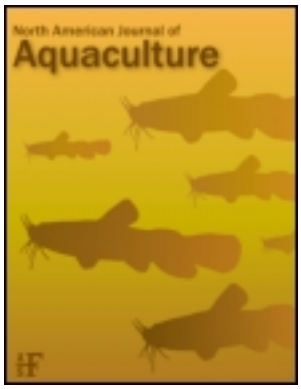

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ARTICLE

Egg Disinfection to Improve Conservation Aquaculture of Leatherside Chub

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Abstract

Leatherside chub *Lepidomeda copei* eggs were obtained as part of separate spawning and life history experiments. This paper summarizes several egg disinfection tests conducted between 2007 and 2010 to develop protocols for controlling fungus and improving hatching success. Several disinfection strategies that have worked for other species did not result in improved hatch rates, namely, formalin (1,000–2,000 mg/L for 15 min), ultraviolet light (10,200 mW/cm^2), or sodium sulfite (1.5% for 5 min). However, petri dish incubation after treatment with copper sulfate ($CuSO_4$) or hydrogen peroxide (H_2O_2) provided high hatch rates. Eggs treated with 40 or 60 mg/L $CuSO_4$ for 2 min had significantly higher hatch rates than untreated controls. Daily or repeated (2-d) treatments using H_2O_2 or $CuSO_4$ did not significantly improve survival to hatch relative to treating only once, but fungal growth was better controlled in the daily H_2O_2 treatments. The highest percentage of hatching (100%) was observed in petri dishes in which eggs were treated daily with either 1,000 or 2,000 mg/L H_2O_2 for 2 min. Disinfection of eggs left on the rock substrate was also successful with 60 mg/L $CuSO_4$ when treated on days 1 and 3 postcollection. These data provide information that will help eliminate fungal infection of eggs and remove an obstacle to the conservation aquaculture of this increasingly rare cyprinid.

Leatherside chub *Lepidomeda copei* (formerly *Gila copei* or *Snyderichthys copei*) were historically found in the Bonneville Basin of Utah (Sigler and Sigler 1987, 1996). Populations of leatherside chub also exist in isolated portions of the Snake River system (Johnson et al. 2004). Recent genetic analysis (mitochondrial DNA; cytochrome *b* sequencing), morphometric analysis, and growth studies at different temperatures have indicated that there are two distinct taxonomic groups of leatherside chub, a northern population and a southern population, which are now considered separate species (Johnson and Jordan 2000; Dowling et al. 2002; Johnson et al. 2004; Belk et al. 2005).

Recent population surveys have indicated reductions in abundance and distribution of leatherside chub, due in part to water withdrawals and to the presence of predatory brown trout *Salmo trutta* (Walser et al. 1999; Wilson and Belk 2001; Belk and Johnson 2007). Both northern and southern leatherside chub species are currently considered "species of concern." In an effort to preclude listing as a threatened or endangered species, an interagency recovery team has identified conservation aquaculture as part of a recovery plan. Unfortunately, basic life history information on leatherside chub on which to base propagation protocols is limited. However, recent efforts have provided some data on habitat preferences (Wilson and Belk 2001; Billman et al. 2008a), maximum age (Johnson et al. 1995), age at first spawning (Johnson et al. 1995; Billman et al. 2008a), effects of temperature on growth and survival (Billman et al. 2008b), and diet (Bell and Belk 2004).

At the Fisheries Experiment Station, Logan, Utah, we have conducted a number of experiments to gain more life history information and to develop methods for reproducing northern leatherside chub (Billman et al. 2008a). However, fungal contamination of eggs has been a significant source of mortality and a major bottleneck in propagation of this species. In this article, we detail several tests exploring egg disinfection options for improving survival to hatching. The selection of chemical treatments were guided by previous efforts with other species

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(Watanabe 1940; Subasinghe and Sommerville 1985; Rach et al. 1998, 2005a, 2005b; Small and Chatakondi 2006; Straus et al. 2009). Tests evaluating the ability of formalin, hydrogen peroxide, iodine, copper sulfate, sodium sulfite, and ultraviolet (UV) light to control fungal growth and improve hatching success of northern leatherside chub eggs are documented in this article.

METHODS

As part of this study 12 separate egg disinfection tests were conducted and the treatments are detailed under the next heading. Eggs for the disinfection tests were harvested from spawning tanks used for other experiments conducted in 2007, 2009, and 2010 (see Billman et al. 2008a for tank details). The tanks held adult northern leatherside chub (4–14 fish per tank) that had been transferred to the hatchery from Deadman Creek and Yellow Creek, Summit County, Utah. Eggs for the disinfection tests were harvested from medium cobble substrates (21–48 mm diameter; mean, 31 mm) in plastic trays kept in the spawning tanks. Temperatures in the spawning tanks were 17.5 ± 0.6°C during the spawning season, and were maintained by flows of 5 L/min per tank. The spawning substrates were screened either every other day (2007, early 2009) or daily (except Fridays and Sundays; late 2009, 2010) from March to September for eggs by removing the plastic tray, rinsing it with well water, then examining the rocks and rinse water for eggs. Between substrate screenings, the rocks were disinfected with 1,200 mg/L benzalkonium chloride and replaced with a second set of trays that had been drying since the last substrate check. When eggs were present, a pipette equipped with a plastic bulb was used to transfer loose eggs and gently pry adhesive eggs off of the rocks. Eggs were collected into a plastic cup for subsequent enumeration and allocation to treatment containers.

Chemicals used in the disinfection tests were iodine, formalin, hydrogen peroxide (H_2O_2), sodium sulfite (Na_2O_3S), and copper sulfate ($CuSO_4$). The iodine was in the povidone-iodine form (Argent Chemical Laboratories, Redmond, Washington) and a 1% concentration of active iodine was assumed per the manufacturer's label. Treatment doses were all based on the active iodine concentrations. Formalin (Argent Chemical Laboratories) was neutralized by addition of NaOH to make the pH about 7 before use. Stock hydrogen peroxide was obtained from a barrel of 35% H_2O_2 (Dyce Chemical, Salt Lake City, Utah). All hydrogen peroxide solutions were buffered with sodium bicarbonate (1.32 g/L; Wagner et al. 2010). Sodium sulfite solutions were made with powdered Na_2O_3S (Sigma Chemical Company, St. Louis, Missouri) dissolved in hatchery well water. Copper sulfate solutions were also made with the powdered form (copper II sulfate, Sigma Chemical Company) dissolved in hatchery well water. The hatchery well water had a total hardness of 222 mg/L as $CaCO_3$, total alkalinity of 222 mg/L, and a pH of 7.6. Temperatures of the well water were consistently between 17°C and 19°C.

Despite differences in the chemical treatments and egg incubation methods, there were many similarities among all the tests. In most instances, not enough eggs were collected during a spawning event to conduct a full, replicated experiment. Instead, most tests were replicated in time with multiple clutches of eggs. Regardless, for each test, at least one replicate of each treatment was tested during each spawning event. If enough eggs were collected to perform multiple replicates with the same clutch of eggs, an equal number of replicates were tested with that clutch for each treatment (i.e., we never tested one replicate of one treatment and two replicates of another treatment with the same clutch, we always tested one replicate of each treatment, two replicates of each treatment, and so on). The tests presented in this study were performed over three spawning seasons with several dozen clutches of eggs. For all tests, survival to hatch (number of fry / initial egg number × 100) was used to compare the efficacy of treatments. Observations of fungal growth on the eggs were also noted.

For all tests, except the in situ treatments (tests 10 and 11), the eggs were removed from the substrate and pooled into a plastic cup. They were then divided into aliquots of equal numbers of eggs, and each aliquot was placed into its own individual cup. The water was then poured out of the cup and 200–300 mL of premixed chemical solution were added. After the appropriate treatment duration, the chemical solution was poured out of the plastic cup and the eggs were rinsed three times with well water. The eggs were then transferred to the appropriate incubation container. For treatments incubated in McDonald hatching jars, hatchery well water was provided at flows ranging between 0.8 and 1.0 L/min. The "mini jars" tested were 500-mL, plastic squirt bottles. The lids of these bottles were replaced with a rubber stopper, the bottoms of the bottles were removed, and the bottles were inverted. Hatchery water was provided to these mini jars at a rate of 0.36 L/min. Both the McDonald and mini jars were placed in larger plastic tubs that collected the effluent. Screens (100 μm mesh size) were installed in these plastic tubs to collect any eggs and fry that left the jars. One plastic tub was assigned to each jar and these tubs were examined for eggs and fry that may have escaped the jars. The eggs in some of our experiments were reared in 100-mm-diameter × 15-mm petri dishes. The petri dishes were filled with 30 mL of hatchery well water. This water was only replaced when the eggs were removed for additional chemical treatment (e.g., for twice and daily treatments in tests 6 and 11). The petri dishes were held at room temperature (16–21°C).

Egg disinfection test treatments.—In test 1, two treatments evaluated the effect of 15 min exposure to buffered formalin: (1) 1,500 mg/L given once at the time of harvest, or (2) 1,500 mg/L formalin daily for 4 d. Eggs were reared in McDonald jars. There were 127–250 eggs per replicate ($N = 3$ replicates). Test 2 further evaluated a 15-min exposure to formalin and tested doses of 0, 1,000, 1,500, or 2,000 mg/L, given at the time of egg harvest. There were three replicates per treatment. Eggs ($N =$ 27–44 per replicate) were incubated in mini jars.

Test 3 compared the timing of a 100-mg/L iodine treatment by treating eggs at the time of harvest, at 24 h, or at 48 h after harvest. Eggs ($N = 102–105$ per replicate, three replicates per treatment) were incubated in mini jars.

Test 4 compared 1-min exposures to hydrogen peroxide concentrations of 0, 5,000, 10,000, 20,000, and 30,000 mg/L H_2O_2 at the time of egg harvest. Eggs ($N = 30–61$ per replicate, three replicates per treatment) were incubated in mini jars. Test 5 used the best treatment from Test 4 (5,000 mg/L) to compare survival between eggs treated once at harvest or daily for 5 d. An untreated egg control was tested as well. Eggs ($N = 50$ per replicate, three replicates per treatment) were incubated in mini jars.

Test 6 evaluated lower doses of hydrogen peroxide as well as petri dish incubation. Treatments were: (1) 1,000 mg/L H_2O_2 for 2 min at harvest and at 24 h, (2) 1,000 mg/L H_2O_2 for 2 min daily for 4 d, (3) 2,000 mg/L H_2O_2 for 2 min at harvest and at 24 h, (4) 2,000 mg/L H_2O_2 for 2 min daily for 4 d, (5) untreated eggs (petri dish control), and (6) untreated eggs (mini jar control). Treatments 1 through 5 were all reared in petri dishes, with 30 eggs per replicate. There were five replicates per treatment except for the mini jar control, which used three replicates.

Test 7 compared the hatching success of eggs in a UV-sterilized water source versus those incubated in regular, nonsterilized well water. The UV-treated water received 10,200 mW/cm^2 UV (QL-8 Lifegard ultraviolet sterilizer). Eggs were provided with either UV-sterilized or nonsterilized water continuously from collection until hatch. Before moving eggs to the UV-treated or untreated mini jars, the eggs were disinfected with 1,000 mg/L H_2O_2 for 2 min.

Test 8 compared sodium sulfite treatment (15,000 mg/L Na_2O_3S for 5 min) with untreated controls. Both treatments were disinfected immediately after the sodium sulfite treatment with 1,000 mg/L H_2O_2 for 2 min. There were four replicates per treatment, two of which were incubated in mini jars, and two were incubated in petri dishes. Test 9 evaluated copper sulfate by comparing 2-min exposures of eggs to concentrations of 0, 40, and 60 mg/L $CuSO_4$. There were five replicates per treatment and 21–37 eggs per replicate.

Tests 10 and 11 evaluated the effect of treating eggs in situ (i.e., left on the rocks), which could potentially reduce the labor required for egg harvest. In Test 10, 100 mg/L of iodine was used (once, at harvest); control eggs were reared in mini jars. There were five replicates per treatment. In Test 11, 60 mg/L $CuSO_4$ was used every other day; untreated control eggs were reared in petri dishes. There were three replicates per treatment. Eggs for the in situ treatment for both tests were incubated in a glass aquarium in which water depth was adjusted to keep the eggs and rocks under several centimeters of water. For these "on rocks" treatments, the spawning substrate was examined and any rocks that did not have adhered eggs were removed before treatment. After these rocks were removed, the tray with the remaining rocks was disinfected by immersing it in 4.0 L of disinfectant. The number of eggs per replicate ranged from 21 to 383 in test 10 and from 16 to 30 in test 11.

Test 12 compared the two best egg disinfectants from the previous tests—copper sulfate and hydrogen peroxide, double versus single chemical treatment, and incubation method (petri dish versus jar). Treatments were (1) 10 mg/L $CuSO_4$ for 2 min once at harvest, (2) 10 mg/L $CuSO_4$ for 2 min at harvest and at 48 h, (3) 40 mg/L $CuSO_4$ for 2 min once at harvest, (4) 40 mg/L $CuSO_4$ for 2 min at harvest and at 48 h, (5) 1,000 mg/L H_2O_2 for 2 min once at harvest, (6) 1,000 mg/L H_2O_2 for 2 min at harvest and at 48 h, (7) untreated control, and (8) McDonald jar control in which eggs were treated once with 1,000 mg/L H_2O_2 at harvest. Treatments 1 through 7 were incubated in petri dishes. There were three to eight replicates per treatment and 17–31 eggs per replicate.

Statistical analysis.—For all tests we used SPSS version 13.0 (SPSS 2005). A significance level of 0.05 was used for all tests. Percent hatch data were analyzed with a *t*-test (UV data) or analysis of variance (ANOVA) after arcsine transformation. When applicable, the least significant difference test was used for subsequent mean comparisons. A paired *t*-test was used for comparing percent hatch between eggs treated in situ and eggs treated after removal from the rocks. For the sodium sulfite test, percent hatch data were examined by using a general linear model in which chemical treatment and incubation method were fixed variables.

RESULTS

Formalin: Effect of Dose and Repeat Treatments (Tests 1 and 2)

In the first test, survival was significantly higher ($P = 0.046$) for eggs given a single formalin treatment of 1,500 mg/L for 15 min upon harvest (41.9 $\pm$ 16.1%, mean $\pm$ SD), than for eggs treated with 1,500 mg/L formalin for 15 min on three consecutive days (one exposure each day; 0.0 $\pm$ 0.0% hatch). In the second test, treatment with 1,000, 1,500, or 2,000 mg/L formalin led to high egg mortality (Table 1). Survival to hatch was significantly better in the untreated controls (54.5 $\pm$ 11.7%) than for eggs treated with formalin ($\leq$3.1%; $P < 0.001$).

TABLE 1. Comparison of the percentage of northern leatherside chub eggs hatched (mean and SD, $n = 3$) after treatment with formalin at various concentrations.

Formalin (mg/L)	Survival to hatching (%)
0	54.5 (11.7)
1,000	3.1 (5.4)
1,500	0.0 (0.0)
2,000	0.8 (1.3)

TABLE 2. Comparison of the survival of northern leatherside chub eggs to hatching (mean and SD, $n = 3$) after treatment with 100 mg/L iodine either on the day of harvest (1) or on either of the two following days (2, 3).

Day of treatment	Survival to hatching (%)
1	60.4 (8.2)
2	57.8 (29.1)
3	30.1 (1.6)

Iodine: Treatment Timing (Test 3)

There was no significant difference ($P = 0.15$) in survival to hatch among eggs treated with 100 mg/L active iodine on days 0, 1, or 2 after harvest. Mean percent survival to hatch ranged from 30.0% to 60.4% among treatments (Table 2). The most variance among treatments was with the second-day iodine treatment (Table 2). By the second day, fungal development on the eggs was already occurring. By the third day, fungal growth had taken over most of the eggs.

Hydrogen Peroxide: Effects of Dose and Repeat Treatments (Tests 4, 5, and 6)

In the fourth test, mean percent hatch values for the 0-, 1,000-, 5,000-, 10,000-, and 20,000-mg/L H_2O_2 treatments were 36.3 ± 42.4 (mean ± SD), 36.9 ± 30.6, 52.6 ± 38.5, 24.0 ± 28.0, and 33.3 ± 29.6%, respectively. The 5,000-mg/L H_2O_2 treatment consistently gave the highest percent hatch, but ANOVA indicated that percent hatch did not significantly differ among the treatments ($P = 0.74$). The percent hatch was highly variable among replicates. For example, among the untreated control replicates, percent hatch ranged from 3.3% to 100%. To provide a clear representation of dose effects, treatments are presented by date (egg lot) rather than showing treatment means (Figure 1).

In test 5, which compared a single exposure against multiple exposures to 5,000 mg/L H_2O_2, there was no significant difference ($P = 0.60$) in percent hatch among treatments. Hatch percentages from control, single, and multiple exposures were 7.33 ± 4.66% (mean ± SE), 13.33 ± 7.42%, and 17.33 ± 7.69%, respectively. In the single-treatment test and controls, substantial fungal growth developed on the eggs before hatch, whereas eggs treated daily with hydrogen peroxide had little to no fungal development.

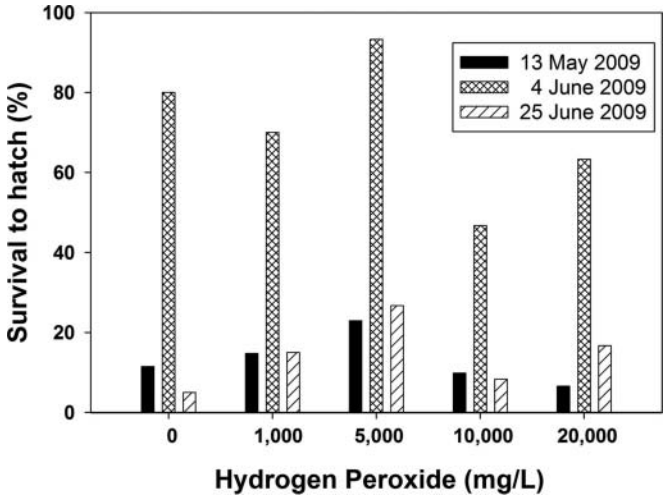

FIGURE 1. Comparison of survival to hatch (%) of northern leatherside chub eggs among three dates and five concentrations of hydrogen peroxide.

TABLE 3. Comparison of mean (SD) percent hatch of northern leatherside chub eggs among varying concentrations and applications of hydrogen peroxide. In the double (2 ×) treatments, eggs were disinfected twice—once at harvest and again the following day. Controls were not given any chemical treatment.

Treatment	Percent hatch
1,000 mg/L H_2O_2, 2 ×	31.3 (22.4)
1,000 mg/L H_2O_2, daily	57.5 (32.6)
2,000 mg/L H_2O_2, 2 ×	38.0 (29.9)
2,000 mg/L H_2O_2, daily	47.3 (40.0)
Petri dish control	38.0 (32.5)
McDonald jar control	8.9 (3.8)

In the sixth test, there was no significance difference in survival to hatch among the hydrogen peroxide treatments and controls ($P = 0.41$; Table 3). There were significant differences ($P = 0.02$) among the three egg batches (treatments pooled). The date effect was partially influenced by the lack of the McDonald jar treatment in the latest batch (1 July 2010); also, there were fewer eggs per dish on the last two dates. If only the data from 19 May 2010 were analyzed (three replicates instead of five), egg survival to hatch was significantly higher ($P = 0.03$) for the 1,000-mg/L H_2O_2 daily treatment (petri dish) than for the McDonald jar treatment; other treatments did not significantly differ from each other ($P > 0.18$). The highest percentage of hatch (100%) was observed in the daily treatments with either 1,000 or 2,000 mg/L H_2O_2. Fungal growth was observed in two replicates of the 1,000-mg/L H_2O_2 double treatment and one replicate of the 2,000-mg/L H_2O_2 double treatment, indicating that in some cases daily treatment is needed to control fungal growth.

Ultraviolet Treatment (Test 7)

No significant difference was observed in survival to hatching of eggs between the UV treatment and control ($P = 0.93$). Hatch rates for eggs in the UV and control treatments were 39.6 ± 18.9% (mean ± SD) and 57.2 ± 19.1%, respectively. One replicate from each treatment had fungus present at the time of fry collection.

Sodium Sulfite Treatment (Test 8)

No significant differences were observed when comparing the percent hatch of eggs treated with and without sodium sulfite ($P = 0.322$; Figure 2). However, there was a significant

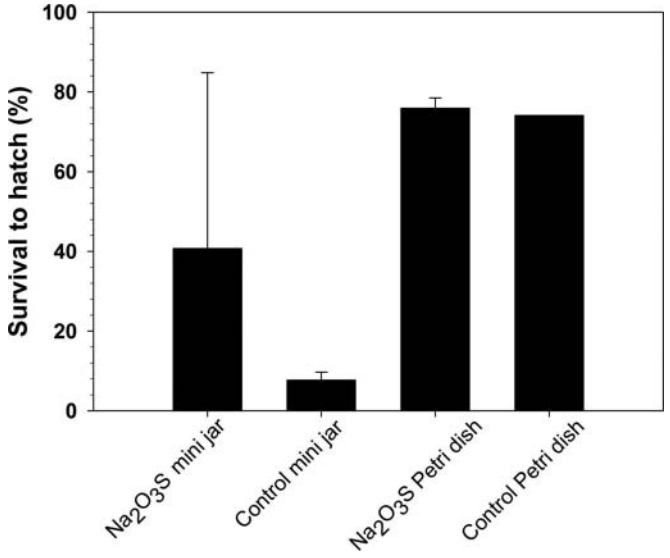

FIGURE 2. Comparison of percent hatch of northern leatherside eggs among eggs incubated by mini hatching jar or petri dish, after treatment with 1.5% sodium sulfite followed by hydrogen peroxide or just 1,000 mg/L hydrogen peroxide.

difference between the incubation methods ($P = 0.030$); the average percent hatch was significantly higher for eggs reared in Petri dishes (75.9 ± 2.6% [mean ± SD]) than in mini jars (40.7 ± 44.1%). The survival to hatching of eggs for controls in the mini jar and petri dish were 7.7 ± 2.0% and 74.1 ± 0.0%, respectively (Figure 2). The consistency of the results was much better within the controlled environment of the petri dish than in the mini jar. The mini jar was more susceptible to fungal infection, which can significantly affect survival. The 5-min exposure to sodium sulfite was effective at breaking down the adhesiveness of the eggs, but it did not result in a definite advantage in the disinfection process.

Copper Sulfate: 40 versus 60 mg/L (Test 9)

The average percent hatch differed significantly ($P = 0.034$) among the 0-, 40-, and 60-mg/L $CuSO_4$ treatments (22.3 ± 20.0% [mean ± SD], 45.7 ± 14.0%, and 49.7 ± 10.3%, respectively). The percent hatch for the two copper sulfate treatments were both significantly greater than for the control ($P \leq 0.035$). No fungus was present in the copper sulfate treatment replicates; however, fungus was present in four of the five control replicates. The higher SD for the average percent hatch in the petri dish control is likely the direct result of the presence of fungus.

In Situ Disinfection with Copper Sulfate or Iodine (Tests 10 and 11)

For the iodine test, the mean hatch rate was significantly higher for eggs disinfected on the rocks (33.9 ± 18.2% [mean ± SD]) than for eggs picked off from the rocks and incubated in an egg jar (22.4 ± 10.0%) (paired *t*-test: $P = 0.024$). By the time the eggs had hatched, fungus had developed on many of the eggs. Since the fungal infection was observed despite the iodine treatment, the difference is probably a result of the benefit of keeping the eggs separated so that fungal infection does not spread from an infected egg to its neighbor. In the copper sulfate test, there was no significant difference among the treatments ($P = 0.447$). The survival to hatch for the $CuSO_4$ treatment in situ, the $CuSO_4$ petri dish treatment, and the control petri dish treatment was 35.8 ± 5.2% (mean ± SD), 29.33 ± 16.90%, and 20.9 ± 18.14%, respectively.

TABLE 4. Comparison of mean (SD) percent hatch (*n* given in last column) of northern leatherside chub eggs at varying concentrations and applications of copper sulfate or hydrogen peroxide followed by incubation in petri dishes ($P = 0.440$). A jar control was included as well.

Treatment	Percent hatch	*N* (Eggs per replicate, range)
10 mg/L $CuSO_4$, 1 ×	68.7 (23.8)	5 (30)
10 mg/L $CuSO_4$, 2 ×	66.2 (43.9)	6 (23–31)
40 mg/L $CuSO_4$, 1 ×	78.7 (18.0)	5 (30)
40 mg/L $CuSO_4$, 2 ×	67.2 (28.3)	6 (23–30)
1,000 ppm H_2O_2, 1 ×	82.6 (13.6)	8 (31)
1,000 ppm H_2O_2, 2 ×	89.7 (9.0)	5 (31)
Petri dish control	60.9 (38.0)	6 (23–30)
McDonald jar control	94.1 (10.2)	3 (17–30)

Copper Sulfate versus Hydrogen Peroxide Treatments (Test 12)

No significant differences were observed when comparing the eight copper sulfate and hydrogen peroxide treatments ($P = 0.440$) or single versus double treatment regimens ($P = 0.425$; Table 4). The hatch rates observed in this test were greater than what was observed in any of the previous tests presented.

DISCUSSION

Our results highlight some of the challenges associated with the culture of northern leatherside chub. The hatch rates observed in our study were generally lower (often <50%, see tables and figures) than what is considered acceptable for most fish species, and considerable variation in hatching success was observed among replicates. This variation often obscured our ability to detect significant differences among treatments. Still, our chemical tests provide important insight on the disinfection of eggs of this species and should aid in conservation efforts.

Formalin

In this study neutral formalin was toxic to the leatherside chub eggs at concentrations of 1,000 mg/L and higher. The concentrations in our tests were similar to those recommended for salmonid or tilapia egg disinfection (Subasinghe and Sommerville 1985; Barnes et al. 2000, 2001). The toxicity was inconsistent, and some single treatments led to acceptable levels of hatching. It is likely that susceptibility to chemical treatment

varies during leatherside chub egg development. As the multiday treatment demonstrated, eggs treated after the first day had higher mortality. The phenomenon has been observed in rainbow trout *Oncorhynchus mykiss* egg incubation, where there is a developmental period before epiboly (70–140 daily temperature units, °C) in which there was greater sensitivity to hydrogen peroxide treatment (Gaikowski et al. 1998; Arndt et al. 2001).

Lower concentrations of formalin are not likely to have an effect on fungi unless longer exposure times are employed (Oláh and Farkas 1978). Rach et al. (2005b) found that a 60-min treatment with 166 mg/L of formalin significantly improved channel catfish *Ictalurus punctatus* egg survival. Rach et al. (1997) improved survival of walleye *Sander vitreus*, common carp *Cyprinus carpio*, white sucker *Catostomus commersonii*, and channel catfish eggs by using 1,500 mg/L of formalin for 45 min. Clearly, some species can tolerate higher concentrations than what was observed in this study for leatherside chub. For example, burbot *Lota lota maculosa* tolerated formalin treatments of up to 5,000 mg/L for 15 min (Polinski et al. 2010). However, Bootsma (1973) noted high mortality in eggs of northern pike *Esox lucius* at concentrations as low as 40 mg/L, although the duration of exposure was 24 h.

The survival rates observed in the formalin tests in this study were relatively low when compared with other species and cyprinids. In a study with common carp, Khodabandeh and Abtahi (2006) recorded a 91.8% hatch rate when 400 mg/L formalin was used. Bestgen and William (1994) disinfected embryos of Colorado pikeminnow *Ptychocheilus lucius* with 1% formalin for 5 min, followed 2 h later with 5 mg/L of malachite green for 30 min; mean hatch was 79% at 18°C. In this study, some egg lots were noticeably poor (whitish-dead eggs or eggs with fungus) at the time of harvest, which would negatively affect the hatching rates.

Iodine

The iodine doses tested in this study were typical of what has been tested in other species (McFadden, 1969; Ross and Smith 1972; Schachte 1979; Bergh and Jelmert 1996). In egg disinfection work with cyprinids, Khodabandeh and Abtahi (2006) found a 27% hatch increase for common carp by using 200 mg/L iodine. However, iodine can be toxic for eggs of some species such as the rainbow smelt *Osmerus mordax* at concentrations as low as 50 mg/L for 15 min (Ayer et al. 2005; Walker et al. 2010).

For single treatments with iodine in this study, the lowest prevalence of fungal development was observed in treatments that occurred immediately after egg recovery. Although not significant, eggs treated with iodine at 2 or 3 d after egg recovery demonstrated a trend towards greater fungal growth and lower hatch success. But, a single treatment of iodine was insufficient to keep eggs free of fungus. Repeated daily treatment could help in this regard, as we observed in the hydrogen peroxide trials, but this protocol should be tested further with iodine. However, Piper et al. (1982) cautioned against treating salmonid eggs within 5 d of hatch with iodine owing to problems with premature hatch. Treating eggs with iodine on subsequent days did not improve survival, but indicated that the stage of development at the time of treatment was not a factor at the concentration used.

Hydrogen Peroxide

Research on hydrogen peroxide treatment of eggs is dominated by work with salmonids, which has indicated that prophylactic treatments can control fungal and bacterial growth (Waterstrat and Marking 1995; Schreier et al. 1996; Gaikowski et al. 1998; Arndt et al. 2001; Wagner et al. 2008, 2010), though toxicity can vary among species (Yamamoto et al. 2001). Rach et al. (1998) noted that the eggs of lake sturgeon *Acipenser fulvescens*, northern pike, and walleye tolerated daily 15-min hydrogen peroxide treatments of 3,000 mg/L. However, Soupir and Barnes (2006) observed that walleye eggs exposed to 200 mg/L hydrogen peroxide for 15 min did not survive as well as eggs treated with 1,667 mg/L formalin, despite controlling fungal growth. Higher temperatures may contribute to higher toxicity (Small 2004); e.g., Roth et al. (1993) noted hydrogen peroxide toxicity to Atlantic salmon *Salmo salar* increased by a factor of five from 6°C to 14°C. For yellow perch *Perca flavescens,* paddlefish *Polyodon spathula*, common carp, and white sucker eggs, survival was best at 1,000 mg/L H_2O_2 (Rach et al. 1998), though common carp eggs tolerated up to 6,000 mg/L with less mortality than several of the other species. Walker et al. (2010) noted that rainbow smelt eggs tolerated up to 2,000 mg/L H_2O_2 in 15-min exposures. Walker et al. (2010) also noted that bacterial growth was controlled at 2,000 mg/L but not at 1,500 mg/L or less. Mitchell et al. (2009) observed fungal growth on channel catfish eggs treated with hydrogen peroxide at concentrations up to 500 mg/L.

In our study, the exposure was for a much shorter period of time (1–2 min versus 15 min), yet daily doses of 1,000–5,000 mg/L H_2O_2 controlled fungal growth without compromising egg survival. Daily treatment with hydrogen peroxide has improved hatching success of other cyprinids, such as the rosy red fathead minnow *Pimephales promelas* (Horne et al. 2010) and golden shiner *Notemigonus crysoleucas* (400 or 800 mg/L for 15 min; Bozwell et al. 2009). Daily treatment was needed in our study to keep fungal growth controlled.

In several of the hydrogen peroxide tests, there was high variability among replicates. The cause of the variability is unknown, but it appears that eggs from some individual lots or spawning events were significantly healthier than others. The data in test 4 suggested that hatch rates are higher later in the season, but additional data from the 12 May 2010 spawn (Table 4), in which survival was high, indicates other factors affect egg quality. Variation could be related to egg quality of particular females, time elapsed between egg deposition and harvest (affecting handling or chemical sensitivity and fungus encroachment), presence or absence of bacterial biofilms (Verner-Jeffreys et al. 2007), or spatial and temporal differences in fungus abundance among egg lots. Verner-Jeffreys et al. (2007) also noted high

variation in egg survival of amberjack *Seriola rivoliana* and Pacific threadfin *Polydactylus sexfilis* eggs treated with hydrogen peroxide.

Ultraviolet Light

Ultraviolet light treatment can be an effective means of controlling bacteria, viruses, and other microorganisms (Qian et al. 2004). For example, doses of 40–480 mJ/cm^2 UV were effective against myxospores of the myxozoan parasite *Myxobolus cerebralis* (Hedrick et al. 2008). Subasinghe and Sommerville (1985) obtained high hatch rates (>88%) of tilapia *Oreochromis mossambicus* by using continuous UV doses of 43,556 or 83,112 μW/cm^2. Lower doses have been effective against viruses (e.g., 4,000 μW/cm^2; Kasai et al. 2005) and bacteria (22,100 μW/cm^2; Kimura et al. 1976).

In this study, UV treatment did not improve hatching success. The well water supply was not turbid, so the light should not have been attenuated. The unit used was new, so bulb life and biofilms on the system should not have been factors either. It is possible that the dose was too high, but given the presence of fungus in one of the treatment replicates, results indicated that fungus control did not occur with continuous UV treatment.

Sodium Sulfite

Horne et al. (2010) found that sodium sulfite was useful for detaching rosy red fathead minnow eggs from spawning substrates. Isaac and Fries (1991) found that sodium sulfite treatment of channel catfish eggs also successfully broke down the glycoprotein matrix of an egg mass. This can facilitate subsequent chemical treatment and removal of dead eggs. In northern leatherside chub culture, egg adhesiveness is a minor concern since eggs typically only adhere in groups of 2–15 eggs. As this test demonstrated, the additional handling and chemical treatment with sodium sulfite did not improve hatching success.

Copper Sulfate

A few studies have documented the efficacy of copper in fungus control on fish eggs (Bailey 1984; Miura et al. 2005; Straus et al. 2009). Concentrations of 10–40 mg/L $CuSO_4$ have successfully treated eggs of channel catfish (Straus et al. 2009). In this study, 10 mg/L $CuSO_4$ was insufficient to control fungal growth, but 40–60 mg/L worked well. The in situ tests indicated that copper sulfate could be used to treat eggs on the rocks, thereby reducing the labor required to hand-pick eggs. Alternatively, the eggs could also be successfully incubated in petri dishes after treatment. The toxicity of copper sulfate can vary with water quality (e.g., temperature, pH, and hardness; Hodson et al. 1979; Schubauer-Berigan et al. 1993; Richards and Beitinger 1995), so care must be taken when applying these results elsewhere.

Petri Dish Incubation

Despite an occasionally high rate of survival in the McDonald jars, our experience with fungal growth in the jars indicated that getting fungus-free eggs was possible, but improbable. The use of petri dishes helped to minimize the risk of fungal infection. The highest hatch rates (100%) were observed in petri dishes after chemical treatment. A variety of other fish species have also been successfully hatched using petri dish incubation (Wedekind and Müller 2004, Avery and Brown 2005; Ayer et al. 2005; Barnes and Durben 2008).

Summary Recommendations for Egg Disinfection

Since research began in 2005, fungal infection of leatherside chub eggs has been a serious impediment to hatchery production of this species. While high levels of survival were observed in some control groups in this study, the probability of fungal infection made chemical treatment necessary to get consistently high survival rates. Traditional methods, such as jar incubation after treatment with standard doses of formalin or iodine, or UV treatment, were not effective at controlling fungal growth or were toxic. Alternative protocols, such as petri dish incubation after treatment with hydrogen peroxide and copper sulfate, significantly improved survival to hatching. We recommend treating leatherside chub eggs daily with 2,000 mg/L H_2O_2 for 2 min or once with 40–60 mg/L $CuSO_4$ for 15 min, followed by petri dish incubation at 16–19°C. Although Barnes and Durben (2008) found that the number of eggs and frequency of water exchanges did not significantly alter hatching success of rainbow trout eggs held in petri dishes, our experience suggested that daily water exchange with sterile well water helped keep the dishes from becoming foul. This practice could reduce bacterial growth and help maintain high dissolved oxygen levels. Removal of dead eggs and those infected with fungus is also recommended while doing the water exchange. We suggest using no more than 20–30 eggs per dish. Disinfecting eggs with 40–60 mg/L $CuSO_4$ while eggs are still on the rock substrate is also recommended, but multiple treatments may be necessary (i.e., day 1 and 3) to keep fungal growth under control. Other practices such as daily inspection of the spawning substrate, removal of waste feed and feces in the broodstock tanks, and periodic disinfection of the wet laboratory will also help reduce fungal development and improve egg survival. Overall these data should provide a means to successfully control fungal growth in leatherside chub eggs, which can lead to greater production for the conservation needs of this increasingly rare species.

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