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Reproductive Ecology and Spawning Substrate Preference of the Northern Leatherside Chub

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Abstract.-Conservation of rare fishes is often hindered by a lack of understanding of their basic life history characteristics. We used captive-breeding studies to determine the preferred spawning habitat and early life history characteristics of the northern leatherside chub Lepidomeda copei, a small cyprinid native to the upper Snake River basin and the Bear River drainage in the Bonneville Basin (Idaho, Utah, and Wyoming). In the first study, wild adult northern leatherside chub were given a choice of four habitats (two shallow riffle habitats and two deeper pool habitats), each with four spawning substrates (large cobble, small cobble, pebble, and a coarse artificial spawning mat) in a large rectangular tank. In the second study, adults were given a choice of three locations with different water velocities (9.5, 12.9, or 19.0 cm/s) but the same substrate size (small cobble). Successful spawning occurred in both studies. Northern leatherside chub spawned almost exclusively over the small cobble substrate (99.6% of total young). Habitat selection was significant, but the patterns were not consistent across tanks; uncovered pool habitat was selected in two tanks and the upper riffle habitat was primarily selected in the third tank. In addition, northern leatherside chub preferentially chose the site with the highest flow (19 cm/s) for spawning. Early life history characteristics (embryo size, time to hatch, larvae size, eggs per spawn, time to swimup, and fry growth) were estimated from the reproductive output of the spawning studies. Small cobble is needed for northern leatherside chub reproduction, and its availability may be among the limiting factors for northern leatherside chub populations.

Effective recovery efforts for threatened or endangered species require an understanding of their life history characteristics, including habitat utilization, growth, and reproduction. Recognition of the need for species conservation, however, often occurs after the populations have become too small to accurately understand or determine life history characteristics (Gaston 1994). Such is the case for minnows (Cyprinidae), a diverse group of fishes that are often viewed as baitfish and thus have received less public and financial support for conservation efforts than have sport fishes (Warren and Burr 1994; Rakes et al. 1999). Recent conservation efforts with a variety of minnow species have demonstrated that captive breeding not only provides individuals to supplement existing or restore extirpated populations but can also provide information on critical life history characteristics to aid in recovery efforts (Blinn et al. 1998; Rakes et al. 1999; Gibson and Fries 2005).

The northern leatherside chub Lepidomeda copei is a small cyprinid endemic to the Bear River drainage in the Bonneville Basin and the upper Snake River drainage of western North America (Sigler and Sigler 1987). The leatherside chub was once believed to be a cohesive species (Snyderichthys copei) that included populations in the Utah Lake drainage in central Utah and the Sevier River drainage in southern Utah, both of which are in the Bonneville Basin. On the basis of genetic, morphological, and ecological differences, however, it was recently separated into two distinct species with a new genus designation, namely, the northern leatherside chub and the southern leatherside chub L. aliciae (Johnson et al. 2004). The number of both species has declined dramatically in the past century, populations becoming extirpated in some locations and the remaining populations becoming increasingly isolated. Habitat degradation (e.g., impoundments, dewatering, and siltation) and introduction of nonnative species, particularly brown trout Salmo trutta, have been implicated in the decline of these species (Walser et al. 1999; Wilson and Belk 2001; Olsen and Belk 2005). Both species of leatherside chub warrant conservation efforts to conserve remaining populations; however, the rarity of the northern leatherside chub indicates a need for immediate conservation and recovery actions for this species (Johnson et al. 2004).

Current conservation strategies for the northern leatherside chub include developing techniques for captive breeding to help recovery efforts by way of supplementation and expansion within the species' historical range. Basic life history information about leatherside chub on which to base propagation protocols is limited, though recent efforts have provided some data (Johnson et al. 1995; Wilson and Belk 2001; Bell and Belk 2004). The objectives of this

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FIGURE 1.—Spawning tanks used to test the habitat and substrate spawning preferences of northern leatherside chub from March to September 2005. The four habitats were (1) upper riffle, (2) lower riffle, (3) uncovered pool, and (4)

study were (1) to propagate wild northern leatherside chub in indoor spawning systems and (2) to examine different techniques for hatching the resulting eggs. In spawning systems, adult fish were provided with different habitat characteristics (flow, depth, and cover) and substrates to determine acceptable spawning habitat.

Methods

Northern leatherside chub spawning studies were conducted at the Utah Division of Wildlife Resources' Fisheries Experiment Station (FES) in Logan, Utah, in 2005 and 2006. Northern leatherside chub used in the first study were collected by electrofishing in Deadman Creek, tributary to Mill Creek in the upper section of the Bear River drainage, Utah. On 4 October 2004, 20 adults were brought to the FES. Before we began the study, the fish were chemically treated with praziquantel (0.67 mg/L for 24 h on 6 October) and formalin (167 mg/L for 1 h on 12 October) to remove any internal and external parasites. The surviving adults (n = 15) were also used in the second study. In addition, for the second study we collected 44 wild adult

northern leatherside chub from Yellow Creek (a tributary to the Bear River) on 2 August 2005 and brought them to FES. These adults were also chemically treated for internal and external parasites before the study was begun.

Substrate and habitat selection.-In this test, we compared northern leatherside chub spawning habitat and spawning substrate selection. Spawning systems were constructed in three rectangular fiberglass tanks, following the design described by Gibson et al. (2004; Figure 1). Tanks 1 and 2 each had a capacity of 890 L, whereas tank 3 had a capacity of 700 L. Each tank had an artificial riffle created by suspending a fiberglass trough $(122 \times 35 \times 18 \text{ cm})$ at an angle on one side of the tank. Water flowed out of the open, downstream end of the riffle. The water velocity in the riffle, however, was too slow to be measured by a handheld flow probe (minimum flow detection, 9 cm/s; Intermountain Environmental, Inc., Logan, Utah), which indicated that the habitat more closely represented shallow habitat than riffle habitat. One set of four substrates was placed in each of four locations throughout the tank: (1) upper riffle, (2) lower riffle, (3) uncovered pool, and (4) covered pool (under riffle). Water depth at these locations was 10 cm for the upper riffle, 15 cm at the lower riffle, and 31 cm for the two pool habitats. The four substrates were pebble (0.5-1.5 cm in diameter; mean, 0.9 cm), small cobble (2.1-4.8 cm; mean, 3.1 cm), large cobble (5.8-11.2 cm; mean, 8.0 cm), and a coarse artificial spawning mat (Enkamat; Colbond, Arnhem, The Netherlands). These substrates were put into clear, plastic trays $(30 \times 16 \times 4)$ cm) such that substrate depth was about 3 cm. Artificial aquarium plants were also placed in the pool area. Well water (18°C) was added at 3.8 L/min at the top of the riffle in each tank. A pump (Mag Drive Model 7; Danner Manufacturing, Inc., Central Islip, New York) recycled water into the head of the riffle through a polyvinyl chloride (PVC) pipe manifold. A fullspectrum fluorescent light (two 122-cm bulbs) was on a timer to deliver a 14 h:10 h (light:dark) photoperiod. Black plastic curtains were hung around each tank to reduce disturbances from other activity in the room.

Before the study, the fish were kept in a circular tank supplied with 13–13.5°C well water and were fed a commercial flake diet (Tetramin Pro; Tetra, Blacksburg, Virginia), which they readily ate. In February 2005, the water temperature was slowly increased by mixing 13.5°C and 18°C well water; the water temperature reached 18°C after approximately 30 d. On 24 March 2005, 20 leatherside chub were transferred into spawning systems—7 fish each into tanks 1 and 2 and 6 into tank 3. Attempts were made to

covered pool.



include putative females in each of the three tanks. Females were identified by the shape and condition of the urogenital papillae and more robust body outline. Two fish from tank 3 died several days after introduction to the tank; one fish from each of the other two tanks was transferred into this tank so that each tank had 6 fish. Total fish weight in tanks 1, 2, and 3 was 43.5, 46.5, and 47.2 g; total lengths (TLs) ranged from 75 to 107 mm, from 79 to 119 mm, and from 71 to 125 mm, respectively. To increase the number of adults and the likelihood of spawning in each tank, all six adults from tank 3 were evenly distributed on the basis of size into tanks 1 and 2 on 13 July. Fish had already spawned in tanks 1 and 3, and reproduction occurred in both tanks 1 and 2 after fish were transferred from tank 3. The study was concluded 4 October 2005. Fish were fed at 3% of body weight per day with two automated feeders (Fish Feeder Model 3581; Eheim Co., Rockenhausen, Germany) in each tank, one delivering feed to the riffle and the other delivering feed at the edge of the cover provided by the riffle trough. Feed was delivered three times per day.

Substrates were visually scanned during weekly tank cleaning for the first 6 weeks after the fish were introduced. For the remainder of the study, each substrate was rinsed in a large plastic container to clean off waste feed and feces. The debris and cleaned substrate were then scanned for eggs and fry. If either were found, they were collected or removed from the substrate with a bulb pipette and enumerated.

Eggs collected before July were incubated in a 4.4-L clear plastic container supplied with well water at 0.1 L/min. After July, unattached eggs were incubated in a clear McDonald-type hatching jar, and eggs attached to the substrate were incubated in a clear plastic container receiving water from the outflow of the hatching jar. After swim-up, fry were enumerated and placed into 4.4-L clear plastic containers supplied with well water. Fry were fed an artificial rotifer diet (Hatchfry Encapsulon, grade 0; Argent Laboratories, Redmond, Washington) for approximately 2 weeks before being switched to frozen brine shrimp Artemia franciscana nauplii. A commercial ground-flake feed (Tetramin Pro) was fed in combination with brine shrimp nauplii after fry were 4 weeks old. For all feed types, fry were fed to excess.

To determine spawning preferences with respect to habitat and substrate, the data were analyzed through a mixed within-subjects analysis of variance (ANOVA; Zar 1999). Tanks were treated as a block, and habitat and substrates within each habitat were fixed factors. The number of young (eggs and fry) recovered within each habitat–substrate was used as the response variable; data were rank transformed before analysis. Fry were included because the majority had not reached the swim-up stage and because they were found in the same substrate as the unhatched eggs from the same cohort. The analysis was conducted in SAS 9.1.3 (SAS Institute 2006), with an α level of 0.05.

The experimental design had a limited sample size because we were constrained by the number of adults of this rare species that were available for the study. Similarly, the independence of the data is limited because adults from one tank were combined into the other two tanks in the middle of the study. However, test conditions were virtually identical in each tank; therefore, each fish was exposed to the same conditions regardless of tank. We also analyzed the data in a reduced design in which the young produced in tanks 2 and 3 were combined because no spawning occurred in tank 2 until the introduction of adults from tank 3. The lack of independence did not invalidate our results; therefore, we report the full analysis as results.

Water velocity .-- In this test, spawning site selection by northern leatherside chub was compared among three sites, each with a different water velocity. Spawning systems were constructed in five rectangular fiberglass tanks, each with a capacity of 890 L (Figure 2). In each tank, sheet PVC was placed vertically and lengthwise at an angle to create a channel that gradually increased in width around the tank. A submersible pump with a PVC pipe manifold forced water to flow around the tank, such that velocity decreased based on the increasing area of the channel (distance of the sheet PVC from the side of the tank). Small cobble substrates (21-48 mm in diameter; mean, 31 mm) were placed in clear plastic trays $(30 \times 16 \times 4)$ cm) in three locations: (1) high flow (19.0 cm/s), (2) medium flow (12.9 cm/s), and (3) low flow (9.5 cm/s). Curtains were placed around each tank to minimize disturbance from activity in the laboratory. A piece of foam insulation was placed over the center of the tank to provide cover for the fish. Full-spectrum fluorescent lights (each with two 122-cm bulbs) were on timers to deliver a natural photoperiod to each tank; timers were periodically adjusted to match the local photoperiod.

Adult northern leatherside chub from Yellow Creek were introduced into the three spawning systems in December 2005; two systems received 15 adults (YC1 and YC2) and the third (YC3) received 14. We were unable to determine sex of the adults; therefore, adults were randomly selected for each tank. The TL of the study fish ranged from 66 to 132 mm, with an average of 103 mm; weights of the adults ranged from 2.7 to 24.5 g, with an average of 11.2 g. We assumed that all fish were reproductively mature based on published data for size at reproductive maturity (Johnson et al. 1995). Deadman Creek adults were already in the tanks



FIGURE 2.—Spawning system used to determine the effect of water velocity on spawning site selection by northern leatherside chub. The letter P denotes the pump used to create the flow of water in the tank, which is indicated by arrows. The substrates are labeled 1–3 from the highest flow (19.0 cm/s) to the lowest (9.5 cm/s).

from the previous study: six adults in one tank (DM1) and nine adults in the other (DM2); systems were modified to match the above specifications. The mean TL of the 15 Deadman Creek adults was 109 mm (range, 97–127 mm); their mean weight was 11.5 g (range, 7.3–18.0 g).

The water temperature in the spawning systems in December 2005—January 2006 was 16.0°C. On 7 February 2006, the water temperature was increased to 19.0°C in all spawning systems by switching well water sources. The water temperature in two tanks (DM1 and YC2) was increased to 24.0°C on 29 March 2006; this temperature was achieved by heating 19.0°C well water in a separate raceway with immersion heaters. The other three tanks (DM1, YC1, and YC3) remained at 19.0°C. Water temperature was maintained at these temperatures for the remainder of the study.

Substrates were scanned biweekly for young northern leatherside chub (eggs and larvae) beginning in March 2006. Substrates were removed from the spawning systems, and each substrate was rinsed in a large plastic container to clean off waste feed and feces. The debris and cleaned substrate were then scanned for eggs and larvae. After being scanned, substrates were placed into new trays and returned to the spawning systems. Any eggs found were collected or removed from the substrate with a bulb pipette and enumerated. Eggs were then incubated in McDonald-type egg jars. After hatching, larvae were enumerated, and placed into grow-out tanks.

To determine whether water velocity affected spawning location, we analyzed the data using a mixed model (Proc MIXED in SAS; significance level, 0.05) in which tank was a random effect and the number of young found in each substrate was the response variable. Because the number of adults in each tank varied, the number of young in each substrate was standardized by dividing it by the number of adults in the tank.

Results

Substrate and Habitat Selection

Spawning occurred between the end of April and early September during this study. Spawning first occurred in Tanks 1 and 3 between the end of April and 1 June. In each tank, we found fry during passive scanning, which led to the switch to more intensive scanning methods. The presence of fry indicated that at least one group or cohort of eggs had been spawned in each tank. Two cohorts of eggs (11 May and 1 June) were found in tank 3 after this change in protocol. We use the term "cohort" to refer to a single spawning event. Some eggs from the 11 May cohort fell from the substrate when it was removed; the following week, 17 fry were found that were at the same developmental



FIGURE 3.—Mean total northern leatherside chub young (eggs and fry) collected in several (a) habitats and (b) substrates in spawning tanks from March to September 2005; the thin vertical lines represent SEs.

stage as fry hatched from the collected eggs. Spawning did not occur again until after fish from tank 3 were distributed to tanks 1 and 2 on 13 July, after which reproduction occurred in both tanks. Tank 1 produced two cohorts of eggs 2 weeks apart in August. The second cohort had already begun hatching when found, and both eggs and newly hatched fry were collected. At the end of August and beginning of September, 979 eggs were produced in tank 2 over a 4-d period. This was the end of production from all tanks.

A total of 2,426 eggs and 125 fry were found in the spawning systems. Significantly more eggs were found in the small cobble substrate than in the other three substrate options (99.6%; $F_{3, 23} = 4.54$; P = 0.012; Figure 3). The other 0.4% of the eggs was found in the substrates adjacent to the small cobble substrate (three eggs each in pebble and large cobble substrates) or in the spawning tanks not associated with a substrate (four eggs), presumably dislodged when the substrates were removed (Figure 3). Habitat choice (covered or uncovered pool or upper or lower riffle) was significant, the uncovered pool habitat being selected over the other habitat types ($F_{3, 23} = 7.61$; P = 0.001;



FIGURE 4.—Growth of juvenile northern leatherside chub at 18°C from hatch through 40 d posthatch.

Figure 3). The differences in habitat selection, although significant, did not follow as clear of a pattern as the substrate selection; adults spawned only in the uncovered pool habitat in two tanks, but primarily spawned in the upper riffle in the third tank.

Fertilized eggs were translucent, whitish, and 1.25-1.5 mm in diameter. Eggs were adhesive, sticking together in clumps of as many as 90 eggs on rocks; however, most eggs were found singly and unattached, perhaps because of the substrate rinsing process. Few eggs, if any, were visible when looking at the top surface of the substrate because they settled into the interstitial spaces before adhering to the substrate. In tank 2, one group of eggs was collected approximately 16 h after substrate had been previously cleaned. These eggs began hatching 4 d after being collected and had all hatched by 6 d after collection, indicating a hatching time of 4-6 d postfertilization at 18°C (72-108 degreedays). Leatherside chub fry were 6 mm TL at hatch. Upon hatching, the fry remained on the bottom of the tank unless disturbed; swim-up occurred approximately 6 d posthatch when fry were approximately 8 mm TL. Fins were first noticeable at 4 d posthatch on some fry and were noticeable on most fry by 8 d posthatch. Fry grew quickly, nearly tripling in size after 40 d at 18°C (Figure 4), which equates to an absolute growth rate of 0.3 mm/d.

Water Velocity

Adult northern leatherside chub reproduced in three of the five spawning systems: DM1, DM2, and YC1. From the 27 adults in these three tanks, a total of 18,416 young (17,234 eggs and 1,182 larvae) were produced. The factors that inhibited spawning in YC2 and YC3 remain unclear (e.g., inadequate spawning habitat, nonreceptive or absent mates, or inadequate



FIGURE 5.—Mean total northern leatherside chub young (eggs and fry) collected in small-cobble substrates at three locations with different water velocities; the thin vertical lines represent SEs.

acclimation time). Given the lack of reproduction in these two tanks, we did not include them in the analysis. Significantly more young were found in the high-flow substrate than the other two substrates ($F_{2, 4} = 8.07$; P = 0.0394; Figure 5). However, adult density appeared to be correlated with spawning over the low-flow substrate; comparatively more young were found in the low-flow substrate in tanks with more adults than tanks with few adults.

Temperature affected the timing of the onset of spawning but did not increase the duration of reproduction. Spawning first occurred in DM1 just 2 d after the water temperature was increased from 19 to 24°C. Reproduction in the other two tanks (both at 19°C) occurred 2 weeks after the reproduction in DM1. Spawning ended earlier in DM1 (30 June) than in YC1 and DM2 (17 July and 31 July, respectively). The duration of spawning ranged from 93 to 118 d, lasting longer in the spawning systems with water temperature of 19°C. Temperature did not appear to affect progeny production (Figure 6), but did affect time to first feeding and larval and juvenile growth (Billman, unpublished data).

Discussion

Through these captive-breeding studies, we were able to provide two important findings that can be used in the conservation of the northern leatherside chub and similar species, particularly other species of the Plagopterini, an endemic tribe of western cyprinids of which the genus *Lepidomeda* is a member (Miller and Hubbs 1960). First, we demonstrated that northern leatherside chub can be propagated in captivity, which can provide individuals to augment existing populations and restore populations in streams where the species has been extirpated. Second, we determined



FIGURE 6.—Combined production (eggs and fry) harvested in 2006 from tanks with northern leatherside chub from two sources (Deadman and Yellow creeks) held at either 19°C or 24°C.

previously unknown life history characteristics that will help managers make better decisions for the management and propagation of this rare species.

We were limited in our studies by small sample sizes, a common problem when dealing with rare species. However, we feel that we have taken appropriate measures to compensate for this in our analyses. Thus, we view our results as robust, and especially so because there were large differences between test means despite the small sample sizes.

Northern leatherside chub almost exclusively spawned over the small cobble substrate. Unlike smaller substrate sizes, the small cobble substrate probably would provide interstitial spaces for the eggs, protecting them from water flow that might wash them downstream. Similarly, the wider interstitial spaces of larger substrates might not provide the eggs adequate protection from predators. Substrate selection appeared to be contradictory to published microhabitat characteristics for leatherside chub species. Wilson and Belk (2001) indicated that leatherside chub abundance was negatively related to coverage of 6.25-74-mm substrates. However, these differences in substrate selection could be a function of activities related to season. Similar species of western stream-dwelling cyprinids, including other plagopterins, are typically found in intermediate water depths (25-65 cm) and low water velocities (2.5-45 cm/s) during nonspawning periods (Greger and Deacon 1982; Blinn et al. 1998; Wilson and Belk 2001). During reproduction, however, males of these other cyprinids occupy riffle habitats (4-15 cm in depth) with 2-100-mm substrates, and females periodically leave pool habitats to join the males in the riffle to spawn (Barber et al. 1970; Greger and Deacon 1982; Blinn et al. 1998). If northern leatherside chub COMMUNICATION

exhibit this movement behavior, adults would shift from run and pool habitats, where substrate sizes are smaller, to riffle habitats with larger substrates at the onset of spawning.

Northern leatherside chub preferentially spawned over substrates with the highest velocity, indicating that the preferred habitat for spawning would probably be riffles or runs. These habitats would provide the eggs with well-oxygenated water and minimal fine sediments. Because our studies did not measure the upper limits of the effect that water velocity has on spawning site selection, we do not prescribe the highest water velocity (19.0 cm/s) as the preferred velocity for spawning sites. Additionally, spawning in locations with minimal flow indicated that the appropriate substrate size is more important than water velocity. For example, in the water velocity study, northern leatherside chub used the low-flow substrate more frequently in tanks with higher adult densities. The increased use of the low-flow substrate might indicate a carrying capacity for the number of adults that could spawn over the substrates provided. With multiple adults attempting to spawn, crowding could have occurred over the narrow substrate trays, forcing some adults to seek out other spawning sites.

The northern leatherside chub used in these studies spawned over a long time period (>100 d), longer than previous estimates of reproductive timing (Johnson et al. 1995). However, the study fish were not subject to natural, seasonal fluctuations in light and temperature, or to other factors (prey availability, interactions with other species, and flow variation) that might affect reproduction (Mills 1991). Spawning late in the summer (August) might not be beneficial to populations because this could greatly influence cohort survivorship and recruitment; juveniles would be smaller during overwintering, increasing the likelihood of overwinter mortality (Black and Bulkley 1985; Durham and Wilde 2005). Prolonged spawning duration and multiple spawns have, however, been suggested for other plagopterins (Barber et al. 1970; Minckley 1973; Blinn et al. 1998) and may be determined by food availability, temperature, and flow, all of which can vary from year to year.

The spawning preferences of northern leatherside chub demonstrated in this study, particularly the substrate preference, have implications that could aid in the management of this species. As is typical of streams and rivers in western North America, the streams northern leatherside chub occupy have become degraded by irrigation practices, including impoundments, dewatering, and channel straightening, and by other anthropogenic disturbances (Minckley and Douglas 1991). These factors often increase sedimentation in these streams, which in turn can negatively affect this imperiled species. Increased sedimentation has been shown to delay the onset of spawning and reduce reproductive success of stream fishes, leading to a decline in fish abundance (Berkman and Rabeni 1987; Burkhead and Jelks 2001). The preference of northern leatherside chub for small cobble substrate suggests that increased sedimentation would have similar effects for this species and might thus be a cause of the species' decline. To the extent possible, managers should prevent habitat alterations that would increase sedimentation and should conduct habitat restoration in streams previously altered by anthropogenic influences.

The observations from these studies provide necessary reproductive and life history information for the northern leatherside chub that had not previously been documented. Not only will the results of this study aid in establishing captive propagation protocols, they also have implications for the conservation or management of northern leatherside chub and other *Lepidomeda* species. These studies demonstrate the capability of captive-breeding studies to provide life history information that is lacking for rare species.

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