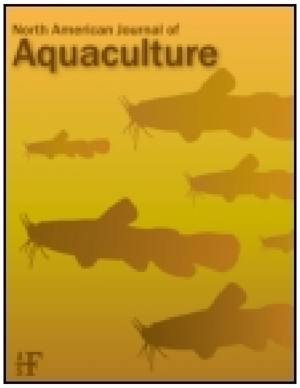

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ARTICLE

Conservation Aquaculture of Northern Leatherside Chub and Effects of Temperature on Egg Survival

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Abstract
We present 4 years of data that refine aquaculture protocols for the northern leatherside chub *Lepidomeda copei*, a species of conservation concern in the Intermountain West. Experiments examined life history traits (age at first spawning and thermal limits to egg hatching success) and aquaculture techniques (brood density, spawning substrate type and surface area, and feeding methods for fry). Tests showed that leatherside chub can reproduce as early as age 2. Multiple spawns per female during a year were also documented. Survival of eggs was compared at incubation temperatures of 18.4, 23.0, 24.6, and 26.8°C. Eggs at 18.4°C had the highest survival to hatching (54.0%); eggs at 26.8°C had significantly lower survival (1.5%). Egg survival at 23.0°C and 24.6°C (32–33%) was significantly lower than survival at 18.4°C. Aquaculture experiments showed that the mean total number of eggs produced did not significantly differ between brood densities of 8.4 (1,246 ± 1,236 eggs [mean ± SD]) or 16.8 (2,224 ± 1,600 eggs) fish/m^3. Studies showed that leatherside chub preferred spawning over natural cobble substrate to spawning over marble substrate. More eggs were recovered from a three-substrate tray treatment (1,350 cm^2) than from a single tray treatment (450 cm^2). Fry given brine shrimp *Artemia* spp. with probiotic bacteria or fed with an automated, more continuous drip feeder did not show any advantages in growth over time. Juveniles at rearing densities of 800, 1,700, and 3,400 fish/m^3 did not differ significantly in growth rates, deformities, or mortalities. This research provides general guidelines for rearing northern leatherside chub and some additional information on the species' life history.

The recovery of threatened or endangered species has been and continues to be an important aspect of fisheries management. Threats to these species include dewatering, increasing average seasonal temperatures, nonnative fish, habitat degradation, and a host of other issues (Brouder and Scheurer 2007; Walser et al. 1999). One of the barriers to management is a lack of knowledge about basic life history traits. A lack of aquaculture techniques for rearing rare species can also be a barrier to species recovery. An understanding of basic life history traits and methods for the aquaculture of a species can aid in conservation efforts for imperiled species (Rakes et al. 1999; Sarkar et al. 2006).

Northern leatherside chub *Lepidomeda copei* (formerly *Gila copei* or *Snyderichthys copei*) is a small cyprinid species found in the Bonneville Basin and the upper Snake River drainage of the western United States (Sigler and Sigler 1987; Wilson and Lentsch 1998; Johnson et al. 2004). Recent population surveys have found reductions in abundance and distribution of northern leatherside chub, resulting in part from water withdrawals and the presence of predatory brown trout (Walser et al. 1999; Wilson and Belk 2001; Belk and Johnson 2007). Northern leatherside chub are currently considered a "species of concern" in Utah.

In an effort to preclude listing this species as threatened or endangered, an interagency recovery team has identified hatchery supplementation as part of a recovery plan. However, basic life history information on northern leatherside chub on which propagation protocols can be based is limited, though recent efforts have provided some data (Johnson et al. 1995; Sigler and Sigler 1996; Wilson and Belk 1996, 2001; Billman et al. 2008a, 2008b). In this study we had two objectives: (1) to provide additional life history data on northern leatherside chub such as

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age at first spawning and upper thermal limits for successful egg incubation, and (2) to provide better protocols for aquaculture of the species. The aquaculture tests explored effects of brood density, fry density, spawning substrate area and composition, automated drip feeders, and probiotic feeds for fry.

METHODS

The following studies were performed at the Fisheries Experiment Station, Logan, Utah. These tests were conducted over 4 years (2006, 2007, 2009, and 2010). Adult leatherside chub were collected from Deadman Creek (October 2004) and Yellow Creek (August 2005), tributaries to Mill Creek in the upper section of the Bear River drainage, Summit County, Utah (2007 studies). Fish were collected again in June 2008 (Yellow Creek only) for the 2009 and 2010 studies.

All the spawning occurred indoors in fiberglass tanks (0.9 m × 1.8 m × 33 cm of water depth) and received 1.7–2.0 L/min of hatchery well water. During winter (before the start of the spawning season), fish were maintained at temperatures between 16.5 and 16.8°C. At the beginning of each spawning season (in late March or April), warmer well water was added to increase temperatures to 18.8°C. This temperature was maintained throughout each spawning season (March through September). A 0.25- hp submersible pump was used to create a current, the output of which was directed though a horizontal polyvinylchloride (PVC) pipe with perforations to create a laminar flow. A range of velocities was created in each tank by putting a sheet of plastic in the center of the tank, perpendicular to the floor, but at an angle to the walls (see Billman et al. 2008a for full description). The resulting velocities provided higher flow areas for the spawning substrate and occasional use and exercise by the fluvial leatherside chub as well as resting areas of lower velocity. Automated feeders (Eheim "Feed-Air" digital automatic feeder and Rena LG100) were programmed to feed Tetramin flakes 4 times per day, at 3% of total body weight per day (about 13 g per week). Phillips full-spectrum lights were set on timers to match the outdoor photoperiod.

Spawning substrates (medium cobble or marbles, depending on the experiment) were made available to the fish. When substrate was screened for eggs, it was done by removing a plastic tray (15 × 30 × 3 cm) containing substrate, rinsing it with well water, and then examining both the rinse water and the substrate for eggs. If any eggs or fry were present, their numbers were determined. All handling of eggs was with a bulb pipette. After each screening, the substrate was replaced with a fresh tray containing disinfected (1200 mg/L benzethonium chloride solution, >15 min exposure) substrate. Egg checks were performed 2–5 times per week, concluding when no more signs of spawning activity were observed.

Egg incubation temperature.—In 2010, survival to hatch was compared among four temperatures: 18, 22, 24, and 26°C. Eggs for the experiment were all derived from a single spawn (18.8°C). Three replicates for each of the four temperature treatments were used, with 44–45 eggs per replicate. The harvested eggs were treated with 60 mg/L copper sulfate solution for 2 min at 18.8°C, and rinsed three times before being placed in the incubation chamber. The incubation chambers were made by cutting a 32-mm-diameter PVC pipe into a 5–cm-long section. The section was capped with mosquito mesh netting secured by rubber bands. This chamber was then placed (without tempering or acclimation) in a plastic tub (11 L) supplied continuously with water (about 1.6 L/min) at the desired temperature. To manipulate water temperature, we used immersion heaters to heat water in a separate tank. Water from this tank was mixed with nonheated water from the same source, and various water mixing ratios (warm/cold) were used to manipulate temperatures in the plastic tubs. For the first 4 days of incubation, the eggs were disinfected by placing the whole chamber into 60 mg/L copper sulfate solution for 2 min. The dead eggs and hatched fry in the chamber were counted after 7 d, when hatching was completed.

Age at first observed spawning.—In 2006, 36 northern leatherside chub were placed into a single fiberglass trough (244 × 61 × 30 cm) to determine the earliest age in which leatherside chub can spawn. At this time the fish were 1 year old, derived from hatchery spawning events the previous year. This tank was supplied with two trays of substrate, small cobble (see above) or medium cobble (3.5 cm diameter) mixed with yellow glass beads (1.8 cm diameter). The tank also contained a portion of a smaller trough (122 × 35 × 18 cm) suspended on a PVC frame above the bottom of the trough to create both cover and a riffle area where the small cobble tray was located (see Billman et al. 2008a for further description). The substrate was checked twice a week between April and September by using the procedure mentioned above. This tank was also monitored in 2007 (when fish were age 2), using the same protocol.

Broodstock density.—This study took place during the 2007 and 2009 seasons. Egg production (total eggs per season and number of spawning events) was compared between two broodstock density treatments: either 4 or 5 (low density; 8.4/m^3) or 9 or 10 spawners (high density, 16.8/m^3) per tank. Broodstock density treatments were limited by the availability of both adult fish and tank space. The low- and high-density treatments were replicated by using two tanks per treatment in both seasons, providing four replicates per treatment for the statistical analysis.

Spawning substrate tests.—Two substrate experiments were conducted, one comparing the preference for either cobble or colored marble substrate, and the second comparing two substrate surface-area treatments. In the first test (2007 spawning season), fish chose between two spawning substrates: small cobble (21–48 mm in size, mean 31 mm) or colored glass marbles (mix of 1.5- and 2.5-cm-diameter marbles). Five spawning tanks (replicates) were used, four of which were also part of the broodstock density experiment; the fifth tank had 14 broodfish present. The substrates were placed in a plastic tray (15 × 30 cm × 3 cm tall), and 1-cm (bar measure) mesh was placed in the plastic tray to create a "false floor" in the trays that collected eggs. The false floor was intended to make egg collection easier and to protect the eggs from predation. Trays for both substrates were placed in the high-velocity area of the tank, side by side. Substrate was screened twice a week. If any eggs were present, the number found was recorded.

In the second experiment (2010 spawning season), egg production was used to compare two different spawning substrate surface-area treatments: 450 or 1,350 cm^2. The substrate trays were the same as used in previous years (plastic trays 15 × 30 × 3 cm of small cobble 21–48 mm; mean 31 mm). For the larger surface-area treatment, three trays (1,350 cm^2 total) were placed adjacent to each other in the tank. The small surface-area treatment (450 cm^2) received only one tray. Six broodfish were put in each of five spawning tanks. Three tanks had a single tray and two tanks had three trays. The substrate was screened daily (except Friday and Sunday) for eggs. Total egg production was recorded for each spawning event and the number of eggs per spawn was compared between the two substrate surface sizes.

Fry diet comparison.—Growth rates of juvenile northern leatherside chub were compared among three feed types: Tetramin flakes, frozen brine shrimp *Artemia* spp. nauplii, and probiotic frozen *Artemia* nauplii. Forty fry (mean weight, 291 ± 16 mg) were used in each of the three replicates of each feed treatment. The fry, pooled from eggs that had hatched during May and June 2007, were placed in nine separate tanks (Rubbermaid plastic tubs, 24 × 34 × 8.5 cm, with 250-μm mesh placed over effluent). The same water source was used for all treatments (24.0°C) and the flow to each tank was 0.4 L/min. The fry were fed four and two times daily on weekdays and weekends, respectively. Waste was siphoned off the bottom of the tanks as needed—usually weekly, sometimes more frequently.

Each tank received a ration that was 8% of the total fish biomass per day. The Tetramin flakes were sieved to provide the fry with consumable sized particles (<425 μm). For the *Artemia*, 2.22 g of dry cysts were incubated in a brine-shrimp hatching cone (Aquatic Ecosystems, Inc., Apopka, Florida). After 24 h the *Artemia* nauplii were separated from the unhatched cysts, sieved (100-μm pore size), and resuspended in 175 mL of well water. The nauplii solution was mixed, and distributed in 15-mL aliquots into individual wells of an ice cube tray and frozen. This same procedure was used for the probiotic *Artemia* except 1 mL of a liquid probiotic (Acidophilus, purchased at a health food store) containing 7 strains of Lactobacilli was added to the *Artemia* water at the 24 h mark. These nauplii were harvested at 48 h and processed as noted above.

The biomass of *Artemia* for each daily ration and feeding was matched to the Tetramin biomass. To estimate the *Artemia* dry weight, we used an equation from a previous unpublished study of ours: $y = 17.745x$, where y is the dry weight of *Artemia* in milligrams and x is the volume of salt water plus *Artemia* suspension in milliliters (that is, the 175 mL of resuspended nauplii solution). We fed one ice cube per tank per feeding. The amount of feed calculated at the beginning of the trial was used for the duration of the study. The study was concluded after 39 d, when individual lengths and total weight were recorded. Any mortality was noted as well.

Automated versus manual feeding.—The objective for this test was to reduce the amount of labor involved with fry feeding and to determine whether more frequent feeding of the same ration over a longer period would result in better growth and survival. Mean length, weight, and number of deformities were compared between juvenile northern leatherside chub (initially $N = 64$/tank, 19.8 ± 3.2 mm mean length, 0.12 g mean weight, 1 deformity) fed either by hand or via an automated drip feeder. Three replicate tanks of fry were subjected to each feeding treatment (manual versus automated). Both methods fed the fry equal amounts of feed but the drip system fed *Artemia* nauplii 8 times daily, whereas hand-fed fish were fed 4 times per day with a plastic bulb pipette. The fry used were hatched in May and June 2007. The experiment began on 7 August 2007 and lasted 42 d, at which time we measured individual length and total biomass for each tank. The tanks (24 cm × 34 cm with a surface outlet at 8.5 cm) were set up similarly to those in the probiotic experiment. The same water source was used for each tub (24.0°C) and flows to each tub were 0.5 L/min.

The *Artemia* were hatched as previously described. After a 24-h incubation period, the hatched *Artemia* were concentrated and divided in two equal parts. Daily, half of the live nauplii went into the automated feeder and the other half was divided into thirds for each replicate of the "manual feed" treatment tanks. Air was pumped in from a separate line at the bottom of the feeding cone to provide an even density among the *Artemia*. The automated feeder consisted of an 8-L hatching *Artemia* cone with clear flexible tubing (8-mm inside diameter, 2-mm-thick wall) attached to the bottom. The tubing was connected to a peristaltic pump, downstream of which irrigation drip fittings (7.6 L/min) were located. A plastic tube from the drip fitting directed the nauplii solution into each replicate of the "automated feed" treatment tanks. The peristaltic pump ran on a programmed timer that ran for 30 min, 8 times a day.

Juvenile rearing density.—Growth of juvenile leatherside chub was compared when grown at three densities: 25, 50, and 100 fish per tank (800, 1700, and 3400 fish/m^3, respectively). Each density had 3 replicates. All tanks were 10-gal glass aquaria, each set up with an outflow standpipe that took water off the bottom. The actual water volume in each tank was 29.5 L. Water temperature among the tanks and over time remained constant at 23.5 ± 1.0°C. At the start of this part of the study, 3 March 2008, the fish used were 6 months old and had an average length of 49.8 ± 7.6 mm and average weight of 0.29 g. Feed (Tetramin flakes, sieved to provide feed <1 mm in size) was provided at a rate of 3–5% of total biomass per day, which resulted in excess feed for all tanks. The tanks were fed manually four times per day and were cleaned as necessary. Mortalities were removed and accounted for in feed calculations. The trial lasted 73 d.

Statistical analysis.—SPSS statistical software (version 13.0) was used for all analyses. A probability of 0.05 was used as the level of significance in all tests. One-way analysis of variance (ANOVA) was used to analyze percent hatch data in the egg temperature test and percent mortality in the fry density test after arc-sine transformation (2 arcsine $\sqrt{x}$; Kirk 1982). ANOVA was also used to compare length and weight among fry density or diet treatments. A *t*-test was used to compare annual egg production and the number of spawning events per year between density treatments, eggs per spawn in the substrate size tests, and growth variables between fry feeding methods. The Mann–Whitney *U*-test was used to compare eggs per spawn in the substrate type test, since the variance was not homogeneous, as determined with the Kolmogorov–Smirnov test.

RESULTS AND DISCUSSION

Egg Incubation Temperature

The final test temperatures were 18.4 ± 0.2, 23.0 ± 0.2, 24.6 ± 0.2, and 26.8 ± 0.4°C (mean ± SD). The mean hatching success at the highest temperature (1.5 ± 2.6%) was significantly lower than at the other temperatures ($P = 0.001$, $F = 14.19$, df = 11). Likewise, at the lowest temperature, mean hatching success (54.0 ± 10.6%) was significantly greater than at the other temperatures ($P = 0.001$, $F = 14.19$, df = 11). Mean hatching rates for the two middle temperatures (23.0°C: 33.3 ± 4.4%; 24.6°C: 32.2 ± 15.0%) were not significantly different from each other (Figure 1).

The data indicated that egg survival decreases at temperatures above 18–19°C, but some survival was possible at temperatures of 23.0–24.6°C. Chronic temperatures approaching 27°C were highly lethal. Marsh (1985) found that for the Colorado River cyprinids *Gila elegans* and *G. cypha* and the pikeminnow *Ptychocheilus lucius*, no eggs hatched when incubated at 5, 10, or 30°C; at 15 and 25°C, the prevalence of deformities was greater than at 20°C, indicating that temperatures around 20°C were optimal for incubation. In another study with Colorado pikeminnow (Bestgen and Williams 1994), however, a 62% average hatch rate was observed for eggs incubated at 26°C, compared with 72% and 67% hatching at 18 and 22°C, respectively. This indicated a higher tolerance to elevated temperature during egg incubation than was observed for leatherside chub in this study.

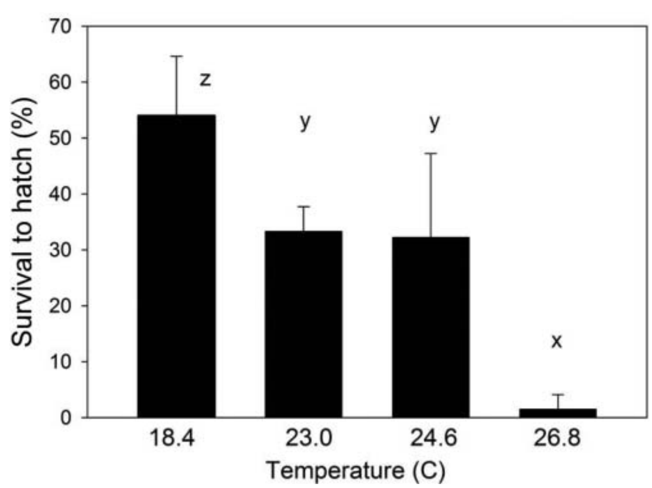

FIGURE 1. Percent hatch (mean ± SD) of northern leatherside chub eggs incubated at one of four different temperatures. Treatments that share a common letter are not statistically different ($P < 0.05$) from one another.

It is possible that diel fluctuations in temperatures that approach the same maxima would be more tolerable. For example, whitefish *Coregonus lavaretus ludoga* eggs subjected to sublethal exposures to 21°C for 2–3 h had increased resistance to subsequent exposure to 25°C (EIFAC 1968). Schrank et al. (2003) found that cutthroat trout *Oncorhynchus clarki* survived in streams with fluctuating temperatures that exceeded the lethal limits established in constant temperature tests. Using 20–40-mm-long spikedace *Meda fulgida,* Carveth et al. (2007) found that diel temperature fluctuations led to better survival and growth than a use of a constant temperature did. Similar observations have been recorded by Geist et al. (2010) for juvenile Chinook salmon *O. tschawytscha*. However, Bestgen and Williams (1994) did not find a significant difference between constant and fluctuating (±2.5°C) temperatures for incubation of Colorado pikeminnow eggs. More research on effects of temperature fluctuation on egg survival is needed on many other species, including northern leatherside chub.

Age at First Observed Spawning

The fish had two spawning events in 2007 with a total egg production of 572 eggs, indicating that northern leatherside chub can spawn at age 2. No spawning was observed at age 1.

Previous to this study, the basis for age of first spawning had been the presence or absence of gonads and had been recorded only for southern leatherside chub *L. aliciae* (Johnson et al. 1995). In this study, we demonstrated that northern leatherside chub can spawn at age 2. The egg production was low but may have been influenced by the relatively high density of fish in the tank and the fact that the fish were from the same age-cohort. Several fish species are able to discriminate between kin and nonkin (McKaye and Barlow 1976; Barnett 1981; Quinn and Busack 1985), preferring to mate with nonkin and showing less aggression toward kin (Brown and Brown 1993). Barber et al. (1970) noted that spikedace begin spawning at age 1. The warmer temperature regimen in southwestern U.S., where spikedace is native, probably gives the species more time for growth and gonadal development than more northern species like northern leatherside chub. Spikedace is a small-bodied species as well, with adults typically less than 65 mm total length and not exceeding 4 years of age (Minckley 1973).

Broodstock Density

The mean total number of eggs produced annually did not significantly differ ($P = 0.58$, $t = -0.58$, df = 7) between the 8.4 (1,246 ± 1,236 eggs [mean ± SD]) and 16.8 fish/m^3 broodstock densities (1,822.8 ± 1,651 eggs). Total production in 2007 was 8,618 eggs from 37 different spawning events. The mean number of spawning events per year per tank did not significantly differ ($P = 0.80$, $t = -0.26$, df = 7) between densities (8.4 fish/m^3: 5.0 events ± 5.1 SD, 16.8 fish/m^3: 5.8 events ± 4.1). The total number of spawning events (summing across all tanks) for the lower density was 17 in 2007 and 3 in 2009, whereas the high-density treatment had 20 spawning events in 2007 and 9 in 2009.

Density effects were not significant, indicating that broodfish densities of 9.3–18.7 fish/m^3 produced comparable numbers of eggs. The ideal density for broodstock is still not clear, but doubling of density did not appear to double production. There was about a 50% increase at the higher density, on average, which could be biologically significant for conservation aquaculture programs. The tank with the age-2 fish had 36 fish and little production, indicating that high density may deter spawning or possibly increase cannibalism of eggs. We have observed leatherside chub cannibalizing their own eggs, so there is a trade-off between having enough fertile broodstock and having so many that they will eat much of any production. For the size of tanks used in this study, a range of 9.3–18.7 fish/m^3 would be sufficient. In larger systems, such as artificial stream reaches, more research should help determine optimal densities. Given that we were unable to identify gender when initiating a broodfish tank, we had to assume a 50/50 male-to-female ratio among the fish on hand and hope that some females were within each group. Individual pairing was not possible so that more fish per tank would provide a higher probability of getting both sexes in a tank.

Evidence from one of the spawning tanks with five fish demonstrated that northern leatherside chub spawn multiple times in a spawning season. Since there were 12 different spawning events in the tank between 7 April and 7 September 2007, and at least one of the five had to be a male because fertile eggs were collected, apparently at least three spawns per female were possible. Johnson et al. (1995) noted that mature ovaries of leatherside chub contain both immature and firm, yolked ova, also suggesting multiple spawns. In our studies, we counted one skein of eggs in a female (107 mm total length) that had jumped from the tank early in the spawning season: She had 510 larger eggs (1 mm diameter) and 334 smaller eggs (0.6 mm). In another 125-mm-long female, the eggs in one skein totaled 550, or an estimated 1100 total per fish. Using the ratio from the one fish dissected (60% ripe) and the range of fecundity provided by Johnson et al. (1995; 938–2,573 eggs/female), the estimated number of eggs per spawning event in the spring would be 563–1,544 eggs. Blinn et al. (1998) similarly reported that Little Colorado spinedace *L. vittata* may spawn up to three times a year, based on observations of mature ovaries and multiple cohorts in captive populations. Evidence in studies of spikedace also indicates that at least two spawns per season are possible (Barber et al. 1970).

Spawning Substrate Type and Surface Area

In 2007, the rocks received significantly more eggs per spawn (217 ± 262 SD) than did the marbles (22 ± 60; $P < 0.001$, Mann–Whitney U-test = 277, df = 1). Total production from marbles and rocks was 894 and 8,870 eggs, respectively. More eggs would rinse off and were easier to see on the marbles, so were significantly easier to collect than the eggs on the rocks. The effect of spawning substrate surface area was assessed in 2010. The larger surface-area treatment with three trays put together produced significantly more eggs per spawning event (231.5 ± 249.5 SD) than a single tray did (90.29 ± 133.4; $P = 0.03$, $t = -2.1$, df = 36).

Although the use of marbles did improve egg collection efficiency as designed, fish still preferred the rock substrate, so we cannot recommend marbles as a spawning substrate. Whether the fish would spawn on marbles if not given a choice remains to be determined. We are not aware of any other reports of using this approach for egg collection. In other substrate preference studies with cyprinids (Gibson et al. 2004; Gibson and Fries 2005), Devils River minnow *Dionda diaboli* preferred gravel to larger rocks, sand, plants or Spawntex. Blinn et al. (1998) noted that Little Colorado spinedace spawned in gravel that averaged 6.4 ± 0.37 mm in diameter. Spawning activity over gravel has been observed in spikedace as well (Barber et al. 1970). However, woundfin *Plagopterus argentissimus* chose larger spawning substrates (5–10 cm diameter), rather than gravel, sand, or larger-diameter rocks (Greger and Deacon 1982).

The success of the larger surface-area treatment could be the result of the dispersal method when the fish spawns; that is, for a broadcast spawner, a bigger target is easier to hit. However, when the fish spawned on the three trays, a majority of the eggs were usually found in just one of the three. More probably, the production was higher for larger surface-area treatments as a result of the reduction in cannibalism because of the greater area in which to hide the eggs. Cannibalism has been observed among other cyprinids such as the spotfin chub *Cyprinella monacha* (Rakes et al. 1999). The rinse method used here collected the loose eggs efficiently and their physical removal with a pipette was easy enough that three trays are recommended for future use. Other studies with cyprinids have used various sizes of substrate to spawn. Clemment and Stone (2004) used substrates with an area of 3496 cm^2 for rosy red fathead minnows. Blinn et al. (1998) used four trays of gravel, each one covering 2500 cm^2, to spawn Little Colorado spinedace.

Fry Diet Comparison

No significant growth difference was observed between fish given the probiotic and the regular *Artemia* ($P = 0.24$ for length and $P = 0.80$ for mean weight). However, growth rates for fish fed Tetramin were significantly higher than for fish fed either *Artemia* or probiotic *Artemia* (all $P < 0.001$, $F \geq 12.0$, df ≥ 8; Table 1). Differences among treatments in cumulative mortality at 39 d were not statistically significant ($P = 0.46$, $F = 0.88$, df = 8). Mean survival percentages were generally high across all treatments (91–100%; Table 1).

Probiotics are live microbial feed supplements that benefit a host animal by improving its intestinal balance (Fuller 1989). In aquaculture, probiotics have been found to increase feed

TABLE 1. Comparison of mean length, weight, and survival of northern leatherside chub fed one of three different diets. Means within a column with a common letter are not significantly different.

Feed	Mean length (mm)	Mean weight (mg)	Survival (%)
Tetramin	39.1 z	550 z	90.8 z
Probiotic *Artemia*	37.0 y	380 y	100.0 z
Artemia	37.5 y	390 y	97.5 z

TABLE 2. Comparison of average (SDs in parentheses) growth and deformity prevalence between northern leatherside chub fed *Artemia* either continuously (7–8 h/d) or manually (4 times/d).

Feeding regimen	Length (mm)	Weight (g)	Deformities (%)
Continuous	32.2 (0.90)	3.8 (0.40)	2.2 (0.85)
Manual	31.7 (1.23)	4.0 (0.06)	1.1 (0.92)

conversion and survivability in cutthroat trout (Arndt and Wagner 2007), turbot *Scophthalmus maximus* (Gatesoupe 1994), and many other species (Gildberg and Mikkelsen 1998; Verschuere et al. 2000). In this study, probiotic *Artemia* did not significantly improve growth or survival. The lack of significant effects on survival is probably the result of high survival rates of the control group and those treated with dry feed. Probiotics may be more beneficial in situations where a bacterial pathogen is limiting survival of fry (Gatesoupe 1994).

The higher growth of the fry on the dry diet after 39 d may indicate some variability in the *Artemia* biomass and energy density. With both *Artemia* treatments it was difficult to get a precise and consistent hatch, and harvest time varied by a few hours. Also, juveniles used in the study may have outgrown the size at which *Artemia* nauplii would be optimal forage. Other fry feed studies have shown that *Artemia* feeding is usually superior to dry feed alternatives (Harzevili et al. 2003; Tesser et al. 2005; Carvalho et al. 2006).

Automated versus Manual Feeding

There were no significant differences in length, weight, or deformity rate for northern leatherside chub fed *Artemia* nauplii either manually four times per day or continuously over 7–8 h via a drip system (Table 2; all $P > 0.18$, $t < 1.61$, df = 4).

Many studies have found that feeding multiple times per day produces higher growth rates, but a threshold is usually reached at which the fish do not benefit from more frequent feedings (Andrews and Stickney 1972; Giberson and Litvak 2003). In this study, the additional time and distribution of the ration did not result in significantly greater growth. However, the automated feeder required just 10 min of set-up time and fed multiple tanks, reducing the overall labor required to feed the fry. One issue with the automated feeder was that unhatched cysts tended to build up in the tubing over time, which could potentially lead to bacteria and fungus problems. However, periodic flushing would help control this problem as well as transfer only hatched nauplii to the system. In this study, pathogens were not a problem. Smaller drip-rate fittings clogged in earlier tests of the system, but the 7.6 L/min fittings alleviated those problems. Some of the same concerns that arose from the probiotic experiment were present in this study, such as fish size and consistency in *Artemia* hatches. Overall, the automated feeder appeared to work well and is recommended for feeding multiple lots of newly hatched fry until they are weaned onto dry feed.

Juvenile Rearing Density

There were no significant differences in length, weight, or percent mortality among the three densities (all $P > 0.11$; Table 3). Final lengths among all the tanks ranged from 45.4 to 49.0 mm; final weights were 1.07–1.25 g. Percent mortality and specific growth rate ranged from 0 to 10% and 1.77–1.99%/d, respectively.

The range of densities for our study was limited to the numbers of fish we had on hand. We found no significant difference in growth or survival in the density range we examined. Our highest density, 3,400 fish/m^3, was still well below or within previously recommended densities for other cyprinid species in intensive aquaculture (Hepher and Pruginin 1981; Horváth et al. 1992; Wagner et al. 2006), but the above densities were typically recommended for pond-reared cyprinids relying on natural foods (Stickney 1979; Horváth et al. 1992; Feldlite and Milstein 2000).

Although the upper limit for juvenile northern leatherside chub rearing density has yet to be determined, densities of at least 3,400/m^3 can be used for intensive aquaculture without compromising growth and survival. As seen with the least chub *Iotichthys phlegethontis* (Wagner et al. 2006), leatherside chub tend to be a schooling species; therefore, high densities are possible. The glass aquaria used for our study were adequate

TABLE 3. Comparison of average (SDs in parenthese) length, weight, percent mortality, and specific growth rate of northern leatherside chub reared at various densities for 73 d.

Fish/L (number)	Length (mm)	Weight (g)	Mortality (%)	Specific growth rate (%/d)
0.8	46.85 (0.87)	1.16 (0.05)	6.67 (4.62)	3.12 (0.18)
1.7	47.71 (0.57)	1.13 (0.03)	4.00 (4.00)	3.07 (0.10)
3.4	47.59 (0.74)	1.14 (0.02)	9.00 (2.64)	3.09 (0.06)

rearing environments for the densities we examined. If the fish are to be raised in rearing ponds, supplemental feed may be necessary.

Summary

The work presented here demonstrated for the first time that northern leatherside chub begin spawning at 2 years of age and produce multiple batches of eggs during a spawning season. In addition, the research supplements previous work (Billman et al. 2008a, 2008b) developing techniques for production of northern leatherside chub. These strategies will probably also be applicable to southern leatherside chub and perhaps other closely related cyprinids. Our indoor and outdoor tank observations (unpublished data) suggest the optimal spawning temperature is around 18.0–23°C. Egg incubation temperatures were optimal at 18.0–19°C. Spawning densities should be kept around 9.3–18.7 fish/m^3. The glass marbles are not recommended, but larger spawning areas of gravel, which recovered more eggs, are. To reduce labor, we developed an automated *Artemia* feeder that worked well. Fry growth was not hindered at 3,400 fish/m^3, and higher densities may be possible. This rough guideline will help to provide the numbers of fish needed to take conservation of the northern leatherside to the next level of stocking and assessment thereof.

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