Ecology and Conservation of the Threatened Blackside Dace, *Chrosomus cumberlandensis*

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Cover Photograph: *Chrosomus cumberlandensis* (Blackside Dace) adults in nuptial coloration, photographed on 25 April 2013 by J.R. Shute at the Conservation Fisheries, Inc. facility in Knoxville, TN. See article on pp. 162—170.

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Spawning and Captive Propagation of Blackside Dace, *Chrosomus cumberlandensis*

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Abstract - *Chrosomus cumberlandensis* (Blackside Dace) is a federally protected stream fish endemic to the upper Cumberland River drainage of Kentucky and Tennessee. Captive propagation of the species has been conducted in only one previous study. Here we report new findings from spawning and rearing the species for the past three years, 2011–2013, at the Conservation Fisheries, Inc. (CFI) facility in Knoxville, TN. Brood stock (n = 80 adults) were collected in 2011–2012 from Big Lick Branch in Pulaski County, KY. Spawning at the CFI facility occurred in April and May of each year when CFI water temperatures were between 16 and 21 °C. We compare CFI spawning dates and temperatures to those measured in a 2006 field study. We also provide video footage of spawning behavior, which conformed to that previously described in the literature. Per capita production of fry (i.e., number of juveniles reared per breeding adult) in 2012 was more than twice that achieved in the previous study. We progressively reduced the presence of (and cues from) other fish species in 2011, 2012, and 2013, with the eventual achievement of having Blackside Dace spawn completely independent from any heterospecific cues. Our results collectively increase the capacity to propagate Blackside Dace in captivity to support ongoing recovery efforts for this threatened species.

Introduction

Captive breeding and reintroduction programs have a number of recognized limitations, yet they often play vital roles in the conservation of endangered species. Among freshwater fishes, there are several examples of successful restoration of species to their native waters (e.g., Mueller and Wydoski 2004, Shute et al. 2005). However, protocols for captive propagation and/or translocation have not been developed for many freshwater fishes, thereby restricting conservation options for those species.

Successful captive propagation of any given fish species is facilitated by advance knowledge of its spawning habits in a natural setting. However, in the diverse minnow family, Cyprinidae, spawning modes are known for only 13 of 46 imperiled species (Johnston 1999). Johnston and Page (1992) reviewed the reproductive strategies of cyprinids and identified eight different categories. Six of the eight strategies involve preparation or use of substrate to form a nest in which eggs are deposited and fertilized. Parental care in the form of nest guarding is provided by males in some instances.

One important aspect of cyprinid reproduction is the nest association behavior displayed by at least 33 species (Johnston and Page 1992). Nest association

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occurs when one species, the host, prepares a nest that another species, the associate, uses for spawning (Johnston and Page 1992). The host species is typically another cyprinid, but some associates use centrarchid or other nests (e.g., Fletcher 1993, Hunter and Hasler 1965, Johnston and Page 1992). Certain cyprinids show flexibility by spawning either (1) as an associate in the nests of hosts or (2) independent of the host by broadcasting or by building their own nests (Johnston and Page 1992). Understanding the degree of host dependence and host specificity can inform captive breeding protocols and establish criteria for selecting reintroduction sites to ensure that appropriate hosts are present.

Chrosomus cumberlandensis (Starnes and Starnes 1978) (Blackside Dace) is a federally protected cyprinid whose known native range is restricted to small tributaries in the upper Cumberland River system in southeastern Kentucky and northeastern Tennessee (Black et al. 2013a [this issue]; Eisenhour and Strange 1998; O'Bara 1988, 1990; Starnes and Etnier 1986; Starnes and Starnes 1978, 1981; USFWS 1988). The species inhabits cool (<20 °C) headwater streams with stable substrates, low water conductivity (<240 μS), and sufficient in-stream cover (Baxter 1997, Black et al. 2013b [this issue], O'Bara 1990).

Blackside Dace reproduction in a natural stream was first reported by Starnes and Starnes (1981), who observed the species using a broadcasting spawning mode over substrate in a nest of *Campostoma anomalum* (Rafinesque) (Central Stoneroller). These authors observed one spawning event on 17 May 1981 at a water temperature of 17.5 °C. Cicerello and Laudermilk (1996) later observed a school of nuptial Blackside Dace over the occupied nest of *Semotilus atromaculatus* (Mitchill) (Creek Chub) on 12 May 1993, although actual spawning was not observed. Mattingly and Black (2013 [this issue]) observed 25 Blackside Dace spawning events from 12 May to 12 June 2006 at water temperatures of 11.9 to 18.2 °C, all of which occurred over Creek Chub nests. These field observations confirm that Blackside Dace is a nest-associating species that uses at least two cyprinid host species. No field reports of Blackside Dace spawning independent of cyprinid hosts are known, although most authors have presumed that independent spawning may occur.

Rakes et al. (1999) conducted the first study of Blackside Dace propagation in captivity. Twenty-four adult Blackside Dace were collected from Buck Creek (Whitley County, KY) on 7 May 1993. The dace were maintained at 16 °C in Knoxville, TN, at the Conservation Fisheries, Inc. hatchery (hereafter CFI facility) in two 75-L aquaria lined with gravel and pebble substrate. Mounds of substrate were arranged in the aquarium to create artificial "chub nests" (20 cm wide, 30 cm long, 5 cm high), thereby simulating a cyprinid host nest. Flowing water was directed over the mounds to mimic natural conditions. The fish spawned upon first arrival at the CFI facility but subsequently ceased reproductive activity. Rakes et al. (1999) then used milt (see Hunter and Hasler 1965) from a reproductively mature male Central Stoneroller and *Nocomis micropogon* (Cope) (River Chub) to induce additional Blackside Dace spawning activity. Three hundred and thirty fertile eggs (230 before addition of milt and 100 after milt) were siphoned from the mounds and moved to incubation trays. Selected early life-history information was recorded, including egg diameter (1.0 mm), egg characteristics (demersal, non-adhesive), egg deposition (among gravel and pebbles of artificial minnow nests), hatchling size (5 mm total length), characteristics of embryos and larvae (benthic approximately 48 h), foods used by larvae (live copepods, brine shrimp nauplii, commercially prepared rotifer-sized powdered food), and survival of fertile eggs to the juvenile stage (87%).

The goal of the present study is to build on the knowledge provided by Rakes et al. (1999) to further advance our understanding of Blackside Dace captive propagation and early life history. We propagated Blackside Dace in captivity for three consecutive years, 2011–2013, to accomplish this goal. Our specific objectives were to (1) refine captive propagation techniques to allow greater production of offspring, (2) determine the duration and survival of early life stages, (3) compare dates and temperatures of spawning in captivity to field observations, (4) determine whether Blackside Dace are capable of spawning independently in captivity without the presence of host fishes, and (5) describe spawning behavior, including the presentation of video footage of spawning.

Methods

Collection of wild brood stock

We collected a total of 80 adult Blackside Dace (*n* = 50 on 17 March 2011 and 3 May 2011, plus an additional *n* = 30 on 7 February 2012) from Big Lick Branch in Pulaski County, KY. Stream water temperatures were 7 °C, 13 °C, and 5.5 °C on the three collection dates, respectively. Big Lick Branch harbors one of the more robust populations known for Blackside Dace (Black et al. 2013a [this issue]). All fish were collected using a fine mesh seine and transported to the CFI facility in bags within insulated containers, then slowly acclimated to laboratory aquaria.

Presence of other fish species in aquaria

Multi-aquaria recirculating systems were utilized to house the brood stock and rear young in 2011, 2012, and 2013 (see Fig. 1 in Rakes et al. 1999). Each year, we progressively reduced the presence of cyprinid host fishes and cues from host fishes as summarized in Table 1. Briefly, in 2011 all 50 Blackside Dace and two reproductively mature male stonerollers (*Campostoma* sp.) were housed in a 380-L spawning aquarium that was part of a larger recirculating system. The stonerollers and then the Blackside Dace suffered a disease outbreak during early May 2011. The infection was successfully eliminated with medications, but only after losing over 50% of the fish. In 2012, brood stock were housed in two 170-L spawning aquaria that were part of a larger recirculating system; one housed the remaining older 20 dace collected in 2011 and the other housed the 30 additional Blackside Dace collected in 2012. Two male Creek Chub were present in another tank in the aquarium system, sharing the same water. In 2013, all 40 surviving Blackside Dace were housed in a single, 170-L aquarium with no contact with other cyprinids (or even water from other cyprinids) for approximately one year. Blackside Dace sex ratios were roughly equivalent in all three years, but precise determination was not made.

Introduction of milt to aquaria

Milt from reproductively mature male stonerollers (in 2011) as well as Creek Chubs and *Moxostoma* sp. "Sicklefin Redhorse" (in 2012) was used to induce spawning in 2011 and 2012. Dates of milt addition are provided in Table 1. Reproductive behavior and intensity of nuptial coloration instantly increased upon exposure to all the introductions of milt, and breeding continued for weeks following this stimulus. However, in 2013 we purposely did not introduce milt or any other chemical or visual cues.

Underwater physical conditions in aquaria

In a field setting, Blackside Dace have only been observed spawning over clean gravel nests constructed by Central Stonerollers and Creek Chubs (Cicerello and Laudermilk 1996, Starnes and Starnes 1981). Further, Mattingly and Black (2013 [this issue]) observed that Blackside Dace only spawned or exhibited spawning behaviors in areas where Creek Chubs had constructed a pit and ridge nest. Therefore, field surveys support the assumption that Blackside Dace may have a strong relationship with nest-building cyprinids. To simulate the nests over which Blackside Dace spawn, we provided our aquaria with flowing water and artificial "chub nests," with dimensions and details remaining the same as described above for the Rakes et al. (1999) study.

Photoperiod and temperature

Blackside Dace were maintained and conditioned in preparation for captive spawning. Photoperiod was controlled with an astronomic timer to mimic natural winter lighting conditions by slowly changing day length on a schedule comparable to the actual season. Food quantities were provided dependent upon water temperature and the accompanying activity levels of the fish. As spring approached, both water temperatures and day length were increased, closely following natural conditions (in Knoxville, TN). Feeding was increased as appetites tracked rising water temperature. High feeding activity and provision of food (2–3 times/day) began in early March as the fish came into breeding condition and feeding activity remained at high levels through mid-May each year.

In 2006, we measured water temperatures at 5 sites in 4 streams where Blackside Dace spawning events were observed by Mattingly and Black (2013 [this issue]). A single temperature datalogger per site was deployed in April 2006 and retrieved in December 2006. In 2011–2013, water temperatures at the CFI facility were measured and recorded by temperature dataloggers (T & D Corporation, Model RTR 500NW and Model RTR 502) and archived in an online storage database. We calculated daily mean temperature values from the hourly field and CFI-facility data from March or April through June each year, and used these mean values in qualitative comparisons between field and hatchery temperature conditions.

Collection and incubation of embryos

Fertilized and unfertilized eggs, as well as detritus, were removed every 2 to 10 days by vacuuming through the artificial pebble nests with an aquarium-cleaning siphon. The few eggs collected in 2011 appeared to be from one spawning event. A total of eight egg collections were performed throughout the spawning period in 2012, with each certainly encompassing multiple spawning events as evidenced by varied egg developmental stages. Likewise, we collected eggs from at least four different spawning events in 2013. Eggs were transferred with a pipette to the incubation trays; water temperature during incubation was 15–20 °C. Unfertilized eggs were discarded and all counts reported below are of fertilized eggs (i.e., embryos).

Our production goal in 2013 was different than in 2011 and 2012. In 2013 we simply wanted to produce ≈700 embryos for use in toxicity studies with collaborating researchers. After reaching our production goal, we stopped collecting additional eggs and ceased monitoring spawning activities of brood stock. We also did not track survival of embryos in 2013.

Results and Discussion

Production and survival of offspring

Only the largest, oldest Blackside Dace collected in 2011 successfully spawned that year, and only the fish collected in 2011spawned in 2012. Though apparently sexually mature, no eggs or larvae were recovered from the younger fish collected in 2012. Post-hatch yolk-sac larval production increased from a low of 71 fry produced from 117 eggs collected in 2011 (60% survivorship) to a peak of 1910 fry produced from the 2855 eggs collected in 2012 (67% survivorship). Production in 2013 was 840 eggs, but additional eggs were not collected and survival was not reported that year.

Our 2011–2012 survival rates (60-67%) were slightly lower than reported previously (87%) by Rakes et al. (1999). However, our per capita production (number of fry reared per breeding adult) in 2012 was 38.2, more than twice that achieved in the previous study (13.75; Rakes et al. 1999). We attribute the increased production capacity in 2012 to a variety of factors, including the 44-day duration of spawning activities that year, the health and condition of the brood stock, an increased variety of foods offered, and the absence of any serious disease outbreaks.

Eggs hatched relatively quickly (≈3 days), producing unpigmented immature yolk-sac larvae that remained benthic for ≈5 days of further development before swimming up to feed. The amount of time spent in benthic development in 2011–2012 was longer than the ≈2 days reported by Rakes et al. (1999). After swim-up, larvae were sufficiently large enough to feed on smaller brine shrimp nauplii, but rotifers (*Brachionus* sp.) and early water flea (*Ceriodaphnia dubia* Richard) instars were also provided, thereby expanding the early-life-stage dietary options reported by Rakes et al. (1999).

Dates and temperatures of spawning

Spawning at the CFI facility began in April when water temperatures warmed to approximately 16 °C and ended in mid-May when water temperatures reached 20 or 21 °C (Fig. 1). Spawning lasted 14 days in 2011, 44 days in 2012, and at least 18 days in 2013 (Table 1). We did not track spawning activities beyond 15 May 2013. Mattingly and Black (2103 [this issue]) observed a 32-day spawning season in their 2006 field study, with spawning events first observed in mid-May and last observed in mid-June, representing a shift of a few weeks later than captive spawning. In both field and captive settings, however, spawning ceased when water temperatures approached 20 or 21 °C (Fig. 1), suggesting that Blackside Dace may generally cease reproduction above that temperature. Water temperature may be a stronger cue than day length for initiation of Blackside Dace spawning, given the notable shift in CFI versus field spawning dates illustrated in each panel of Figure 1.

Independent spawning

Blackside Dace spawned completely independent of any cues from other host fish species in 2013, representing the first observation of independent spawning by the species in captivity. Independent spawning has never been reported in field studies, but we now know the species is capable of independent spawning in a captive setting. It remains unknown whether spawning and captive propagation is enhanced by independent spawning, because we did not measure survival in 2013 as we did in 2011 and 2012. Nevertheless, we do know that captive propagation is not entirely reliant on obtaining and introducing milt from other fish species.

Table 1. Hatchery conditions and results obtained during 2011, 2012, and 2013 spawning and rearing of Blackside Dace, *Chrosomus cumberlandensis*, at the CFI facility in Knoxville, Tennessee. NM = not recorded or not measured, *C* = *Campostoma* sp., *Sa* = *Semotilus atromaculatus*, *M* = *Moxostoma* sp.

	Year		
Characteristic	2011	2012	2013
Number of adult Blackside Dace brood stock	50	50	40
Number and size of spawning aquaria	1, 380-L	2, 170-L	1, 170-L
Other fish species in the same spawning aquaria	Yes (*C*)	No	No
Other fish species in shared recirculating system	Yes (NM)	Yes (*Sa*)	No
Addition of milt from other fish species	Yes (*C*)	Yes (*M, Sa*)	No
Dates of milt addition	26 April	2 April, 10 April	None
Date of first spawning observation	28 April	2 April	28 April
Date of last spawning observation	11 May	15 May	NM
Water temperatures during spawning (°C)	17—20	16—21	17—21
Number of fertile eggs collected	117	2,855	840
Time to hatching	3 d	3 d	NM
Post-hatching time spent as benthic	5 d	5 d	NM
Number of fry produced	71	1,910	NM
Survival from embryo to fry stage	60%	67%	NM

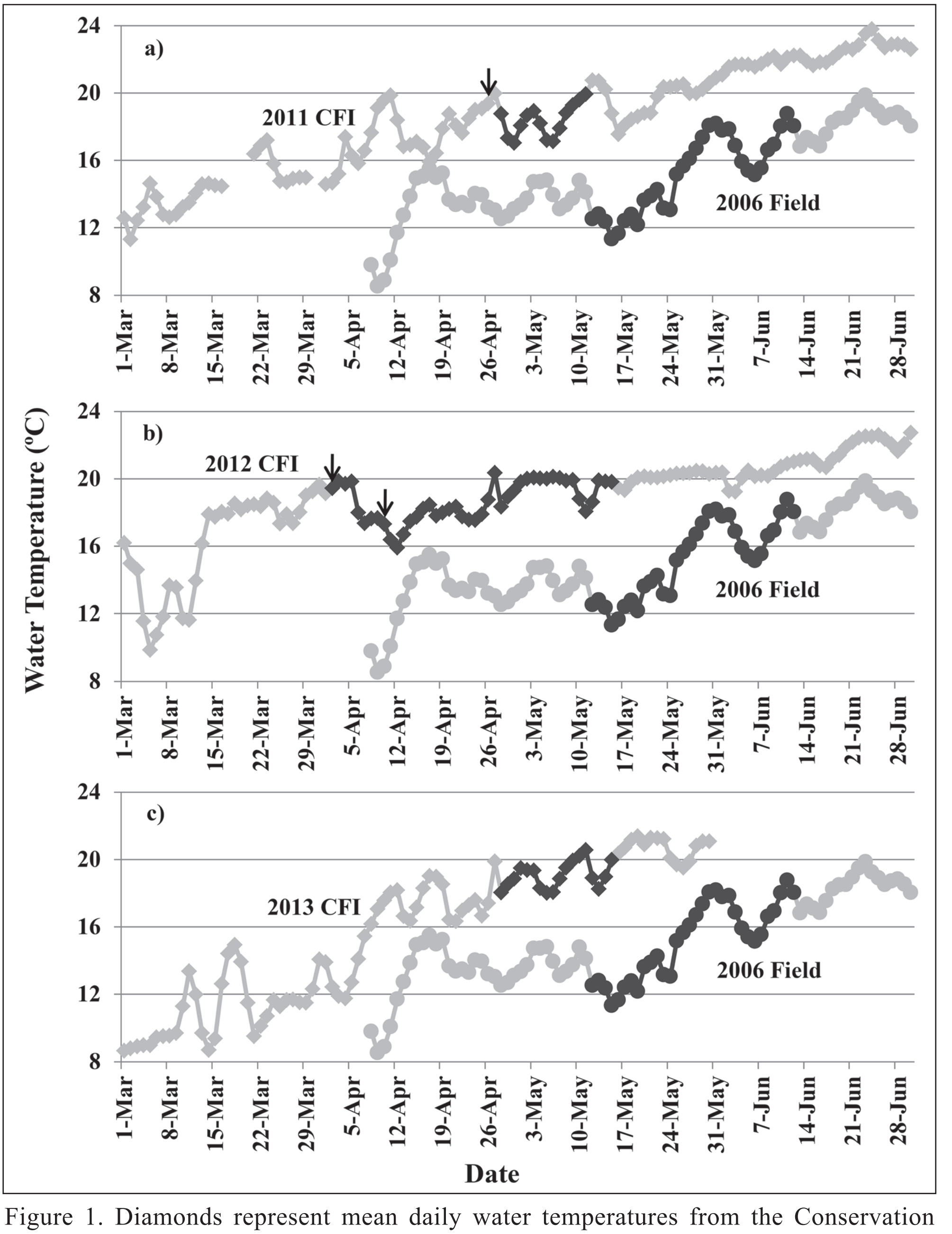

Figure 1. Diamonds represent mean daily water temperatures from the Conservation Fisheries, Inc. (CFI) facility in Knoxville, TN measured during a) 2011, b) 2012, and c) 2013. Circles represent mean daily water temperatures averaged from five sites in four *Chrosomus cumberlandensis* (Blackside Dace) streams in 2006 to generate a single field-water-temperature profile to visually compare to CFI temperatures. Black diamonds and circles indicate when spawning was observed in each setting, and gray diamonds and circles indicate when spawning was not observed. Arrows indicate when heterospecific milt was added in 2011 and 2012 to stimulate Blackside Dace spawning activities. Milt was not added in 2013.

Spawning behavior

Spawning behavior generally conformed to that described by Starnes and Starnes (1981). Spawning activity and nuptial coloration peaked in the morning hours (in the absence of milt additions), and usually "pods" of 2–3 males were observed following or courting a gravid female. We observed spawning events taking place on the mounded substrate "chub nests" provided in the spawning aquaria. Although presumed spawning acts were observed via closed circuit video (see Supplemental Video File 1, available online at http://www.eaglehill.us/SENAonline/suppl-files/s12-Sp4-1040i-Rakes-s1, and, for BioOne subscribers, at http://dx.doi.org/10.1656/S1040i.s1), actual egg releases were difficult to observe and searches of the substrate immediately afterwards only occasionally yielded eggs. At the peak of breeding when water temperature was approximately 18 °C, brilliant coloration was displayed by males and females during spawning and was most pronounced in late April. Males had a golden dorsum and bright yellow paired fins and anal fin, while females exhibited more subdued coloration and lacked yellow or gold.

Conclusions

We successfully spawned and reared Blackside Dace in captivity for the past three years, with desirable rates of juvenile production in 2012 and 2013. Water temperature and heterospecific milt appear to be strong abiotic and biotic cues for induction of spawning, with egg deposition in captivity beginning in April when water temperatures exceeded 16 or 17 °C. Spawning in a 2006 field study by Mattingly and Black (2013 [this issue]) started later in May when water temperatures were generally cooler than at CFI. Spawning in both settings ended as water temperatures approached or reached 20–21 °C in May (CFI) or June (field). Captive spawning of reproductively mature and conditioned Blackside Dace can be triggered within 0–2 days by addition of milt from other fishes, as also noted by Rakes et al. (1999). However, as we observed in 2013, captive spawning also can be initiated independently without the presence of other fishes or their milt. Our observation of independent spawning by Blackside Dace in captivity is the first reported for this species, yet it remains unknown whether the species practices independent spawning in a wild setting.

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Supplemental Video File 1 is available online at http://www.eaglehill.us/SENAonline/suppl-files/s12-Sp4-1040i-Rakes-s1, and, for BioOne subscribers, at http://dx.doi.org/10.1656/S1040i.s1.