neonates, *Brachionus* rotifers, and powdered foods (50–400 microns). Diamond Darter larvae did not feed in captivity, except for cannibalizing other larvae. Larvae survived for a maximum of 11 days. Larvae had relatively large gapes and teeth, suggesting possible larval piscivory and a need for alternative food sources during captive propagation. During the *Nothonotus* study, the length of spawning period and associated range of water temperatures for the Wounded Darter (89 days, 16.0–24.0°C) exceeded those of the Spotted Darter (46 days, 17.0–22.5°C) and Boulder Darter (48 days, 17.0–22.5°C). Eggs produced per female were least in the Boulder Darter (163), intermediate in the Spotted Darter (191), and highest in the Wounded Darter (345). Diameters of eggs at deposition and prior to hatch were least for Spotted Darter (1.9–2.0 mm, and 1.9–2.1 mm), intermediate for Wounded Darter (2.1–2.2 mm, 2.4–2.5 mm), and largest for Boulder Darter (2.2–2.4 mm, 2.4–2.6 mm). Lengths of larvae at hatch and at the start of first fin development varied in a similar pattern: Boulder Darter (8.5–9.1, 14.0–15.0), Wounded Darter (7.7–8.0, 13.5–14.0), and Spotted Darter (6.5–7.0, 12.0–13.0 mm). Overall production per female was lowest in Boulder Darters (61 juveniles/ female), intermediate in Spotted Darters (86 juveniles/ female), and highest in Wounded Darters (90 juveniles/ female).

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Chapter 1. Literature Review

Darters of the Family Percidae represent a diverse group of fishes including four genera (*Percina*, *Etheostoma*, *Ammocrypta*, and *Crystallaria*; Simons 1991), over 25 subgenera (Page 1983, Boschung and Mayden 2004), and approximately 198 species (Page and Burr 2011). Darters are native to North America with most species occurring in the southeast United States (Page 1983). Many species of darters have small or disjunct distribution ranges owing to endemism or habitat loss and fragmentation. Consequently, darters are often species of conservation concern. For many darter species, a lack of life history information limits the effectiveness of conservation efforts (Rakes et al. 1999).

Captive propagation studies of darters provide valuable information for species conservation and management. The reproduction of darters is partly controlled by the synchronization of temperature and photoperiod (Hubbs 1985), and captive propagation studies allow for an increased understanding of how these variables influence reproduction. For example, captive propagation has benefited conservation efforts for the federally endangered *Etheostoma wapiti* (Petty et al. 2012). The Boulder Darter occurs downstream of Tims Ford Dam, Elk River, TN. Tailwater releases from this hydroelectric dam cause drastically low, seasonally abnormal water temperatures as well as widely ranging flows and depths (Burkhead 1992). Data from captive studies on life history and water temperature preference of the Boulder Darter have assisted managers with balancing power generation and water releases relative to times of Boulder Darter spawning and larval development (Ferry 2008).

Darters employ a wide range of reproductive strategies from primitive egg attachers and buriers to the more derived egg clumpers, egg clusterers, and nest guards (Page 1985). Reproduction and early life history of many species of darters, however, are poorly understood.

Important reproduction and early life history parameters include the following: 1) male courting and behavior, 2) female reception, 3) the mode of egg deposition, 4) spawning period and temperature ranges during this time, 5) number of clutches and eggs per clutch; egg produced per female, 6) larval survivorship, and 7) development time. An understanding of life history parameters, such as clutch size, number of clutches, egg size, and egg survival are critical to the understanding of reproductive success. Also, an understanding of life history parameters, such as hatch time, larval size, and larval survival, are critical for the understanding of the early life history of fishes.

This thesis focuses on the reproductive biology and early life history of four species of darters: *Crystallaria cincotta* (Diamond Darter), *Etheostoma wapiti* (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter). The thesis is organized as (1) a literature review chapter, (2) a manuscript on the reproductive biology and early life history of the Diamond Darter, and (3) a manuscript on the reproductive biology and early life history of three darters of the subgenus *Nothonotus*: Boulder Darter, Wounded Darter, and Spotted Darter. The following review summarizes information on reproductive biology, early life history, captive propagation, and conservation concerns of these four species.

Diamond Darter

The Diamond Darter, described as a new species in January 2008, was previously recognized as *Crystallaria asprella* (Crystal Darter; Welsh and Wood 2008). Currently, only a single population of the Diamond Darter is known from the lower 37 km of the Elk River, Kanawha County, WV. Based on museum specimens, this species occurred previously over a wider range within the Ohio River drainage, but populations are considered extirpated from Kentucky, Ohio, and Tennessee (Welsh et al. 2009). In November 2009, the Diamond Darter

was included as a candidate for listing as endangered or threatened (USFWS 2009). Recently, USFWS (2012) published a rule in the Federal Register proposing the Diamond Darter for listing as endangered with critical habitat under the U.S. Endangered Species Act.

Reproductive biology and early life history have not been documented for the Diamond Darter. However, reproductive biology is known, in part, for the Crystal Darter (George et al. 1996, Simon et al. 1992, Simon and Wallus 2006). George et al. (1996) reported a reproductive season from late January through mid-April for the Crystal Darter in the Saline River, Arkansas. Clutch sizes were positively correlated with fish length, ranging from 106–576 mature oocytes per female (George et al. 1996). Mature or ripening oocyte diameters ranged from 1.0–1.2 mm (George et al. 1996). Simon and Wallus (2006) reported that the Crystal Darter is an egg burier that spawns from February through April. They also noted that 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.

Boulder, Wounded, and Spotted Darters

Darters in the subgenus *Nothonotus* are small (35–65 mm TL) laterally compressed fish occupying primarily swift riffles in medium to large streams and rivers. *Nothonotus* classification includes: complete lateral line, complete head canals, high scale and fin ray counts, separate gill membranes, six branchiostegal rays, dorsal saddles nine or more or not apparent, and frenum well developed. Sexual dimorphism is typical within the clade and eggs are attached beneath rocks or buried in gravel (Zorach and Raney 1967; Etnier and Williams 1989). *Nothonotus* comprises 21 species, including three species within the *Etheostoma maculatum* species group that are concerned in this study: *E. wapiti*, *E. vulneratum*, and *E. maculatum*

(Etnier and Starnes 1993, Near and Keck 2005). Many darters within this species group are of conservation concern because of small or fragmented distribution ranges. The *E. maculatum* species group is unified, in part, by a unique reproductive mode (Etnier and Williams 1989); spawning females clump eggs in a male guarded nest (Page 1983). Another unifying feature of the *E. maculatum* species group is all of the members have a few scales associated with the post orbital spot on the upper cheek. Typically, the male members of this species group select a rock or slab that is perched at an angle on top of another rock or slab, usually found within a riffle or at the head of a riffle. The wedge that is formed between these two rocks is the site of egg deposition by females. Like many other nest guarding species, multiple females contribute eggs to a single nest (Constantz 1985, Etnier and Starnes 1993, Layman 1991). Although the general reproductive strategies are known, little information is available for many life history parameters such as spawning time of year, temperatures associated with spawning time, nest size, and female contribution.

From an ecological perspective, the *Nothonotus* research of this thesis compares life history parameters among three species that differ in having northern and southern distribution ranges, small to relatively wide distribution ranges, and small to large population abundances. The Spotted Darter has the most widespread range with isolated populations within five states. The Wounded Darter is only found in the Tennessee River drainage, and spans only two states, however it is locally abundant within its range. The Boulder Darter has the most limited range of the three species. Extant populations are found only in the Elk River, TN, and two tributaries running into the Elk River: Richland Creek and Indian Creek (Etnier and Williams 1989, Etnier and Starnes 1993). Observations and among-species comparisons of life history will aid conservation and management efforts for these three species.

Boulder Darter

Captive spawning studies of the Boulder Darter were conducted in 1995 (Rakes et al. 1999). The Boulder Darter was once thought to be restricted in small fragmented sections of Elk River, TN, and two tributaries to Elk River, Indian Creek and Richland Creek (Etnier and Williams 1989, Burkhead and Williams 1992). Subsequently, two specimens were found in an 1884 specimen collection from Shoal Creek, Florence, Lauderdale County, AL, which verified its presence in Shoal Creek during a time prior to major pollution and siltation (Etnier and Williams 1989). In 1988, Richard Biggins of the U.S. Fish and Wildlife Service fought to list the Boulder Darter as an endangered species. Subsequently water regulations in Lawrenceburg have improved the water quality in Shoal Creek, providing suitable conditions for the Boulder Darter (Biggins 1988). Biggins (1989) proposed a recovery plan for the Boulder Darter, suggesting the use of captive propagated Boulder Darters as a source for stocking extreme reaches of the Elk River, and possibly Shoal Creek. In 1994, Conservation Fisheries Inc. (CFI) used *E. sanguifluum* (Bloodfin Darter) as a surrogate species to develop captive propagation protocols for Boulder Darters. After developing propagation protocols for Bloodfin Darters, a small group of Boulder Darters were collected during fall 1994 from Elk River, TN, and successfully spawned in 1995 and again in 1996 (Rakes et al. 1999). From 1997–2005, CFI released 2263 Boulder Darters in several sites in the Elk River to help recover the population (Rakes and Shute 2005). In 2006, CFI started recovery efforts in Shoal Creek, TN. This creek was selected because of the historic records of Boulder Darters (Etnier and Williams 1989). During 2005–2012, CFI released 2896 Boulder Darters into Shoal Creek (Petty et al. 2012).

Since the early stages of developing propagation protocols for the Boulder Darter, CFI has continued to learn and modify techniques. Conservation Fisheries, Inc. has propagated the Boulder Darter for reintroductions over a span of 18 years (1995–2012) and continues to work

with the species in Shoal Creek, TN and AL. Conservation Fisheries, Inc. reported that the initiation of spawning correlated with temperatures reaching an average of 15–18°C while the cessation of spawning correlated with temperatures consistently staying over 23°C. Typically nests are first collected the second week of April through the end of June, a 6-week span of time. Two exceptions have occurred over the years that have yielded longer breeding seasons for the Boulder Darters. In 2009 and 2012 there was a warmer than normal late winter/early spring conditions and for both of these years spawning started the first of April. In correlation to this early warm up, the 2009 spawning season extended for a total of 9 weeks. With additions in environmental controls installed over the past 3 years, CFI was able to maintain lower temperatures through the late spring and summer. These lower temperature regimes during the late spring and summer 2012 likely explain an extended Boulder Darter spawning period through mid-July, yielding a 10 week spawning season (Petty et al. 2012).

Wounded Darter

The Wounded Darter is established in the upper Tennessee River downstream to Whites Creek and Little Tennessee River, Clinch River, and Little River. In Tennessee the Wounded Darter has no state-level protection status, but in North Carolina the Wounded Darter is listed as a species of concern. The range of this species is also restricted and impeded by impoundments throughout the Tennessee River drainage (Etnier and Starnes 1993). Data on the reproductive ecology and early life history of this species are needed, particularly because conservation efforts for this species are currently underway. The TAPOCO Project Relicensing Settlement Agreement (RSA), filed with FERC in May 2004, established two funds, the Tallassee Fund and the North Carolina Resource Management and Enhancement Fund (now the Cheoah Fund) to support restoration, recovery, and conservation efforts in Tennessee and North Carolina,

respectively. In accordance with the RSA, for the next 40 years seasonal and base flow regimens are being regulated in the Cheoah River, along with gravel augmentations and efforts to restore indigenous fish and other fauna that were extirpated when the river was bypassed by a hydroelectric diversion flume. Conservation Fisheries, Inc. has been contracted to develop captive propagation techniques for several species that would be too difficult to translocate in significant numbers. The Wounded Darter is among these species being propagated at CFI and released into the Cheoah River. For propagation studies, 12 Wounded Darters were collected from Little Tennessee River, North Carolina, on 1 May 2007 from the bend below Wiggins Branch. On 13 August 2007 two additional males were collected from near the mouth of Tellico Creek in Little Tennessee River, and another male and female were collected from above Dean Island (Petty et al. 2011).

Simons and Wallace (2006) reported on 16 egg masses of Wounded Darters that were collected from the Little River, TN. These egg masses averaged 48 eggs per nest, with as few as 17 and as many as 166 eggs. Eggs varied in development within each nest suggesting that each nest is comprised of several spawning events. Simon and Wallace (2006) also reported that most Wounded Darters took two years to come to full sexual maturity. Simon and Wallace (2006) confirmed that the Wounded Darter is a nest egg clumper with a male guarding the nest under wedged rocks in moderate current areas at heads of riffles. Males defend spawning territories for several weeks. The female enters the male territory, orients in a sideways or upside down position, and deposits eggs, while the male fertilizes the eggs. Simons and Wallace (2006) described the eggs as demersal, adhesive, and spherical. The eggs averaged 3.0 mm in diameter, and had a pale yellow yolk, an oil globule, a narrow perivitelline space and an unpigmented chorion. Eggs incubated at 24°C hatched in 7 days. Larvae ranged from 8.5–9.3 mm TL at hatch.

Stiles (1972) studied the Wounded Darter in Little River, TN under its subspecies name *Etheostoma maculatum vulneratum*. Stiles (1972) reported the Wounded Darter as typically using habitat at the head or tails of riffles, primarily in transitional zones with intermediate water velocity and clean swept substrate that typically lacked vegetation except in the swiftest part of the riffle. Substrates in these areas were typically imbricated cobbles and boulders (8 inches or greater in diameter) with fines interspersed. Young sub-adult Wounded Darters were typically found in slower current areas than the adults.

Stiles (1972) observed territorial behavior in the Wounded Darters in Little River, TN. Stiles (1972) described these territories as areas with a radius of up to 30 cm. Territorial males escorted other darters out of the preferred habitat, displayed dominance by raising dorsal fins and posturing perpendicularly to the intruding fish, and sometimes chased or bit at other darters within the territory. Territorial behavior was observed for both sexes, but was greatly increased in the males during the spawning season. From fall 1970 through summer 1971, a tagging study by Stiles (1972) did not document the movement of Wounded Darters out of their home range (head of riffle or tail of riffle). Possibly, most movements of this species involve larval dispersal through drift and habitat shifts as the juveniles mature.

Stiles (1972) documented spawning starting mid-May or June and lasting through July (8–10 weeks). Water temperatures during this time were 16–20°C. Stiles also reported that the Wounded Darter laid eggs in a wedge-like formation between two stacked rocks. These eggs were not laid in a single layer, but instead were laid over top of each other. Several nests were investigated and it was determined that at least two of the nests contained eggs of two different ages, suggesting multiple females contributed to the nest. Stiles (1972) witnessed increased spawning activity in the morning hours from dawn through noon. Actual spawning acts consisted

of the female positioning herself towards the crevice or upper portion of the rock while the male was positioned over her back at the same angle. When the eggs were being deposited both fish vibrated. Males remained with the nest after the spawning event. Stiles (1972) observed a particular nesting site to contain a consistent male for over 15 days. It is possible that intruding males, current velocity changes, or even water depth variability could cause males to abandon a nest.

Spotted Darter

The Spotted Darter, *E. maculatum*, the namesake to the *E. maculatum* group, has a disjunct distribution across the states of New York, Pennsylvania, Ohio, Indiana, Kentucky, and West Virginia (Mayasich et al. 2004). In West Virginia, the Spotted Darter is restricted to the Elk River and is associated with large rocks (over 20 cm) in glides adjacent to and upstream of riffles, and to a lesser extent, in the head of riffles (Osier and Welsh 2007). There are many land use and water quality impacts that threaten the Spotted Darter in its small range in the Elk River, WV. A population of the Spotted Darter upstream of Sutton Dam on the Elk River is thought to be extirpated, but the population in the middle section of the Elk River below Sutton Dam is currently considered as stable. In Ohio the Spotted Darter is state listed as endangered. It has disappeared from three central Ohio streams, remaining only in parts of Big Darby Creek (Mayasich et al. 2004). The Spotted Darter is also listed as state endangered in Indiana where it occurs in the upper Wabash River, East Fork White River, and Blue River (Simon 2005). In New York the Spotted Darter is considered threatened, but given it only occurs in French Creek it could warrant status elevation to endangered. In Pennsylvania the Spotted Darter is also listed as threatened (Mayasich et al. 2004). In Kentucky the Spotted Darter occurs in the Green and Barren River, and a recent discovery of the Spotted Darter was made in the North Fork Kentucky

River (Simon 2006).

Reproductive ecology and early life history data are not available for the Elk River population of *E. maculatum*, but this information is needed for the conservation and management of this species. It is likely that knowledge of the life history of other populations of the Spotted Darter will apply to the West Virginia population. Raney and Lachner (1939) reported on observed populations in French Creek at Mill Village, Erie County, PA in June of 1938. Nests were found in all parts of the riffle, but mainly concentrated in the head of the riffle. Nests were collected from depths of 15 cm to 60 cm. Nest rocks were anywhere from 10–20 cm in diameter, and although larger rocks were available they were not utilized. Vegetation was not common at nest sites through the spawning season, but later in the summer filamentous algae were noted on these rocks. Nest density was low despite appropriate habitat. Possibly, intraspecific or interspecific competition influenced nest density. Also, unmeasured variables, such as rock size, placement, degree of sedimentation, and impactedness, may have influenced nest density. Spawning season was reported to start during the last week of May and span through June (5–6 weeks). Eggs in nests were packed tightly into wedge-shaped masses. Egg masses included up to four stacked rows of eggs. Nest rocks were always on a base of another rock. Eggs were reported to average 2 mm in diameter (in formalin). Nests included from 140–352 eggs at various ages and stages of development. The different stages of eggs in a single nest suggested multiple females adding to the nest over time.

Raney and Lachner (1939) examined ova of collected females during and before spawning season. Only a certain portion of ova in each female were mature at any given time (ranged from 47–76 mature ova in 8 females examined). Two females were found with 63 and 83 eggs massed just inside the external genital opening, suggesting these females were ripe and immediately

ready to spawn. The average ova count per female (mean SL=50 mm) collected in March was 400. Female size was highly correlated with number mature ova, smaller females averaging 40–45 cm in standard length only had half as many ova. It was surmised that females take two years to reach an average standard length of 44 mm before they spawn based on average growth and maturity of tracked juveniles. It was also surmised that females may continue spawning up to four years after reaching sexual maturity. Males were 50 mm at sexual maturity and were typically 2 years old before they were large enough to spawn. An individual's body size, which influences its ability to defend a territory, may influence sexual maturity in males. Males normally faced upstream while guarding nests. When a nest was uncovered the male would stay nearby. Eggs were found in the stomachs of males, possibly resulting from male grooming of infertile or aborted eggs. Egg grooming is documented in many nesting fish species (Layman 1991, Constantz 1985).

Eyed eggs of *E. maculatum* collected on 7 June 1938 hatched overnight (Raney and Lachner 1939). Increased activity within the egg was observed preceding hatching. The hatching larvae had heavy yolk sacs and were only 5 mm TL. After 48 hours, the larvae were 8 mm TL and the yolk sac was greatly absorbed. Melanophores were developing more as the larvae aged. Raney and Lachner (1939) reported that juvenile males grow faster than females.

Life history studies and captive propagation

Life history studies on fish species have often required the collection of hundreds of specimens across all seasons in order to estimate age, growth rate, breeding time, sexual dimorphism characteristics, seasonal pigmentation, and gonadosomatic index (Page and Smith 1971, Page and Smith 1970). With rare species, sacrificing a large number of individuals is not only unwise, but in many cases against state or federal regulations. As an alternative to field

collection, captive propagation methods provide a less environmentally intrusive way to track life history of a species.

As mentioned previously, some fishes spawn in nests, which allows for weekly inspection of nests. Weekly sampling designs work well within captive propagation studies, where data can be collected for many life history parameters, such as total numbers of eggs per female produced, eggs per clutch or per week, total egg production, spawning time (days/weeks), and preferred temperature ranges. Although captive propagation studies of eggs may not be directly comparable to a gonadosomatic index, it might be more informative in some cases, such as estimating the number of ova per female in a season. The gonadosomatic index classifies weight of ova as a proportion of total weight, and some may count the number of mature ova per female, but it doesn't give information of the actual number of eggs laid per female in a season.

As larvae hatch during a captive propagation study, further data can be collected pertaining to the percent egg survivorship to larval stage. As larvae grow and transform into adults, percent survivorship from larval to juvenile stages can be calculated. Although percent survivorship and overall transform rate may not directly translate to what happens in the wild, it could still be valuable information as to the overall hardiness of the eggs and larvae of all three species. By using photography to track larval development, estimated age and size can be calculated at crucial points in development such as first and second dorsal fin formation, size at this transformation, and estimated age and size at juvenile transformation. This information could be applied to collected larvae in the field.

Summary

This literature review is provided in support of Chapters 2 and 3 of this thesis. Chapter 2 focuses on reproduction and early life history of the Diamond Darter. Currently, the only known

extant population of the Diamond Darter is restricted to the lower Elk River, WV, and information on reproduction and early life history of this species was not available prior to this thesis research. Chapter 3 is a manuscript on reproduction and early life history of three darters: Boulder Darter, Wounded Darter, and Spotted Darter. These three species have small or fragmented populations, and data from this thesis research on reproduction and early life history are relevant to current management and conservation efforts.

Literature Cited

- Amano, M., T. Yamanome, H. Yamada, K. Okuzawa, and K. Yamamori. 2004. Effects of photoperiod on gonadotropin-releasing hormone levels in the brain and pituitary of underyearling male barfin flounder. *Fisheries Science* 70: 812–818.
- Biggins, R. G. 1988. Endangered and threatened wildlife and plants; determinations of threatened status for the boulder darter. Fed. Reg. 53: 33996–33998.
- Biggins, R. G. 1989. Recovery plan for boulder darter (*Etheostoma* sp.). U.S. Fish & Wildlife Service, Southeast Region, Atlanta. 15pp.
- Boschung, H. 1976. An evaluation of the slackwater darter, *Etheostoma boschungii*, relative to its range, critical habitat, and reproductive habitat in the Cypress Creek watershed and adjacent stream systems. U.S.D.A. Soil Conservation Service, Auburn, Alabama. 50 pp.
- Bryan J. L., M. L. Wildhaber, and D.B. Noltie. 2006. Influence of water flow on Neosho madtom (*Noturus placidus*) reproductive behavior. *American Midland Naturalist* 156: 305–318.
- Burkhead, N. M. and J. D. Williams. 1992. The boulder darter: a conservation challenge. *Endangered Species Technical Bulletin* 13: 3–8.
- Clark, R. W., A. Henderson-Arzapalo, and C. V. Sullivan. 2005. Disparate effects of constant and annually-cycling daylength and water temperature on reproductive maturation of striped bass (*Morone saxatilis*). *Aquaculture* 249: 497–513.
- Constantz, G. D., 1985. Allopaternal care in the tessellated darter, *Etheostoma olmstedi* (Pisces: Percidae). *Environmental Biology of Fishes* 14 No. 2/3, 175-183.
- Etnier, D. A. and J. M. Williams. 1989. *Etheostoma (Nothonotus) wapiti* (Osteichthyes: Percidae), a new darter from the southern bend of the Tennessee River system Alabama and Tennessee. *Proceedings of the Biological Society of Washington* 102:987–1000.
- Etnier, D. A. and W. C. Starnes. 1993. *The Fishes of Tennessee*. The University of Tennessee Press, Knoxville. 689pp.
- Ferry, D. H., 2008. Finding of No Significant Impact, TVA Unpublished report. http://www.tva.gov/environment/reports/timsford2/TimsFord_FONSI.pdf
- Hubbs, C., 1985. Darter reproductive seasons. *Copeia* 1985: 56–68.

- Johnston, C. E., and W. R. Haag. 1994. Life history of the Yazoo Darter (Percidae: *Etheostoma raneyi*), a species endemic to north-central Mississippi. *Tulane Studies in Zoology and Botany* 30: 47–60.
- Knouft, J. H., L. M. Page, and M. J. Plewa. 2003. Antimicrobial egg cleaning by the fringed darter (Perciformes: Percidae: *Etheostoma crossopterygion*): implications of a novel component of parental care in fishes. *Proceedings of the Royal Society B* 270: 2405–2411.
- Layman, S. R., 1991. Life history of the relict, duskytail darter, *Etheostoma (Catanotus)* sp. in Little River, Tennessee. *Copeia* 1991: 471-485.
- Mattingly, H. T., J. Hamilton, and D. L. Galat. 2003. Reproductive ecology and captive breeding of the threatened Niangua Darter *Etheostoma nianguae*. *American Midland Naturalist* 149: 375–383.
- Mayasich, J. M., D. Grandmaison, and D. Etnier. 2004. Spotted darter status assessment. Unpublished Natural Resources Research Institute Technical Report to U.S. Fish and Wildlife Service Region 3. 27 pp.
- Near, T. J., and B. P. Keck. 2005. Dispersal, vicariance, and timing of diversification in *Nothonotus* darters. *Molecular Ecology* 14: 3485–3496.
- Osier, E.A. and S.A. Welsh. 2007. Habitat use of *Etheostoma maculatum* (spotted darter) in Elk River, West Virginia. *Northeastern Naturalist* 14:447–460.
- Page, L. M. 1983. *Handbook of Darters*. T.F.H. Pub., Inc., Neptune City, NJ. 271pp.
- Page, L. M. 1985. Evolution of reproductive behaviors in percid fishes. *Illinois Natural History Survey Bulletin* 33:275–295.
- Page, L.M. and B.M. Burr. 2011. *Peterson field guide to freshwater fishes of North America north of Mexico*. Houghton Mifflin Harcourt, Boston. 663 pp.
- Paine, M. D. 1990. Life history tactics of darters (Percidae: Etheostomatiini) and their relationship with body size, reproductive behaviour, latitude and rarity. *Journal of Fish Biology* 37: 473–488.
- Petty, M. A., C. L. Ruble, P. L. Rakes, and J. R. Shute. 2011. Propagation and Reintroduction of Wounded Darters, *Etheostoma vulneratum*, in the Cheoah River, North Carolina. Unpublished report to North Carolina Division of Water Resources, Cheoah Fund.
- Petty, M. A., P. L. Rakes, J. R. Shute, and C. L. Ruble. 2012. Captive Propagation and Population Monitoring of Rare Southeastern Fishes in Tennessee: 2012. Unpublished report to TWRA, USFWS Cookeville and Asheville, Cherokee National Forest, and TVA.

- Rakes, P. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. *Environmental Biology of Fishes* 55: 31–42.
- Rakes, P. L., J. R. Shute. 2005. Captive Propagation and Population Monitoring of Rare Southeastern Fishes in Tennessee: 2005. Unpublished Final Report to Tennessee Wildlife Resources Agency.
- Raney, E.C. and E.A. Lachner. 1939. Observations on the life history of the spotted darter, *Poeciliichthys maculatus* (Kirtland). *Copeia* 1939(3):157-165.
- Simon, T. P., 1987. Description of eggs, larvae and early juveniles of the Stripetail Darter, *Etheostoma kennicotti* (Putnam) and Spottail Darter, *E. squamiceps* Jordan (Percidae: Etheostomatini) from tributaries of the Ohio River. *Copeia* 1987: 433–442.
- Simon, T. P., and N. J. Garcia. 1990. Description of eggs, larvae, and early juveniles of the stippled darter, *Etheostoma punctulatum* (Agassiz), from a tributary of the Osage River, Missouri. *Southwestern Naturalist* 35: 123–129.
- Simon, T. P., and J. B. Kaskey. 1992. Description of eggs, larvae, and early juveniles of the bigscale logperch, *Percina macrolepida* Stevenson, from the West Fork of the Trinity River basin, Texas. *Southwestern Naturalist* 37: 28–34.
- Simon, T.P., E.J. Tyberghein, K.J. Scheidegger, and C.E. Johnston. 1992. Descriptions of protolarvae of the sand darters (Percidae: *Ammocrypta* and *Crystallaria*) with comments on systematic relationships. *Ichthyological Exploration of Freshwaters* 3: 347–358.
- Simon, T.P. and R. Wallus. 2006. Reproductive Biology and Early Life History of Fishes in the Ohio River Drainage. Percidae - Perch, Pikeperch, and Darters – Volume 4. CRC Press. pp 52–57.
- Simon, T.P., 2005. Conservation Assessment for the Spotted Darter (*Etheostoma maculatum*), Unpublished report for USDA Forest Service, Eastern Region. 18pp.
- Simons, A. M. 1991. Phylogenetic relationships of the crystal darter, *Crystallaria asprella*, (Telostei: Percidae). *Copeia* 1991: 927–936.
- Storey, C. M., B. A. Porter, M. C. Freeman, and B. J. Freeman. 2006. Analysis of spawning behavior, habitat, and season of the federally threatened *Etheostoma scotti*, Cherokee Darter (Osteichthyes; Percidae). *Southeastern Naturalist* 5: 413–424.
- USFWS (U.S. Fish and Wildlife Service). 2009. Endangered and threatened wildlife and plants; review of native species that are candidates for listing as endangered or threatened; annual notice of findings on resubmitted petitions; annual description of progress on listing actions. *Federal Register* 74: 57804–57878.

- USFWS (U.S. Fish and Wildlife Service). 2012. Endangered and threatened wildlife and plants; endangered status for the diamond darter and designation of critical habitat; proposed rule. Federal Register 77: 43906–43939.
- Welsh, S. A., and S. Perry. 1998. Habitat partitioning in a community of darters in the Elk River, West Virginia. Environmental Biology of Fishes 51: 411–419.
- Welsh, S.A. and R.M. Wood. 2008. *Crystallaria cincotta*, a new species of darter (Teleostei: Percidae) from the Elk River of the Ohio River drainage, West Virginia. Zootaxa 1680: 62–68.
- Welsh, S.A., R.M. Wood, K.R. Sheehan. 2009. Threatened fishes of the world: *Crystallaria cincotta* Welsh and Wood 2008 (Percidae). Environmental Biology of Fishes 84:191–192.
- Zorach, R. and E. C. Raney. 1967. Systematics of the percid fish, *Etheostoma maculatum* Kirtland, and related species of the subgenus *Nothonotus*. American Midland Naturalist 77: 296–322.

Chapter 2. Captive propagation, reproductive biology, and early life history of the Diamond Darter (*Crystallaria cincotta*)

Abstract

Reproductive biology and early life history data are critical for the conservation and management of rare fishes such as the Diamond Darter *Crystallaria cincotta*. During 2008–2012, a captive propagation study was conducted on the Diamond Darter, a rare species with a single extant population in the lower Elk River, WV. 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 days. Diamond Darters were 6.7–7.2 mm TL at hatch. Larvae ranged from 9.0–11.0 mm TL following a 5–10 day period of yolk sac absorption. Larvae were provided brine shrimp *Artemia sp.*, *Ceriodaphnia dubia* neonates, marine *Brachionus* rotifers, and powdered foods (50–400 microns). Diamond Darter larvae did not feed in captivity, except for cannibalizing other larvae. Larvae survived for a maximum of 11 days. Larvae had relatively large gapes and teeth, suggesting possible larval piscivory and a need for alternative food sources 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 from the lower 37 km of the Elk River, Kanawha County, WV. Based on museum specimens, this species occurred previously over a wider range within the Ohio River drainage, but populations are considered extirpated from Kentucky, Ohio, and Tennessee (Welsh et al. 2009). In November 2009, the Diamond Darter was included as a candidate for listing as endangered or threatened (USDOJ 2009). Recently, USFWS (2012) published a rule in the Federal Register proposing the Diamond Darter for listing as endangered with critical habitat 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 propagation 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, an understanding of egg size and development, and egg viability and survival are critical to understanding reproductive success. Also, an understanding of the development, growth rate, and survival rate of larvae are critical for understanding 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, 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 that the Crystal Darter, a close relative of the Diamond Darter, is an egg burier that spawns from February through April. They also noted that 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 August 2008 (n = 1, female), 8 October 2008 (n =2 juveniles), 23 September 2009 (n = 2, female and male), and 13–14 September 2011 (n = 12, 8 females and 4 males). Diamond Darters were successfully transported to Conservation Fisheries, Inc. (CFI), Knoxville, TN 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 reduced water temperatures 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-L aquarium, which was part of a larger recirculation system of approximately 900 L as described by Rakes et al. (1999). A mixture of sand and gravel (up to 25 mm) 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/hr. The

recirculating aquaria system was treated with salt (~2%) to reduce fish stress and fight parasitic infections.

During March 2012, the breeding set up was modified, in part, to accommodate the larger number of brood fish. Diamond Darters were moved to an 1140 L opaque plastic oval vat (PRE 328; Behlen Country Poly Stock Tank). A centrifugal water pump, rated at 3500 L/hr, generated substantial flow within the oval vat. A reverse-flow undergravel filter was installed within the substrate in $\frac{1}{4}$ 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 recorded individual interactions, including territorial behavior of males, 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 L (~50 x 70 cm with ~15-cm depth). A rearing tub with a slower flow turnover was also set up for swimming larvae. This tub had a 70-cm diameter, a 30-cm depth, and approximately a 100-L capacity. Water from the recirculation system was piped to the rearing tub, entering through a PVC end cap with a 4-mm hole, and creating circular flow. A swift circular surface flow reduced overall water turnover, and increased food retention within the rearing tub. Vertical center standpipes, which were topped

with 250–500 micron screens, drained both capture and rearing tubs. Airstones at the base of standpipes prevented larval escape or impingement on the screens.

Collection and rearing tubs were modified in 2011 and 2012, respectively. The collection tub was replaced with a shortened 50 L 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 x 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 helped to maintain a directional flow pattern of the water. To precisely control flow and water exchange within the 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 in the rearing tub, 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-cm 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 with the amount of air going through the lift tube. The collection tub was monitored daily for larvae from April through July. An overhead shop light positioned near the water surface presumably attracted 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.

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 (comprised of a reservoir, timer, and solenoid) dispensed food to the rearing tub. The feeding reservoir was an 11.4-L 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®, ReedMarineculture Inc.), newly hatched brine shrimp nauplii, and *Ceriodaphnia dubia* neonates. The timer-controlled solenoid dispensed food for 8–10 seconds every 2 minutes. Initially, food was provided to larvae during daytime, but the automated feeder also provided food at nighttime in 2012, 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 fed 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 could be obligatory larvae eaters. Also, given the possibility that larval feeding may 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 and Discussion

Behavior and reproduction of three Diamond Darters were examined during the first period of study (fall 2008–spring 2009), including one large adult female (~97 mm TL), and two smaller individuals (55–65 mm TL). Throughout the fall/winter, the three Diamond Darters were often buried in the sand during the day and active at night. Diamond Darters buried less as water temperatures increased above 15°C, and were consistently above the sand substrate during day and night once water temperatures exceeded 21°C. By May, the large adult female was noticeably gravid, but no eggs or larvae were observed from May to mid-August. By mid-August, the previously-gravid female had absorbed her eggs. At this time, it was assumed that the two smaller individuals were juveniles or females.

Prior to the second study period (fall 2009–spring 2010), two additional Diamond Darters

(a male and female) were collected from the lower Elk River, WV, in September and transported to CFI on 1 October 2009. As temperatures decreased below 15°C, Diamond Darter activity decreased and individuals were often buried in the sand. Low temperatures of about 3°C were obtained during the wintering conditioning period. On 10 March 2010, the two individuals from 2009 were added to the tank with the other three Diamond Darters. At this time, several individuals remained out of the sand. On 31 March 2010, the largest female was visibly gravid.

Spawning during spring 2010 occurred with all individuals in one tank. However, egg production and egg viability were low, owing possibly to stress of females from excessive solicitation by males. In an attempt to make mating events more productive and less stressful on females, males were removed from the tank for several days to give females time to recover condition. When the females appeared gravid again, the largest male was returned to the tank and immediately initiated spawning with the smaller female. 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 come up beside the female and vibrate in an attempt to start a spawn. Females would either swim away, or accept the courtship display by vibrating with the male down into the substrate. During this process the female would be buried about 1/3–1/2 of the way down into the sand and the male would be positioned along her side, tilted almost at a 45° angle. Vibrations and burial into the sand usually coincided with a wide gape of the female's mouth. Total spawn time was 7–8 seconds (CFI 2010a, 2010b).

For the third study period (fall 2010–spring 2011), an inventory of the Diamond Darters accounted for all 5 individuals during March 2011, but the condition of the fish for the 2011 breeding season was markedly poorer than 2009 or 2010. By 1 April, only a smaller female was

gravid and a larger female succumbed possibly to disease or senescence. The age of the larger female at time of death is unknown, but she was approximately 2 years old when collected in the fall of 2009, putting her at about 4 years old.

In 2012, the breeding group consisted of a male (collected in 2009), a female (collected in 2010), and four males and eight females collected in September 2011. Interactions of these 14 individuals in the 250 gallon vat differed from those of the previous breeding groups in the smaller breeding tank. In 2012, a dominant male maintained a territory in the middle of the tank toward the back portion of the undergravel filter. The male chased away other individuals, except for receptive females. Receptive females would swim into the territory and accept the male's vibration solicitation for spawning. This was markedly different from observations in 2010 and 2011, when male territoriality was not observed during the spawning season in the smaller tank. Dominant territoriality lessened during the latter part of the 2012 breeding season, even though fish were still spawning based on passive collections of larvae.

The spawning period in 2010 lasted approximately 53 days, beginning near 31 March and ending on 21 May. Water temperature (mean±SE, °C) averaged 19.1 ± 0.21 , ranging from 14.9 to 21.2. Diamond Darter eggs and larvae were first observed on 8 April 2010, when 27 eggs and 3 larvae were recovered with a substrate siphon. Based on an estimated hatch time of 7–9 days, it is likely that the larvae were from eggs spawned near 31 March (14.9°C water temperature, Fig. 2.1). Of the 27 eggs, only 3 were fertile, eyed and developing, 18 were infertile, and another 6 were of uncertain status based on opaqueness of the chorion. The fertile eggs were well developed and measured 1.9–2.0 mm (Figs. 2.2A–B). Each embryo had a clear chorion and a very light tint of yellow to almost clear yolk sac with a moderate amount of melanophores

present. The oil droplets on embryos and larvae were large with a dark yellow coloration. Larvae were 6.7–7.2 mm TL with heavy yolk sacs (Figs. 2.2C–D).

Viable eggs and yolk-sac larvae were placed in a small shoebox-sized tray measuring 30 cm x 18 cm nested in a 76-L aquarium with a flow through screen in the same system as the adults. The larvae had an initial swim up burst of several minutes to possibly an hour, but then settled to the bottom. These early swim-up larvae did not maintain a particular position in the water column and were positively phototactic. They would occasionally swim up when disturbed. Yolk-sac larvae began swimming consistently in 1–2 days, and changed from being positively to negatively phototactic (i.e., photophobic). Protolarvae had 22–23 post anal myomeres and 23–24 preanal myomeres. Pigmentation at hatch consisted of moderate melanophores concentrated on the ventral surface of the yolk sac, but pigmentation was also prevalent posterior to the anus both on ventral and dorsal areas along the myomeres.

Several more larvae hatched in the tray and others were passively-collected from the collection tub, resulting in an addition of 10 larvae to the 65-L rearing tub by 13 April. Measurements of first swim up larvae were 6.8–7.2mm TL. The rearing tub was supplied with a regulated source of live zooplankton during daylight hours, but larval feeding was not observed. On 13 April, only 3 larvae were found in the tub, and two were recovered and photographed. Possibly, larvae were transferred to the rearing tub before they were able to swim against the current properly. The two photographed larvae were 9.7–10.0 mm TL, and were assumed to be the oldest larvae transferred to the rearing tub (Fig. 2.2E). Surprisingly, these older larvae, estimated to be 5 days post hatch, still had a large yolk sac (Fig. 2.2E). In general, most other pelagic darter larvae absorb the yolk sac within a few days of hatch and swim-up. These yolk-sac larvae displayed pigment along myomere edges (Fig. 2.2E). Head and mouth morphology were

also more developed with an elongated snout and increased gape size to almost 1 mm (Fig. 2.2E). With this gape size, larvae were capable of consuming the food items provided, but guts were empty. On 16 April, the 8-day old larvae died, never showing signs of feeding.

On 16 April 2010, an additional 25 eggs and larvae were collected, including 11 infertile eggs and three recently hatched larvae. Only 10 of these survived to transfer to the rearing tub. Many apparently viable eggs succumbed to fungus. Over a time span of 7 days all eggs hatched and were transferred to the tub. Larvae mortalities were noted on 25, 26, and 30 April. By 3 May, only one individual remained and it was photographed to document size, yolk-sac presence, and general appearance (Fig. 2.2F). This larva exhibited a large mouth and teeth, but no gut contents. The larva measured 10.8 mm TL and had a gape length of over 1 mm, with a total lower jaw length of 1.4 mm. The larva failed to feed on invertebrates, prompting speculation that the relatively large gape size and teeth might be adaptations for predation on other fish larvae. On 26 April, another 45 eggs were collected, but all appeared to be infertile. On 28 April, another 27 eggs were collected, but only one egg appeared viable, exhibiting vertebral development, as well as a fungal infection on the chorion. Spawning was also documented on 7 May (11 eggs), 14 May (2 eggs), and 21 May (17 eggs), but all eggs exhibited fungal infection. Most viable eggs were considerably developed, precluding an estimate of incubation time. Hatch time at 15°C was estimated to be 7–9 days.

During 2011, water temperatures within a 29-day spawning period (1–29 April) ranged from 14.7–20.1°C (mean±SE, 17.7±0.28, Fig. 2.1). During this time, the total egg count was 15, and five of those eggs hatched into larvae. The first larva was passively collected and transferred to the rearing tub on 9 April, but was not found on the following day. Two dead eggs were gravel-siphoned from the tank on 11 April, and two larvae were passively-collected on 21 April

The larvae were observed for at least two days, but were not found on 25 April. On 29 April, 10 eggs were recovered, 6 of which were dead. Two eyed eggs with vertebral development remained on 2 May, hatching by 4 May, and dying by 8 May. No additional larvae were passively collected, and video observations did not show breeding activity of adults. The previously-gravid female no longer appeared to have eggs, and her condition was poor. An adult was found dead on 31 July, and a second dead adult was found 5 days later, leaving only the two youngest individuals. Possibly, the older individuals succumbed to senescence.

In 2012, a 111-day spawning period ranged from 13 March to 1 July. Water temperatures during the spawning period ranged from 15.9 to 23.3°C (mean±SE, 19.5±0.15, Fig. 2.1). A total of 154 fertile eggs and larvae were collected during 2012. In addition to 154 fertile eggs and larvae, another 25 infertile or dead eggs and larvae were collected during three gravel wash collections. In 2012, the number of gravel-siphoning events was lowered in an effort to reduce disturbance and increase larval survival. Most dead eggs (n=18) were collected by gravel siphon during the first collection from the holding tank, before fish were moved to the breeding vat with reverse-flow filtration. These eggs possibly succumbed to disease or low oxygen availability in the substrate. Infertile or bad eggs were rarely collected with gravel siphon from the breeding vat relative to those from previous years. Oddly, very few fertile eggs were collected via gravel siphoning. Possibly, fertile eggs adhere to sand grains and are not easily recovered with a gravel siphon in such a large set up. Also, Instant Algae[®] was added to larval tubs in 2012, and although orientation of Diamond Darter larvae improved in green water conditions, feeding was not observed.

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 11 days. The large gape width and large teeth of larvae were important findings, which suggest that larvae may be obligatory piscivores. Interestingly, larval mortality was influenced by starvation and cannibalism, where Diamond Darter larvae did not feed on invertebrates, but were observed cannibalizing younger larvae.

Territorial aggression in male Diamond Darters occurred only during the breeding season. In 2009–2011, 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 2009–2011, but a dominant male solicited females in his territory during 2012. 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 habitat.

The duration of spawning periods of Diamond Darters and associated water temperatures differed among study periods. The longer spawning period of 2012 (111 days) was associated with higher temperatures during early spring and lower temperature during late spring relative to those of 2010 (52 days) and 2011 (28 days). 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 with the larger breeding group of 2012 may explain differences in spawning period duration. Also, females were possibly cycling into condition at varying times through the spawning period, lengthening the overall observed spawning time.

Differences in egg 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 cannibalism. Two females produced 89 eggs each during the 53-day spawning period of 2010. During spring 2011, the advanced age and poor condition of females may have reduced spawning success. During this relatively short 28-day spawning season, only one female was gravid, and only 3 larvae and 12 eggs were found. Adults were slightly emaciated during winter months prior to spring 2011 even though food quantities provided were similar to the previous more productive year. In 2012, the 179 eggs from nine females averaged out to only 20 eggs per female. 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 big proportion of larvae in the larger vat were cannibalized just after swim up and before being passively collected in the overflow.

In 2010, fecundity decreased during the spawning season. Diamond Darters continued to deposit eggs, but fewer of the eggs were fertile as the spawning season progressed. 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 increased 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.

Several interesting findings concerning Diamond Darter larvae involved yolk-sac persistence, swimming behavior, and reaction to light. Diamond Darter larvae had a very heavy yolk sac and many were collected via gravel siphoning, suggesting that they were not yet swimming. Also, Diamond Darter larvae tend to swim up early following hatch, then settle 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. In 2012, orientation of Diamond Darter larvae improved in green water conditions after adding Instant Algae®. This could be a visual or chemical change in orientation, and possibly the limited sight range in green water improved focus on prey items. Diamond Darter larvae darted frequently and purposefully in and out of the flow, which may indicate flow avoidance or prey-seeking behavior.

Other interesting results were the discoveries of large gape size and large teeth of Diamond Darter larvae (Fig. 2.3). Large teeth are not present in adult Diamond Darters; hence, larval teeth are lost during early development. Ontogenetic tooth loss also occurs in other fishes, including members of elopiformes and anguilliformes (Fahay 1983), acipenseriformes (Nelson 2006), and siliformes (Huyssseune and Sire 1997, Kakizawa & Meenakarn 2003). To my knowledge, larval teeth and ontogenetic tooth loss have only been previously reported for a very few number of darters. Simon and Wallace (2006) noted small teeth in *Etheostoma bleunioides pholidotum* and *Percina caprodes*. Larval teeth were also noted in *Etheostoma variatum*, *Etheostoma cf sp. zonale* “Jade Darter”, *Percina macrolepida*, *Percina rex*, and *Percina austoperca* (Personal communication, Thomas P. Simon). These larval teeth, however, were never as extensive as the teeth 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, Huyssseune et al. 2009).

Survival of Diamond Darter larvae was influenced by starvation and cannibalism, and possibly by nutrition and condition of the adult breeders. Larvae did not eat *Artemia sp.* or *Ceriodaphnia dubia*. Larvae typically have an orange or dark gut after feeding on these two food items, but Diamond Darter larvae always had clear guts. 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-day old larvae suggest the possibility for predation on other smaller fish larvae. In 2012, a larva was observed cannibalizing a smaller larva. Possibly, older larvae commonly cannibalized newly hatched larvae, which may explain why only older larvae were found in the rearing tub after several days. During 2012, larvae of

Sawfin Shiners and Redline Darters were provided as forage, but Diamond Darter larvae did not feed on this alternative food source. 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 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 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 captive propagation of Diamond Darter larvae. Age classes of larvae may require segregation every few days to prevent cannibalism among Diamond Darter larvae.

Captive propagation of Diamond Darters was hindered by low survival of eggs and 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 create bacterial blooms that in turn cause larvae to succumb to infections. Disease did not appear to affect survival of Diamond Darter larvae, but was negatively associated with egg survival.

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 egg survivorship, 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, such as the larvae of other fishes. 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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Literature Cited

- Bonner, T. H., T. M. Brandt, J. N. Fries and B. G. Whiteside. 1998. Effects of temperature on egg production and early life stages of the fountain darter. *Trans. Am. Fish. Soc.*, **127**:971–978.
- Brandt, T. M., K. G. Graves, C. S. Berkhouse, T. P. Simon and B. G. Whiteside. 1993. Laboratory spawning and rearing of the endangered fountain darter. *Prog. Fish Cultur.*, **55**:149–156.
- CFI (Conservation Fisheries, Inc.). 2010a. Diamond darters spawning at CFI. Retrieved from <http://www.youtube.com/watch?v=7ggPui-rq2I>.
- CFI (Conservation Fisheries, Inc.). 2010b. Diamond darters spawning at CFI 2010. Retrieved from http://www.youtube.com/watch?v=tQsvqXX_az8&NR=1
- Davit-Béal, T., A. S. Tucker, and J-Y. Sire. 2009. Loss of teeth and enamel in tetrapods: fossil record, genetic data and morphological adaptations. *J. Anat.*, **214**:477–501.
- Fahay, M. P. 1983. Guide to the early stages of marine fishes occurring in the western North Atlantic Ocean, Cape Hatteras to the southern Scotian Shelf. *J. Northwest. Atl. Fish. Soc.*, **4**:1–424.
- Fisher, W. L. 1990. Life history and ecology of the orangefin darter *Etheostoma bellum* (Pisces: Percidae). *Am. Midl. Nat.*, **123**:268–281.
- Hubbs, C. 1985. Darter reproductive seasons. *Copeia*, **1985**:56–68.
- Huysseune, A., and J-Y. Sire. 1997. Structure and development of teeth in three armoured catfish, *Corydoras aeneus*, *C. arcuatus* and *Hoplosternum littorale* (Siluriformes, Callichthyidae). *Acta Zool.*, **78**:69–84.
- Huysseune, A., J-Y. Sire, and P. E. Witten. 2009. Evolutionary and developmental origins of the vertebrate dentition Witten. *J. Anat.*, **214**:465–476.
- James, P. W. and O. E. Maughan. 1989. Spawning behavior and habitat of the threatened leopard Darter, *Percina pantherina*. *Southwest Nat.*, **34**:298–301.
- Kakizawa, Y., and W. Meenakarn. 2003. Histogenesis and disappearance of the teeth of the Mekong giant catfish, *Pangasianodon gigas* (Teleostei). *J. Oral Sci.*, **45**:213–221.
- Mattingly, H. T., J. Hamilton, and D. L. Galat. 2003. Reproductive ecology and captive breeding of the threatened Niangua Darter *Etheostoma nianguae*. *Am. Midl. Nat.*, **149**:375–385.
- Nelson, J. S. 2006. *Fishes of the world*, 4th edition. John Wiley and Sons, Inc., Hoboken, New Jersey.
- Page, L. M. 1983. *Handbook of Darters*. TFH Publications, Neptune City, New Jersey.

- Rakes, P. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. *Env. Biol. Fish.*, **55**:31–42.
- Simon, T. P. and R. Wallus. 2006. Reproductive Biology and Early Life History of Fishes in the Ohio River Drainage. Percidae - Perch, Pikeperch, and Darters – Volume 4. CRC Press, Boca Raton, Florida.
- Simon, T. P., E. J. Tyberghein, K. J. Scheidegger, and C. E. Johnston. 1992. Descriptions of protolarvae of the sand darters (Percidae: *Ammocrypta* and *Crystallaria*) with comments on systematic relationships. *Ichthyol. Explor. Freshw.*, **3**:347–358.
- Stock D. W. 2001. The genetic basis of modularity in the development and evolution of the vertebrate dentition. *Philos. Trans. R. Soc. Lond. B.*, **356**:1633–1653.
- USDOJ (U.S. Department of the Interior). 2009. Federal Register, Candidate Notice of Review, Part III, Vol. 74, No. 215, Monday, November 9, 2009. Retrieved from <http://www.fws.gov/endangered/esa-library/pdf/CNOR%2011-9-09.pdf>
- Welsh, S. A. and R. M. Wood. 2008. *Crystallaria cincotta*, a new species of darter (Teleostei: Percidae) from the Elk River of the Ohio River drainage, West Virginia. *Zootaxa*, **1680**:62–68.
- Welsh, S. A., R. M. Wood, K. R. Sheehan. 2009. Threatened fishes of the world: *Crystallaria cincotta* Welsh and Wood 2008 (Percidae). *Env. Biol. Fish.*, **84**:191–192.

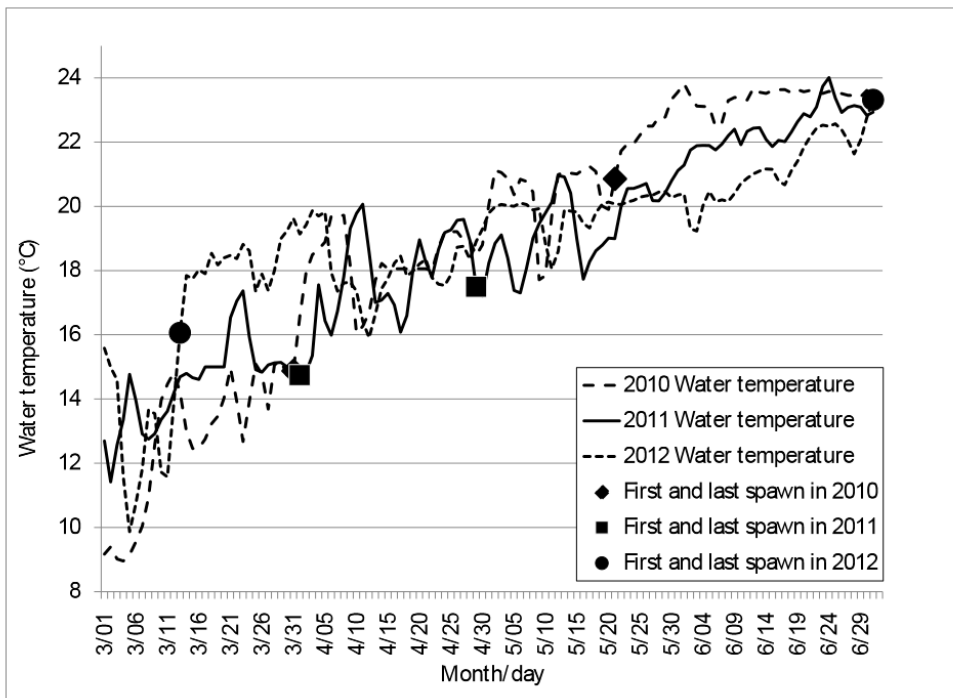


Figure 2.1. Aquarium water temperatures for spring 2010, 2011, and 2012 including dates of the first and last spawning events of captive Diamond Darters.

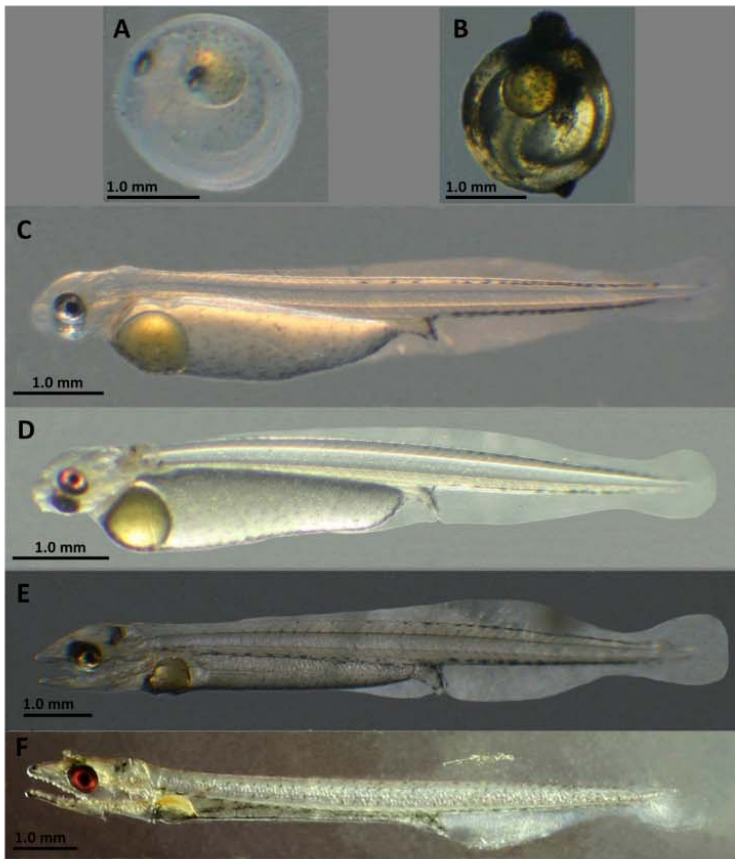


Figure 2.2. Photographs of eggs and larvae from a captive propagation study of the Diamond Darter: (A) eyed egg, (B) well-developed egg, (C) one-day old larva with heavy yolk sac, (D) two-day old larva, (E) five-day old larvae, (F) 10-day old larva with large gape size and large teeth.

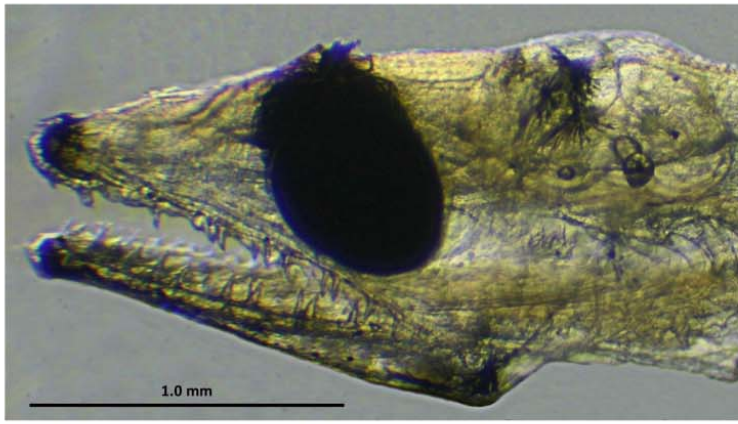


Figure 2.3. Large gape size and teeth of a 10-day old Diamond Darter larva photographed during a captive propagation study.

Chapter 3. Captive propagation, reproductive biology, and early life history of *Etheostoma wapiti* (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter)

Abstract

Reproductive biology and early life history data are important for understanding the ecology of fishes, and can be used for the management and conservation of rare species. In 2008, captive propagation studies were conducted on three species of darters of the subgenus *Nothonotus*: *Etheostoma wapiti* (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter). The length of spawning period and associated range of water temperatures for the Wounded Darter (89 days, 16.0 – 24.0°C) exceeded that of the Spotted Darter (46 days, 17.0 – 22.5°C) and Boulder Darter (48 days, 17.0 – 22.5°C). Eggs produced per female were least in the Boulder Darter (163), intermediate in the Spotted Darter (191), and highest in the Wounded Darter (345). Egg diameters at deposition and prior to hatch were least for Spotted Darter (1.9–2.0 mm, and 1.9–2.1 mm), intermediate for Wounded Darter (2.1–2.2 mm, 2.4–2.5 mm), and largest for Boulder Darter (2.2–2.4 mm, 2.4–2.6 mm). Lengths of larvae at hatch and at the start of first fin development varied in a similar pattern: Boulder Darter (8.5–9.1, 14.0–15.0), Wounded Darter (7.7–8.0, 13.5–14.0), and Spotted Darter (6.5–7.0, 12.0–13.0 mm). Overall production per female was lowest in the Boulder Darter (61 juveniles/ female), intermediate in the Spotted Darter (86 juveniles/ female), and highest in the Wounded Darter (90 juveniles/ female).

Introduction

Etheostoma wapiti (Boulder Darter), *E. vulneratum* (Wounded Darter), and *E. maculatum* (Spotted Darter) are members of the *E. maculatum* species group of the darter subgenus *Nothonotus* (Etnier and Williams 1989, Near and Keck 2005). Species within this group are of conservation concern, in part, because of small or fragmented distribution ranges. The Boulder Darter occurs only within the Elk River drainage, Tennessee. In comparison, the Wounded Darter is found within the upper Tennessee River drainage including North Carolina, Tennessee, and Virginia, and the Spotted Darter occurs in isolated populations within six states: Indiana, Kentucky, Ohio, New York, Pennsylvania, and West Virginia. These darters typically occur within or near riffle habitat of medium to large rivers. Conservation of these species would benefit from studies on reproductive biology, such as captive propagation studies which examine

water temperature ranges of the spawning period, clutch sizes, and egg and larval survival and development (Rakes et al. 1999).

General reproductive strategies are known for the *E. maculatum* species group. Spawning females clump eggs in a male-guarded nest (Page 1983, Etnier and Williams 1989). Typically, the male-selected spawning site occurs within a riffle or near a riffle head and often includes two large rocks, one perched at an angle on top of another. A “V-shaped” space is formed between these two imbricated rocks providing an egg deposition area for females. The female clumps eggs on the ventral surface of the top rock and further in towards the “V.” Like many other nest guarding species, many females contribute to a single nest (Etnier and Starnes 1993). Although the general reproductive strategies are known, little information is available for many early life history parameters associated with eggs and larvae.

Captive propagation studies can provide valuable information for the conservation and management of rare fishes. Some studies have used large numbers of preserved specimens for estimating parameters of reproductive biology, such as the gonadosomatic index (Page and Smith 1971, Page and Smith 1970). Although these studies provide valuable information, sacrificing large numbers of individuals is not feasible for rare species. Captive propagation of rare species, however, can be used to spawn species providing a less environmentally intrusive way to track life history of a species. An understanding of life history parameters, such as clutch size, number of clutches, egg size, and egg survival are critical to the understanding of reproductive success. Also, an understanding of life history parameters, such as hatch time, larval size, and larval survival, are critical for the understanding of the early life history of fishes as it applies to conservation and management.

The primary goal of this study was to estimate life history parameters associated with the reproductive ecology and early life history of *E. wapiti*, *E. vulneratum*, and *E. maculatum*. Because these species spawn in nests, weekly collections enable data collection for several life history parameters: total numbers of eggs per female produced, eggs per clutch or per week, total egg production, spawning time (days/weeks), preferred temperature ranges and daylight cycle. As larvae hatch, further data can be collected pertaining to the percent egg survivorship to larval stage. From an ecological perspective, this research compares life history parameters among three species that differ in having northern and southern distribution ranges, and small to relatively wide distribution ranges.

Methods

For this study, captive propagation of Boulder Darters, Wounded Darters, and Spotted Darters occurred in 2008 at Conservation Fisheries Inc. (CFI), Knoxville, TN. At the start of April 2008, the captive Boulder Darter population consisted of 27 breeder adults. Boulder Darter breeders were previously collected from Elk River and Richland Creek, TN. Four more individuals were collected on 16 April from Richland Creek to add to the breeding population. Another eight were collected from the Elk River near the Alabama-Tennessee state line, too late to participate in 2008 breeding. Boulder Darters were housed in a 1500-L (net volume) recirculation system consisting of twenty-seven 76-L aquaria with a 190-L sump. Several propagated Boulder Darters were also added to the breeding stock from previous years of spawning. Wounded Darters were collected from Little Tennessee River, NC, during spring and summer 2007, included 12 individuals from the bend below Wiggins Branch, two males near the mouth of Tellico Creek, and a male and female from above Dean Island. Wounded Darters were housed in a 1500-L recirculation system consisting of twenty-seven 76-L long aquaria with a

190-L sump. During February 2008, 18 Spotted Darters were collected from Elk River, WV, and transported to CFI for captive propagation. Spotted Darters were housed in an 850-L recirculating system of fourteen 76-L long glass aquaria with a 190-L sump.

During winter months, water temperature and photoperiod were manipulated for conditioning breeders for the upcoming spring spawning period. Photoperiod was controlled by an astronomic timer (Intermatic Next Generation Year Long Double Circuit Electronic Timer) operating 48" fluorescent shoplights (with Daylight Deluxe and Cool White bulbs). The astronomic timer mimicked natural winter 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.

For all species, tanks were set up with appropriate substrate and cover items consisting of ceramic slates, PVC pipes, and natural stone slabs. For spawning sites, tiles or terra cotta slates (28.0–30.5-cm long and 15.3-cm wide) were stacked together to form an inverted "V", spaced apart on the open end with 1.9-cm or 2.5-cm diameter PVC tubes. Filtration included individual tank sponge filters, airstones, and system biotower filters. Recirculating aquaria systems were treated with salt (~2%) to reduce fish stress and parasitic infections.

Breeding groups were arranged with different male/female ratios. The Boulder Darters were set up in eleven breeding groups: five with one male to two females and six with one male to one female. Multiple males were not used due to territorial aggression witnessed with multiple

males in one tank (P. Rakes and J.R. Shute, personal communication). The Wounded Darters were set up in three breeding groups each containing two males and two females. The Spotted Darters were set up in three breeding groups varying in proportion and number of males and females: two males and three females, one male and one female, one male and two females. Four extra males were housed in a separate tank. Four other fish (one male and three young females), which were presumed to be juveniles, were placed in a fifth tank.

Incubation tanks (76-L long aquaria), oval catch tubs (63.5-cm length by 53.3-cm width, and 17.8-cm depth), and circular rearing tubs (70-cm diameter, 30-cm depth, and approximately a 100-L capacity) were set up separately for each species. Vertical center standpipes, which were topped with 250–500 μm screens, drained both capture and rearing tubs. Water from incubation tanks (which were in line with the recirculation system) flowed into catch tubs through a valve at a rate slow enough to prevent overwhelming of the overflow screen in the collection tub. The rearing tub with a slower flow turnover was used for swimming larvae. Water from the recirculation system was piped to the rearing tub, entering through a PVC end cap with a 4-mm hole, and creating circular flow. A swift circular surface flow reduced overall water turnover, and increased food retention within the rearing tub. Flexible airstones, which encircled the base of center standpipes, created water movement with rising air bubbles, thereby preventing larval escape or impingement on the standpipe screens.

Adult breeders, larvae, and juveniles required different feeding regimes. Adult darters were fed live blackworms, live *Daphnia pulex*, 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. The rearing of tiny pelagic larvae required a balance between providing adequate planktonic food densities while simultaneously maintaining

adequate water quality and avoiding too high larval densities. An automatic feeder with a reservoir, timer, and solenoid dispensed food into the rearing tub during the day. The feeding reservoir was an 11.4-L 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® produced by ReedMarineculture Inc.), newly hatched brine shrimp nauplii, and *Ceriodaphnia dubia* neonates. The timer-controlled solenoid dispensed food for 8–10 seconds every 2 minutes during daylight hours. To supplement the reservoir feeding, several powders were lightly dusted on top of the rearing tub several times daily. The powders fed were all in equal parts from a premixed batch: A.P.R. (Artificial Plankton – Rotifer) by O.S.I., Larval AP 100 (<100 µm and 100–150 µm), by Zeigler Bros., Inc., and Spirulina by Salt Creek, Inc. Twice weekly it was necessary to take apart the feeding reservoir for cleaning. Routine cleaning of the rearing tub was done with either a large baster, or a small flexible siphon tube (drained to a bucket). Larvae captured during the cleaning process were returned to the rearing tub. As larvae grew and transformed to benthic juveniles they were subsequently separated from younger larvae and transferred to 76-L long tanks for further grow out. Feeding of these juveniles was supplemented with larger food items such as chopped blackworms, and later frozen chopped bloodworms. Brine shrimp nauplii and *Ceriodaphnia dubia* adults were still offered for undersized individuals.

The duration and water temperatures of spawning periods, and egg and larval characteristics were recorded for each species during 2008. Spawning substrates were examined and any nests collected nearly every week in all tanks set up with breeders. Egg production and egg size, including clutch sizes, average number of eggs per female, number of eggs per breeding group, egg size at deposition, egg size prior to hatch, and percent of eggs hatched were recorded.

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, developing larvae, and transformed juveniles. Myomeres were also counted using these photos. Additionally, larva length (mm) at hatch and larva length (mm) at the start of first fin development were measured. Also, age (days) at the start of first fin development was documented. For juveniles the percent transformation of pelagic larvae to benthic juveniles was estimated, as well as the length (mm TL) and age of fully transformed juveniles.

Results

Boulder Darter

Boulder Darters spawned for 48 days (25 April–11 June 2008) within a water temperature range of 17–22.5°C (Table 3.1). From the eleven breeding groups eleven batches of nests were collected, comprised of 43 clutches, numbering approximately 2614 eggs. Average eggs produced per female were 163 (Table 3.1). Breeding groups that were set up with one male and two females produced a total of 1317 egg averaging 132 eggs per female. Breeding groups set up with only a single male to a single female produced a total of 1297 egg averaging 216 eggs per female. Ages of breeders were largely unknown given that they were all wild collected, but many individuals have been retained for breeding for several years at CFI and could be as old as four to five years. A total 1779 larvae survived to hatch (88.8%, Table 3.1). A total of 968 juveniles were produced in 2008, a transformation rate of 54.4% from pelagic larvae to benthic juveniles and a total survival rate from eggs to juveniles of 48.3% (Table 3.1). The average of juveniles survived per female was 61.

Egg size at spawn was 2.2–2.4 mm, and prior to hatch was 2.4–2.6 mm (Table 3.1, Figs. 3.2A–B). Larval Boulder Darters at hatch ranged from 8.5–9.1 mm TL with a yolk sac, but not a very heavy yolk sac as compared to those of Spotted Darters and Wounded Darters (Table 3.1, Fig. 3.2C). Larval size at yolk sac absorption was 9.5–9.6 mm TL. Transformation of fins on larvae started 14–16 days post hatch. Larval size ranged from 14.0–15.0 mm TL at the time of initial development of first dorsal fin (Table 3.1, Fig. 3.2D). Full juvenile transformation of larvae including a fully-developed first dorsal fin was completed by 19–21 days post hatch. At this time, larvae ranged from 16.0–17.0 mm TL and were fully benthic (Table 3.1, Fig. 3.2E). Dorsal myomere count for larvae ranged from 37–39 (N=17) with a mode of 38 (Table 3.1).

Wounded Darter

Wounded Darters spawned for 89 days (21 April–18 July 2008) within a water temperature range of 16.0–24.0°C. The three breeding groups consisting of two males and two females each varied in production from 187 to 448 eggs per female (average of 345; Table 3.1). Number of clutches per breeding group ranged from 13 to 17, with 29 eggs per clutch in the former and 47 to 53 in the latter. Clutch sizes, examined every 3–7 days, ranged from 2–100 eggs. Age of females was unknown given all females were wild caught and adults, but was estimated at 2–3 years. Egg size at spawn was 2.1 – 2.3 mm, roughly spherical in shape (Fig. 3.2F). At hatch, eggs were oblong with the short diameter of 2.2 mm and the long diameter of 2.5 mm (Fig. 3.2G). Hatch times for eggs laid in late April were 7–9 days, however, hatch times generally decreased as temperatures increased during the spawning season.

Larval size at hatch was approximately 7.8 mm TL (Table 3.1, Fig. 3.2H). Larval size at yolk sac absorption was 9.7–9.8 mm TL. Transformation of fins on larvae started 12–14 days

post hatch. Larval size ranged from 13.5–14.0 mm TL at the time of initial development of first dorsal fin (Table 3.1, Fig. 3.2I). Full juvenile transformation of larvae including a fully-developed first dorsal fin was completed by 18–22 days post hatch. At this time, larvae ranged from 16.5–17.5 mm TL and were fully benthic (Table 3.1, Fig. 3.2J). Dorsal myomere counts on larvae ranged from 35–39 (N=19) with a mode of 37 (Table 3.1).

Out of 2070 eggs produced, 709 larvae were transferred from the catch tub to the rearing tub, representing a 34.3% hatch rate (Table 3.1). Of these 709 larvae, 539 transformed into juveniles and were transferred to tanks for grow out, representing a 76.0% survival rate from pelagic larvae to benthic juveniles (Table 3.1). Survivorship from egg to juvenile was 26.0%. Unlike both the Spotted Darter and Boulder Darter there was some juvenile loss after transformation given that only 494 survived to be released. The average of juveniles survived per female was 90.

Spotted Darter

Spotted Darters spawned for 46 days (29 April–13 June 2008) within a range of water temperatures of 17.0–22.5°C. An average of 191 eggs was produced per female (Table 3.1). Water temperature during this initial spawning event was 17.0°C (Fig. 3.1). Eggs from the two nests were approximately the same age, because eggs were not observed in tanks on the previous day. Tiles with attached eggs were placed in the incubation tank, where passive capture of hatched larvae was expected during swim up. Several eggs, however, were removed with a razor from the first nest slate and placed in a flow through tray for ease of access and tracking for photographs of development. Egg size at lay was 1.9–2.0 mm, and egg shape was oblong just prior to hatch with approximate size of 1.9-mm width and 2.1-mm length (Table 3.1, Figs. 3.2K–

L). Larval Spotted Darters at hatch were approximately 6.7–7.0 mm TL and had heavy yolk sacs (Table 3.1, Fig. 3.2M). Size at yolk sac absorption was 8.0–8.4 mm TL. Transformation of fins on larvae started 14–16 days post hatch. Larval size ranged from 12–13 mm TL at the time of initial development of first dorsal fin, and dorsal myomere count was 33–35 (N=17) with a mode of 35 (Table 3.1, Fig. 3.2N). Full juvenile transformation of larvae including a fully-developed first dorsal fin was completed by 19–21 days post hatch. At this time, larvae ranged from 16–17 mm TL and were fully benthic (Table 3.1, Fig. 3.2O).

The Spotted Darters produced 1124 eggs, approximately 187 eggs being produced per each female. The number of eggs per female differed among spawning groups; two males and one female produced an average of 211 eggs per female, one male and two females produced an average of 128 eggs per female, and one male and one female produced an average of 116 eggs per female. A total of 714 larvae were transferred from the catch tub to the rearing tub, representing a 63.5% hatch rate (Table 3.1). Of these 714 larvae, 517 transformed into juveniles resulting in a 72.4% survivorship of larvae after initial swim up (Table 3.1). The total survivorship of Spotted Darters from egg to juvenile was 45.7% (Table 3.1). The average of juveniles survived per female was 86.

Discussion

Boulder Darter

Based on observations of *E. wapiti* at CFI during 1995–2012, spawning initiates with temperatures reaching 15.5–17.5°C and ends with temperatures consistently staying over 23.5°C (Rakes et al. 2001, 2002, 2005, 2006, 2008, 2009; Petty et al., 2010, 2011, 2012). From 1995 through 2008 the Boulder Darters spawned only five to six weeks consistently each year (Rakes

et al. 2001, 2002, 2005, 2006, 2008). In 2008 the temperature range during the Boulder Darter spawning was 17.0–22.5°C. A few exceptions to the six week breeding window have occurred over the past few years yielding longer breeding seasons. In 2009, 2010, and 2012 there were warmer than normal late winter/early spring temperatures causing spawning to start at the first of April (Petty et al. 2010, 2012, Rakes et al. 2009). The 2009 spawning season was a total of nine weeks and the 2010 season was 10 weeks (Rakes et al 2009, Petty et al 2010). With additions in environmental controls installed in late 2008, CFI was able to maintain lower temperatures through the late spring, therefore extending the breeding season of the darters by three weeks (Rakes et al. 2009, Petty et al. 2010, 2012). Maintaining temperatures under 23°C likely contributed to longer breeding seasons of the Boulder Darter than observed in the past. This suggests temperature could be more influential than photoperiod to induce and terminate spawning.

The very high 2008 hatch rate (88.8%) of Boulder Darter eggs is consistent with those observed from 2000–2012 with very few exceptions (Table 3.1) (Rakes et al 2001, 2002, 2005, 2006, 2008, 2009; Petty et al. 2010, 2011, 2012). The relatively low larval transformation rate in 2008 (54.4%) was below average for the species (Table 3.1) (Rakes et al 2001, 2002, 2005, 2006, 2008, 2009; Petty et al. 2010, 2011, 2012). The low larval transformation rate was possibly due to poor water quality and excess food build up within the feeding tubs causing a disease issue (Table 3.1). Past larval transformation percentages have ranged from 37% in 2000 to 90% in 2005. The 2008 larval losses that were noted from 15 May through 6 June 2008 in the first of four rearing tubs might account for this slightly lower than average juvenile transformation. The total survival rate from eggs to juveniles (48.3%) was relatively high compared to the total survival rate of 22.3–59.4% from 2000–2012 (Table 3.1) (Rakes et al. 2001, Petty et al. 2012).

Conservation Fisheries Inc. has modified their breeding and incubating protocols over the years and the percent survivorship has been increasing accordingly.

Of the three species in this study the Boulder Darter had the largest eggs just prior to hatch (2.4–2.6 mm) as well as the largest larvae at hatch (8.5–9.1 mm TL) (Table 3.1). This may partly explain the increased egg hatch rate and larval survivorship rate of this species as compared to the other two species. Boulder Darter larvae swam up with a much reduced yolk sac as compared to both the Wounded Darter and the Spotted Darter larvae, suggesting that the larvae are more developed than the other two species at a critical time of swim up and first feeding. The eggs laid per female were lowest with the Boulder Darter (163 per female) as compared to the Wounded Darter and the Spotted Darter (Table 3.1). This could be because there is more investment in each egg as compared to the other two species, suggesting that the Boulder Darter's reproductive advantage is producing fewer, but more viable eggs and larvae.

Wounded Darter

Wounded Darter propagation protocols, as expected, were found to be essentially identical to those developed and refined since 1995 for a close relative, the Boulder Darter, *E. wapiti* (Rakes et al. 1999). The Wounded Darters spawned for more than 12 weeks in 2008 and this pattern has held consistent through 2012 (Petty et al. 2009, 2010, 2011, 2012). CFI observations (2008-2012) found the initiation of *E. vulneratum* spawning correlates with temperatures reaching an average of 15.0–16.0°C, while termination of spawning correlates with temperatures consistently staying over 24.5°C (Petty et al., 2009, 2010, 2011, 2012). In 2008 the prime temperature range during the Wounded Darter spawning was 16.0–24.0°C (Table 3.1). In 2009, 2010, and 2012 there were warmer than normal late winter/early spring temperatures causing spawning to start at the very first of April and extending the spawning season to as much

as 16 weeks (Petty et al. 2010, 2012, Rakes et al. 2009). As reported previously for the Boulder Darter, CFI was able to maintain lower temperatures through the late spring, therefore extending the breeding season of the Wounded Darters several weeks (Rakes et al. 2009, Petty et al. 2010, 2012).

The relatively low hatch rate (34.3%) of Wounded Darter eggs was influenced by egg infertility and possibly water quality (Table 3.1). Of the 2070 eggs, some were infertile, but many heavy yolk sac hatchlings succumbed before swimming up during a period of poor water quality. This poor water quality issue was likely due to heavy organics building up in the system from the automatic feeder. However, this poor survival pattern has held consistent with Wounded Darter eggs and larvae, with hatch rates ranging from as low as 22.8% in 2012 to as high as 48.2% in 2011 (Petty et al. 2009, 2010, 2011, 2012). Thus, the 2008 hatch rate was within the range of those from other Wounded Darter studies at CFI from 2009–2012. Juvenile transform rates for the Wounded Darter range from 61.0% in 2012 to 76.9% in 2008, showing that this species' larval survival is usually much higher than the egg survivorship (Petty et al. 2009, 2010, 2011, 2012). Overall egg to juvenile survival rate for this species is very low ranging from low of 13.9% in 2012 to a high of 32.5% in 2011, therefore the total survival rate from egg to juvenile in 2008 of 26% was average for the species (Table 3.1).

In 2008 the survivorship of larvae was high. Losses that were noted occurred during early June, likely due to water quality declines during peak automatic feeding time. After a water change and system medication of formalin semi-daily, losses stopped. It is also possible that some cannibalism took place during late larval transfers in July. Growth of larvae greatly increased as water temperatures increased, making it hard to transfer older larvae and juveniles

out of the larval rearing tub fast enough to maintain similar-sized larvae. Some older larvae and juveniles were seen chasing new larvae, and we have observed cannibalism in other species.

Of the three species, the Wounded Darter had intermediate sized eggs just prior to hatch (2.4–2.5 mm) as well as intermediate sized larvae (7.7–8.0 mm TL) (Table 3.1). Unlike both the Boulder Darter and Spotted Darter the average number of eggs laid per female was very high (345) (Table 3.1). It was also noted that the Wounded Darter larvae swam up with the largest yolk sac as compared to that of both the Boulder Darter and the Spotted Darter, suggesting that the larvae are the least developed at the critical time of hatching and first swim up possibly making them the most susceptible to mortality during this stage. This could be partly due to a much reduced investment in each egg as compared to the other two species, suggesting that the Wounded Darter's reproductive strategy is producing a greater number of eggs, although possibly at the sacrifice of overall survivorship rate.

Spotted Darter

Due to the close relatedness of the Spotted Darter to both the Boulder Darter and the Wounded Darter all the same propagation protocols were applied to this species. As for the Boulder Darter, in 2008 the temperature range during Spotted Darter spawning was 17–22.5°C (Table 3.1). The total spawn time was just less than six weeks. Spawning ceased when water temperatures exceeded 22.5°C, suggesting that, like the Boulder Darter, water temperatures have a strong influence on both the initiation and cessation of spawning.

Out of 1124 eggs produced, 714 larvae were transferred over to the rearing tub from the catch tub. This is a 63.5% hatch rate (Table 3.1). Of these 714 placed in the rearing tub, 517 were transferred to tanks for grow out after they had transformed into juveniles. This yielded a 72.4%

survivorship of larvae to juvenile stage and an overall survivorship of eggs to juvenile stage of 45.7% (Table 3.1). This overall egg to juvenile survival rate was very similar to that of the Boulder Darter. It is possible that most of the losses of larvae occurred in the last month when temperatures were higher. Both excessive feeding and water quality issues might have played a part in poor larval survivorship in late May through early June. Up until this time transferred larval numbers were consistent. Another influence on larval survivorship may also be connected to predatory behavior of the oldest larvae on the youngest.

Of the three species, the Spotted Darter had the smallest eggs just prior to hatch (1.9–2.1 mm) as well as the smallest larvae (6.7–7.0 mm TL) (Table 3.1). Numbers of eggs produced per female Spotted Darters were higher (191 per female) than those of the Boulder Darter. Although the eggs were smaller than both other species, the egg hatch rate of 61.0% and larval survivorship to juvenile stage of 72.4% was intermediate (Table 3.1).

From an ecological perspective, this research compared life history parameters among three species that differ in having northern and southern distribution ranges, and small to relatively wide distribution ranges. Although all three species employed the same K-selected reproductive strategy of male guarded nests, they varied in the number of eggs produced per female, size of eggs and larvae, hatch rate, and total larval survivorship when compared via captive propagation. The Boulder Darter and Spotted Darter produced far fewer eggs, but these had a much higher survivorship rate to juvenile stages than Wounded Darter eggs, illustrating reproductive variability among these closely related species.

Literature Cited

- Amano, M., T. Yamanome, H. Yamada, K. Okuzawa, and K. Yamamori. 2004. Effects of photoperiod on gonadotropin-releasing hormone levels in the brain and pituitary of underyearling male barfin flounder. *Fisheries Science* 70: 812–818.
- Biggins, R. G. 1988. Endangered and threatened wildlife and plants; determinations of threatened status for the boulder darter. *Fed. Reg.* 53: 33996–33998.
- Biggins, R. G. 1989. Recovery plan for boulder darter (*Etheostoma sp.*). U.S. Fish & Wildlife Service, Southeast Region, Atlanta. 15pp.
- Boschung, H. 1976. An evaluation of the slackwater darter, *Etheostoma boschungi*, relative to its range, critical habitat, and reproductive habitat in the Cypress Creek watershed and adjacent stream systems. U.S.D.A. Soil Conservation Service, Auburn, Alabama. 50 pp.
- Bryan J. L., M. L. Wildhaber, and D.B. Noltie. 2006. Influence of water flow on Neosho madtom (*Noturus placidus*) reproductive behavior. *American Midland Naturalist* 156: 305–318.
- Burkhead, N. M. and J. D. Williams. 1992. The boulder darter: a conservation challenge. *Endangered Species Technical Bulletin* 13: 3–8.
- Clark, R. W., A. Henderson-Arzapalo, and C. V. Sullivan. 2005. Disparate effects of constant and annually-cycling daylength and water temperature on reproductive maturation of striped bass (*Morone saxatilis*). *Aquaculture* 249: 497–513.
- Etnier, D. A. and J. M. Williams. 1989. *Etheostoma (Nothonotus) wapiti* (Osteichthyes: Percidae), a new darter from the southern bend of the Tennessee River system Alabama and Tennessee. *Proceedings of the Biological Society of Washington* 102:987–1000.
- Etnier, D. A. and W. C. Starnes. 1993. *The Fishes of Tennessee*. The University of Tennessee Press, Knoxville. 689pp.
- Hubbs, C., 1985. Darter reproductive seasons. *Copeia* 1985: 56–68.
- Johnston, C. E., and W. R. Haag. 1994. Life history of the Yazoo darter (Percidae: *Etheostoma raneyi*), a species endemic to north-central Mississippi. *Tulane Studies in Zoology and Botany* 30: 47–60.
- Knouft, J. H., L. M. Page, and M. J. Plewa. 2003. Antimicrobial egg cleaning by the fringed darter (Perciformes: Percidae: *Etheostoma crossopterum*): implications of a novel component of parental care in fishes. *Proceedings of the Royal Society B* 270: 2405–2411.
- Mattingly, H. T., J. Hamilton, and D. L. Galat. 2003. Reproductive ecology and captive breeding of the threatened Niangua Darter *Etheostoma nianguae*. *American Midland Naturalist* 149: 375–383.
- Mayasich, J. M., D. Grandmaison, and D. Etnier. 2004. Spotted Darter status assessment. Natural Resources Research Institute Technical Report to U.S. Fish and Wildlife Service Region 3. 27 pp.

- Near, T. J., and B. P. Keck. 2005. Dispersal, vicariance, and timing of diversification in *Nothonotus darters*. *Molecular Ecology* 14: 3485–3496.
- Osier, E.A. and S.A. Welsh. 2007. Habitat use of *Etheostoma maculatum* (spotted darter) in Elk River, West Virginia. *Northeastern Naturalist* 14:447–460.
- Page, L. M. 1983. *Handbook of Darters*. T.F.H. Pub., Inc., Neptune City, NJ. 271pp.
- Page, L. M. 1985. Evolution of reproductive behaviors in percid fishes. *Illinois Natural History Survey Bulletin* 33:275–295.
- Page, L.M. and B.M. Burr. 2011. *Peterson field guide to freshwater fishes of North America north of Mexico*. Houghton Mifflin Harcourt, Boston. 663 pp.
- Paine, M. D. 1990. Life history tactics of darters (Percidae: Etheostomatiini) and their relationship with body size, reproductive behaviour, latitude and rarity. *Journal of Fish Biology* 37: 473–488.
- Petty, M. A., C.L. Ruble, P.L. Rakes, and J.R. Shute. 2009. Hatchery Propagation and Reintroduction of Wounded Darters, *Etheostoma vulneratum*, in the Cheoah River, North Carolina. Unpublished report to NC Division of Water Resources, Cheoah Fund.
- Petty, M. A., C.L. Ruble, P.L. Rakes, and J.R. Shute. 2010. Propagation and Reintroduction of Wounded Darters, *Etheostoma vulneratum*, in the Cheoah River, North Carolina. Unpublished report to NC Division of Water Resources, Cheoah Fund.
- Petty, M. A., P.L. Rakes, C.L. Ruble, J.R. Shute, and R.A. Xiques. 2011. Propagation and Reintroduction of Wounded Darters, *Etheostoma vulneratum*, in the Cheoah River, North Carolina. Unpublished report to NC Division of Water Resources, Cheoah Fund.
- Petty, M. A., P.L. Rakes, C.L. Ruble, J.R. Shute, and R.A. Xiques. 2012. Propagation and Reintroduction of Wounded Darters, *Etheostoma vulneratum*, in the Cheoah River, North Carolina. Unpublished report to NC Division of Water Resources, Cheoah Fund.
- Petty, M.A., C.L. Ruble, P.L. Rakes, and J.R. Shute. 2010. Hatchery propagation and reintroduction of Wounded Darters, *Etheostoma vulneratum*, into the Cheoah River, North Carolina. Final report to NC Division of Natural Resources, Raleigh, NC.
- Petty, M.A., P. L. Rakes, J. R. Shute, and C. L. Ruble. 2010. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2010. Unpublished report to Tennessee Wildlife Resource Agency.
- Petty, M.A., P. L. Rakes, J. R. Shute, and C. L. Ruble. 2011. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2011. Unpublished report to Tennessee Wildlife Resource Agency.
- Petty, M.A., P. L. Rakes, J. R. Shute, and C. L. Ruble. 2012. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2012. Unpublished report to Tennessee Wildlife Resource Agency.

- Rakes, P. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. *Environmental Biology of Fishes* 55: 31–42.
- Rakes, P. L., P. W. Shute, and J. R. Shute. 2001. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2001. Unpublished report to Tennessee Wildlife Resource Agency.
- Rakes, P. L., and J. R. Shute. 2002. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2002. Unpublished report to Tennessee Wildlife Resource Agency.
- Rakes, P. L., and J. R. Shute. 2005. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2005. Unpublished report to Tennessee Wildlife Resource Agency.
- Rakes, P. L., and J. R. Shute. 2006. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2006. Unpublished report to Tennessee Wildlife Resource Agency.
- Rakes, P. L., J. R. Shute, C. L. Ruble, and M. A. Petty. 2008. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2008. Unpublished report to Tennessee Wildlife Resource Agency.
- Rakes, P. L., M. A. Petty, J. R. Shute, and C. L. Ruble. 2009. Captive Propagation and Population Monitoring of Rare Southeastern Fishes: 2009. Unpublished report to Tennessee Wildlife Resource Agency.
- Simon, T. P., 1987. Description of eggs, larvae and early juveniles of the Stripetail Darter, *Etheostoma kennicotti* (Putnam) and Spottail Darter, *E. squamiceps* Jordon (Percidae: Etheostomatini) from tributaries of the Ohio River. *Copeia* 1987: 433–442.
- Simon, T. P., and N. J. Garcia. 1990. Description of eggs, larvae, and early juveniles of the stippled darter, *Etheostoma punctulatum* (Agassiz), from a tributary of the Osage River, Missouri. *Southwestern Naturalist* 35: 123–129.
- Simon, T. P., and J. B. Kaskey. 1992. Description of eggs, larvae, and early juveniles of the bigscale logperch, *Percina macrolepida* Stevenson, from the West Fork of the Trinity River basin, Texas. *Southwestern Naturalist* 37: 28–34.
- Storey, C. M., B. A. Porter, M. C. Freeman, and B. J. Freeman. 2006. Analysis of spawning behavior, habitat, and season of the federally threatened *Etheostoma scotti*, Cherokee Darter (Osteichthyes; Percidae). *Southeastern Naturalist* 5: 413–424.
- Zorach, R. and E. C. Raney. 1967. Systematics of the percid fish, *Etheostoma maculatum* Kirtland, and related species of the subgenus *Nothonotus*. *American Midland Naturalist* 77: 296–322.

Table 3.1. Reproductive biology and early life history parameters of Boulder Darters, Wounded Darters, and Spotted Darters from a captive propagation study during 2008.

Parameter	<i>E. wapiti</i>		<i>E. vulneratum</i>		<i>E. maculatum</i>	
	N	Value	N	Value	N	Value
Spawning period (days)		48		89		46
Spawning water temperatures (C°)		17.0–22.5		16.0–24.0		17.0–22.5
Average eggs/female	16	163	6	345	6	191
Percent egg hatch		88.8%		34.3%		63.5%
Percent larval transform		54.4%		76.0%		72.4%
Percent total survive		48.3%		26.0%		45.7%
Average juvenile transform per female	16	61	6	90	6	86
Egg diameter at deposition (mm)	4	2.2–2.4	3	2.1–2.3	2	1.9–2.0
Egg diameter prior to hatch (mm)	7	2.4–2.6	8	2.2–2.5	10	1.9–2.1
Larvae length at hatch (mm)	3	8.5–9.1	2	7.7–8.0	4	6.7–7.0
Larvae length at yolk sac absorption (mm)	4	9.5–9.6	3	9.7–9.9	6	8.0–8.4
Myomere count	17	37–39	19	35–39	17	33–35
Larvae length at start of first fin development	5	14.0–15.0	5	13.5–14.0	5	12.0–13.0
Age at first fin development (days)	5	14–16	5	12–14	5	14–16
Length of juvenile (mm)	5	16.0–17.0	5	16.5–17.5	5	16.0–17.0
Age of full transformation (days)	5	19–21	5	18–22	5	19–21

