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Whirling Disease-Resistant Rainbow Trout Program Begins

INSIDE THIS ISSUE:

As the parasite *Myxobolus cerebralis*, the cause of whirling disease, spreads in Utah, the need for some remedy to the problem becomes more acute. Already three state fish hatcheries have become infected, leading to the destruction of thousands of fish. Additional impacts have been felt even more severely in the private sector.

One promising development in the whirling disease research arena is the discovery of rainbow trout strains resistant to the parasite. The Hofer strain has been exposed to the parasite since it was exported from Colorado to Germany in the late 1800s. Through efforts spearheaded by Ron Hedrick at the University of California, Davis, the Hofer strain was imported back into the US via California. It was later sent back to Colorado where Dr. George Shisler and others with the Colorado Division of Wildlife (CDOW) have begun a broodstock program to develop a resistant strain of rainbow trout. Research by Hedrick et al. (2003) has shown that the Hofer strain is resistant to infection, with spore loads 10- to 100-fold less than more susceptible strains. The Hofer strain has been domesticated and adapted for commercial aquaculture, so it grows very well in the hatchery with little fright response. Good growth is a desirable attribute, but more 'survival savvy' is desirable for a fish that is stocked into waters where it must fend for itself.

A Harrison Lake rainbow trout shows the beauty and wild characteristics prized by anglers

Fortunately, parallel research with another strain of rainbow trout, the Harrison Lake (HL) strain, has unveiled another whirling disease resistant stock that is derived from wild, lake-adapted stock. Harrison Lake was stocked with fish from Lake DeSmet, WY. Tests here at the Fisheries Experiment Station (FES) have shown that HL exposed to the parasite have significantly reduced spore loads and a high percentage of fish still uninfected after exposure. Not every fish is resistant, however, so selection for resistance is needed in this strain. Unreplicated observations here at the FES indicate that the HL have slower growth than our Utah strains, likely due to the wild nature of the strain and the negative effects of stress on growth. Currently at FES we have two year classes of HL (2004, 2005) that will be the initial vanguard of a broodstock producing whirling disease resistant progeny for stocking in Utah waters.

Based on what we know about each strain's strengths and weaknesses, we decided that a cross between the two would be desirable. This would incorporate the wild aspects of the HL and the growth and superior resistance attributes of the Hofer. With the help of George Schisler, Phil Schler, and Eric Hughes from the CDOW, we obtained two separate batches of eyed eggs (received 12-13-05, 12-27-05) derived from a backcross of the Hofer x HL (F1) with Hofer females. The Fisheries Experiment Station received 13,000 eggs in the first batch and 31,680 in the second, for a total of 44,680 eyed eggs.

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Our plan is to compare the hatchery performance of this strain compared to a Utah hatchery strain (TenSleep) as well as the field survival and catchability. The hatchery performance portion of the study was completed at the end of May 2006. Hyrum and Porcupine reservoirs, both positive for *M. cerebralis*, were stocked with both strains. The two strains were stocked in May into Hyrum (15,105 TS and 16,012 HH) and Porcupine (11,479 TS and 11,805 HH) reservoirs for further evaluation of catchability and post-stocking survival. Each strain was marked with a pelvic fin clip prior to stocking (right = HH, left = TS). Prior to stocking, fish were sampled from each reservoir to establish baseline levels of *Myxobolus cerebralis* prevalence. Post stocking prevalence rates will be evaluated in subsequent fall and spring gill netting. A creel survey is also planned, using additional funding provided by the Whirling Disease Foundation.

Eric Wagner

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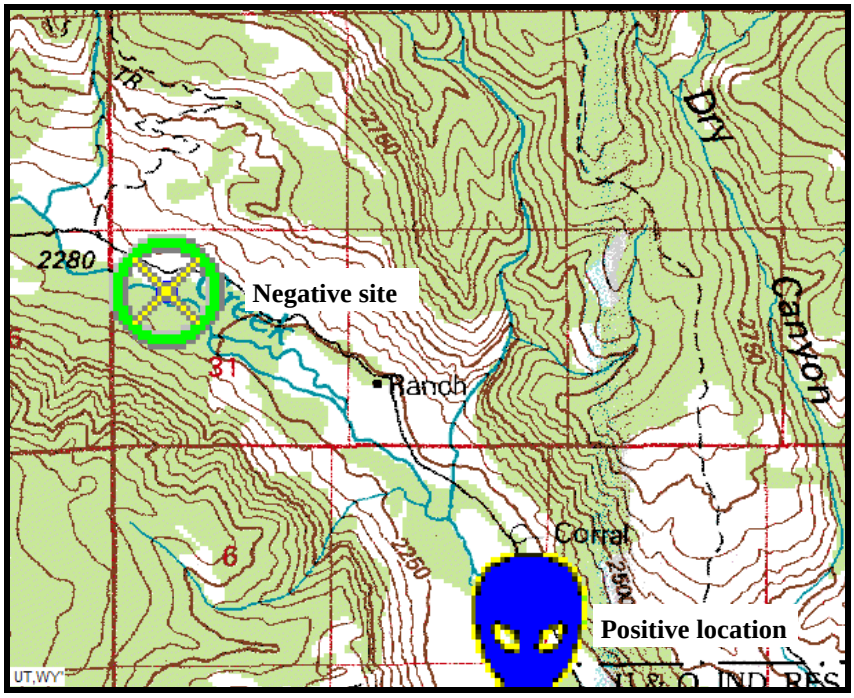
Myxobolus Cerebralis Detected in Rock Creek in Uintah Mountains

Fish pathologists at the Fisheries Experiment Station have announced the discovery of *Myxobolus cerebralis* (MC), the parasite which causes whirling disease, in trout from Rock Creek, a small stream which flows south along the south slope of the Uintah Mountains in the northeast corner of Utah.

The samples were collected by fisheries biologist from the Northeastern region of UDWR on 9/28/2005, and were processed at the Fish Disease Diagnostic Laboratory in June 2006 by single round PCR testing. Brook, rainbow and brown trout from this site tested positive for the parasite. Biologists did not report the presence of any clinical signs of disease in the fish.

Fish from two locations on the stream were sampled, and those samples from the upstream location tested negative, while those downstream were positive for the parasite.



The finding is concerning to fisheries biologists for a number of reasons. Genetically pure Colorado River cutthroat are found upstream of the sampling sites, and are vulnerable. Rock Creek itself drains into the Duchesne River and eventually the Strawberry River, causing biologists concern whether the infection has already spread into the watershed.

Plans are underway to sample surrounding streams to determine the extent of the infection.

Chris Wilson

Evaluation of Substrate Type as a Factor in Optimizing Growth of Least Chub, *Iotichthys phlegethontis*

In 2002, a refuge population of least chub was established at the Utah Division of Wildlife Resources Fisheries Experiment Station, Logan, Utah, using fish from Mona Springs, near Mona, Utah. These fish, and their progeny, were used to conduct several experiments aimed at developing aquaculture techniques for rearing least chub. The objective of the two experiments detailed in this article was to determine if a simulated pond environment (aquaria with silt substrate) improved survival and growth of least chub relative to indoor or outdoor aquaria with no silt.

Methods

Substrate Study 1

Six aquaria were placed outdoors two weeks prior to introducing fish, which allowed for algae and invertebrate colonization. Into three randomly selected aquaria, we added 2 to 3 cm of silt that had been dried since the previous year. The remaining three aquaria were used as controls in which no substrate was added. An additional three aquaria were kept indoors to control for variables associated with outdoor rearing in no-substrate tanks, such as algae production. Well water (13.5°C) was supplied to each tank at a rate of 0.8 ± 0.1 L/min in a flow-through system. Each outdoor tank had a 200 W heater within the tank to warm the water to 19 ± 1.1°C.

Fry used for the study were 4 to 7 months old when the study began on 26 April 2005. These were progeny from fish in a 2004 experiment examining least chub spawning. A total of 17 fish were randomly assigned to each of the nine aquaria. Initial weights were obtained by placing all 17 fry into a pre-weighed beaker of water, measuring the increase in total weight, and dividing by the number of fish. Care was taken to avoid introducing water with the fish.

The fry had been adapted to a dried flake diet (Tetramin®) which was the diet used for the study. The flake crumble was fed three times per day on weekdays, and 1 to 2 times per day on weekends. A total of 0.5 g feed was fed per week per tank for the first 10 weeks, then 1.06 $g \cdot wk^{-1} \cdot tank^{-2}$ thereafter.

After 8 (midpoint) and 16 weeks (end), pictures were taken of the fish in a shallow (<1 cm) pan with a ruler for reference. Total lengths were measured later from the digital photograph. Final weights were determined as noted above for the initial weight measurement, as well as by weighing individual fish. At the end of the study, water quality measurements were taken. Dissolved oxygen was measured with a calibrated probe (YSI, Yellow Springs, Ohio). Total alkalinity was measured with a commercial kit (Hach Chemical Co., Loveland, Colorado). Ammonia-nitrogen was determined by the Nesslerization method using five standards and a spectrophotometer (APHA et al. 1989).

Plants and invertebrates within the tanks at the end of the study were identified with the aid of taxonomic keys and guides (Needham and Needham 1962, Prescott 1980, Pennak1989, Thorp and Covich 1991, Patterson 1996, Wehr and Sheath 2003).

Substrate Study 2

This second evaluation of substrate was to determine if the significant mortality that occurs 5 to 15 days after hatching could be reduced by cultivating newly hatched fry in small ponds. For the test, 6 plastic tanks (32 x 18 cm) were located in an outdoor raceway covered with bird netting (2.5 cm mesh). Tarpaulins over the bird netting were also used to help shade the tanks. Three tanks had 1 cm of silt substrate in them, and three controls had no substrate. The tanks were put into a larger pan in which water flowed at a higher rate than through the tanks; this was to control temperatures in the tanks so they were less influenced by fluctuations in air

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temperature and insolation. The tanks were established 2 weeks prior to introducing fish in order to enhance invertebrate and plankton colonization. Flow rates of hatchery well water were 39 to 100 mL/min to each tank. A covered plastic headbox was used to heat the water with an immersion heater prior to delivery to the experimental tanks via a manifold.

On 11 August 2005, 50 least chub fry that were harvested the same day from a spawning experiment (≤ 4 days old) were placed into each of the 6 tanks. Fry were fed an artificial Rotifera diet (Hatchfry Encapsulon Grade 0, 30 µm, Argent Chemical Laboratories, Redmond, WA) four times a day (144 mg/day) until the study ended, 28 days later.

At the conclusion of the test, fish lengths were measured from digital photographs as noted above. Mortality rates were calculated based on the survivors recovered at the end of the study. Plants and invertebrates within the tanks at the end of the study were identified with the aid of taxonomic keys and guides (Needham and Needham 1962, Prescott 1980, Pennak1989, Thorp and Covich 1991, Patterson 1996, Wehr and Sheath 2003).

Results and Discussion

Substrate Study 1

There were significant differences in growth among the three substrate treatments after the first 8 weeks (P = 0.038), in the second 8 weeks (P = 0.032), or over the entire 16 weeks (P = 0.004; Table 1). Significantly larger fish developed in the silt substrate treatment than in the two no-substrate treatments. The indoor and outdoor no-substrate treatments did not significantly differ in growth in the first 8 weeks, second 8 weeks, or overall (P > 0.52).

Mortality rates averaged from 9.8 to 21.6% across treatments and did not significantly differ among the three treatments (P = 0.328). The coefficient of variation (CV) for total length was significantly higher (P = 0.013) for fish in the indoor aquaria (12.6% ± 1.61 S.E.) than for fish in outdoor aquaria with (5.7% ± 0.32) or without (8.0% ± 1.68) a silt substrate. CV in outdoor treatments did not significantly differ from each other.

Total ammonia in all the aquaria was barely above detectable limits and was similar among treatments. Total alkalinity ranged from 205 to 222 mg/L among tanks. Dissolved oxygen was 8.0 to 8.1 mg/L in the indoor aquaria, 9.2 to 9.8 mg/L in the outdoor aquaria, and 10.0 to 10.5 mg/L in the silt substrate treatments. Algae in the outdoor treatments likely contributed to the higher dissolved oxygen values. There was a wide variety of organisms identified in the outdoor tanks at the end of the study. The green filamentous algae *Ulothrix* and the diatom *Diatoma* were dominant, but 14 other organisms were found as well (Table 2).

Substrate Study 2

The mortality rate was significantly higher in the aquaria treatment (95.3% ± 2.3 SD) than in the silt substrate treatment (45.3% ± 23.1; P = 0.009). Final length was also significantly higher in the simulated ponds (6.2 mm ± 2.7 SD) than in the aquaria with no sediment (10.2 mm ± 1.2; P < 0.001).

In the few weeks in which there was an opportunity to colonize the tanks, a remarkable number of plants and invertebrates were able to invade the new habitat. The diatom *Diatoma* and the filamentous algae *Ulothrix* dominated the cultures, but 20 other organisms were observed as well .

	Specific growth rate (%/d)	Final weight (mg)	Final length (mm)	Survival (%)
Outdoor				
Silt substrate	3.38 ± 0.03 a	458.7 ± 4.51 a	35.0 ± 0.25 a	90.2 ± 2.0 a
No substrate	2.18 ± 0.29 b	318.0 ± 31.1 b	31.2 ± 0.94 b	78.4 ± 8.5 a
Indoor				
No substrate	2.06 ± 0.12 b	306.4 ± 11.8 b	30.3 ± 0.32 b	90.2 ± 2.0 a

Table 1. Mean (n = 3; ± SE) growth and survival over 16 weeks for least chub in aquaria with silt substrate or no substrate (indoor and outdoor). Significant differences between means within a column are noted with different subscript letters.

Morrison and Burtle (1989) achieved a 73% survival rate for golden shiner (*Notemegonus crysoleucas*) fry transferred to fertilized rearing ponds. Least chub in this study had a 55% survival rate in simulated ponds if stocked right after hatching. If stocked after 4-6 months, least chub survival was 90% in simulated outdoor ponds and in indoor aquaria as well. The variation in size among least chub in indoor aquaria was significantly higher than those held outdoors, which did not differ from each other. This indicated that natural invertebrate prey items in the outdoor tanks provided more uniform growth of fry. Despite finding a variety of invertebrates and algae in both outdoor tanks, fish in the silt substrate tanks were significantly larger. The aquaria with substrate had more vegetation present, including *Typha* and *Chara.* In one aquaria, plants covered 75% of the surface area. This created a habitat that would likely have attracted more insects. Ostracods were also favored in the silt substrate, which would have provided nutrition as well. Least chub are known to consume a wide variety of prey items, including algal and diatomaceous material associated with chironomid consumption, mosquito larvae, copepods, amphipods, ostracods, cladocerans, and aquatic insects (Pendleton and Smart 1954, Workman et al. 1979). The higher growth in the outdoor silt substrate treatment also indicated that the artificial fry diets, or our application of them, still do not match natural feeds.

Growth in silt substrates indicated that fertilized fry ponds would likely provide better growth and survival than in aquaria and that further research is needed in artificial feeding strategies. The growth data should provide metrics for comparing growth in new least chub populations and existing populations subjected to various environmental pressures.

By Eric Wagner, Eric Billman, and Ronney Arndt

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Egg Disinfection: Comparison of the Efficacy of Iodine, Formalin, Salt, and Hydrogen Peroxide

The standard protocol for salmonid egg disinfection in Utah state hatcheries is a 10-15 min bath in 100 ppm free iodine (1% povidone iodine). This was largely due to the work by McFadden (1969) who showed that 50 to 120 ppm free iodine was superior to acriflavine or merthiolate for controlling the growth of the fish pathogen *Aeromonas liquefaciens*. McFadden's tests were conducted both in-vitro (bacteria in water and disinfectant followed by centrifugation and washing three times, then culturing) and in-vivo (disinfected eggs in a broth culture). In vitro tests by Ross and Smith (1972), in which bacteria culture broth was mixed with a 50 mg/L iodine solution, also indicated successful suppression of many principal bacterial pathogens (*A. liquefaciens, A. salmonicida, Vibrio anguillarum, Flavobacterium psychrophilum*). Tests with viruses indicated that IHNV (infectious hematopoietic necrosis virus) on the surface of 'green' or eyed rainbow trout eggs treated with 100 ppm iodine for 10 or 60 min was still recovered, but 99.98% of the virus was destroyed (Goldes and Mead 1995). Eskildsen and Vestergaard-Jorgensen (1974) found similar mortality rates for IPNV (infectious pancreatic necrosis virus, 90%) and VHS (viral hemorrahagic septicemia virus, 99.9%) after treatment of eggs with 80-100 mg iodine/L. Recently however, Kumagai et al. (1998) noted that 50-1,000 mg/L of iodine failed to disinfect salmonid eggs of *F. psychrophilum,* although the eggs in that study were homogenized; i.e., external disinfection would not reach internally located bacteria.

Problems in Utah with coldwater disease, caused by the bacterium *F. psychrophilum*, have prompted a renewed examination of disinfection efficacy. Recent work here has also shown bacteria persist despite disinfection. So, research was conducted with the objective of finding a disinfectant that would kill 100% of external bacteria without harming the egg.

Methods

Two experiments were conducted recently to determine just how effective various disinfectants were at killing bacteria on the outside of eyed rainbow trout eggs, relative to standard methods. In the first experiment, ten different treatments were evaluated

1. 100 ppm iodine
2. 500 ppm iodine
3. 500 ppm formalin
4. 1000 ppm formalin
5. 1667 ppm formalin
6. 500 mg/L hydrogen peroxide
7. 1000 mg/L hydrogen peroxide
8. 2000 mg/L hydrogen peroxide
9. 30 ppt NaCl
10. Untreated control

The chemicals used were Argentyne (10% povidone-iodine or 1% free iodine), Paracide F (37% formaldehyde, 6-13% methanol; Argent Chemical Co.), and hydrogen peroxide (34% stock solution). Three replicates of 30 eggs each were each treated with 500 mL of the test disinfectant solution in a beaker. After 15 min, the eggs were rinsed 3X with sterile water.

For the bacteriology portion, two media were used: enhanced Ordahl's with the antibiotic Tobramycin (EOT) and trypicase soy agar (TSA). The EOT media was targeted at specifically growing *F. psychrophilum* (Kumagai et al. 2004). TSA media was used to get prevalence and abundance for all other culturable bacteria and fungi. For each media, six eggs were used per replicate to infect three agar plates with a divider that split the plate in two. Each egg was removed from the beaker with sterile forceps, rolled three times across the Petri dish, and discarded. Uninocculated plates ($n = 3$) served as media controls.

Plates were incubated at 15ºC and examined 2, 4, 7, 9, and 15 days after inoculation. Counts were made of each colony-forming unit (CFU) where possible, though many plates were classified as 'too numerous to count'(TNC). Colony descriptions were also recorded. For statistical analysis, the CFU counts were classified into four categories: 0, 1-100, 101-300, >300. Categorical data analysis was used to compare the CFU classification among the treatments. In addition, rank transformed CFU data for each media type were analyzed by ANOVA to compare among the ten chemical-dose treatments.

Results and Discussion

The chi-square analysis and ranked CFU analysis both indicated that there were significant differences between treatments for both media ($P < 0.001$; Table 1). For EOT plates, when each treatment was compared to the control, all the iodine, salt, and hydrogen peroxide treatments had significantly fewer bacteria. At 1667 ppm, formalin significantly reduced bacterial loads, but CFU counts for 500 or 1000 ppm formalin did not significantly differ from controls. For TSA plates, all chemical treatments significantly reduced CFUs relative to controls except salt and 500 ppm formalin.

Table 1. Number of eggs in each total colony-forming-unit (CFU) category for rainbow trout eggs after 15 min exposure to various chemical treatments and rolling on either EOT or TSA media. Means ranks that are significantly different among chemical treatment-dose combinations within a media type are followed by different letters.

		CFU category				
Media Treatment	Dose (ppm)	0	1 to 100	101-300	>300	Mean rank
EOT						
Iodine	100	0	7	1	10	74.2 bcd
	500	3	5	0	10	67.9 bcd
Salt	30,000	0	8	2	7	62.3 c
Formalin	500	0	0	0	18	115.0 a
	1000	0	0	0	18	115.0 a
	1667	0	0	4	14	99.7 ab
Hydrogen peroxide	500	0	1	5	12	90.3 be
	1000	0	3	3	12	87.5 de
	2000	1	6	1	10	71.4 e
Control	0	0	0	0	18	115.0 a
TSA						
Iodine	100	2	15	1	0	27.9 d
	500	2	5	3	8	74.7 c
Salt	30,000	0	0	0	18	128.5 a
Formalin	500	0	0	0	18	128.5 a
	1000	0	5	2	11	100.6 b
	1667	0	10	2	6	78.3 bc
Hydrogen peroxide	500	0	10	0	8	80.0 bc
	1000	0	10	0	8	75.8 c
	2000	0	9	0	9	82.1 bc
Control	0	0	0	0	18	128.5 a

Iodine proved to be among the best disinfectants tested, though on EOT, 10 of 18 eggs had plates that were TNC and only 3 eggs had zero CFUs (Table 1). On TSA, only 2 eggs had zero CFUs, and 8 of 18 were TNC. On EOT, 500 ppm iodine was significantly better than salt ($P = 0.043$) or 1667 ppm formalin ($P = 0.003$), but did not significantly differ from either 100 ppm iodine or 2000 ppm hydrogen peroxide. On TSA, eggs treated with

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500 ppm iodine had significantly fewer CFUs than those treated with either salt ($P < 0.001$) or 2000 ppm hydrogen peroxide ($P = 0.043$), but did not differ from the 1667 ppm formalin treatment.

Differences among doses within a treatment were significant for formalin (EOT, $P = 0.009$; TSA, $P = 0.003$), but not hydrogen peroxide (EOT, $P = 0.154$, TSA, $P = 0.928$). CFUs did not differ between the two iodine doses on EOT plates, but there were significant differences in the counts on TSA.

The data showed that plenty of bacteria are still present after disinfection using the standard protocol of 100 ppm iodine. Unfortunately, the other treatments evaluated did not completely disinfect the eggs either. The data suggest that eggs brought into the hatchery from wild sources bring whatever bacteria are present in the wild, albeit at lower concentrations. Therefore it appears wise to have certain hatcheries dedicated to particular wild brood programs, rather than scattering the egg take among several locations. Continued monitoring of prohibitive pathogens in the wild population via annual disease inspections is also an obvious recommendation.

Egg disinfection Experiment II

Methods

The results of the first study indicated that there were still plenty of bacteria after chemical treatments. One hypothesis was that the point where eggs are touching each other is less likely to be affected by the chemical. Therefore, eggs need to be 'rolled', i.e., kept in suspension, during chemical treatment to assure equal exposure of the outside of the egg. So, in the second experiment, eggs were rolled within the egg jar to see if this would reduce CFUs relative to controls that were not rolled. Treatments evaluated were:

1. 100 ppm iodine not rolled
2. 100 ppm iodine rolled
3. 500 ppm iodine not rolled
4. 500 ppm iodine rolled
5. 2000 ppm formalin rolled
6. 2000 ppm hydrogen peroxide rolled
7. control not rolled
8. control rolled

The tests were conducted in clear acrylic egg jars. A recirculation system was devised in which a submersible pump delivered solution to the center inflow pipe of the egg jar; overflow from the jar returned to a bucket in which the pump was located; a valve controlled flow to the jars, which was adjusted to gently roll the eggs. Total volume of the chemical treatment solution was 12 L (fresh for each rep, T = 13ºC). For each replicate, 2227 eggs (180 mL) were exposed for 15 min. About 15 eggs were collected into sterile vials and covered; the remaining eggs were transferred to an egg tray for further monitoring of hatch and crippling rates. The survival to hatch was calculated as a percentage of the initial number of eyed eggs (2227 eggs) and percent deformity was calculated as a percentage of the number surviving at hatch.

The methodology used in the first study (i.e., rolling the egg across the media) made it difficult to get good estimates of bacterial abundance. In this second experiment, a different approach was taken. Following the methodology of Barnes et al. (2005), eggs were vortexed in 2 mL of sterile peptone-salt diluent (0.1% proteose peptone and 0.8% NaCl) and 100 uL of the solution was plated on TSA or EOT. In addition, 100 uL of a ten-fold dilution was also plated on both media. Plates were incubated at 15ºC and CFUs were counted at 3, 5, 7, and 11 days after inoculation. Plates with too many bacteria to count or that were overgrown, were noted as 'too numerous to count' (TNC). Observations of colony morphology were recorded and Gram stains were made of representative colony types.

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For statistical analysis, TNC plates were assigned the value 10,000 CFUs. Since this was an arbitrary value, total CFUs were rank transformed for analysis. Separate analyses were conducted for each level of dilution and each media type. Since the formalin and peroxide treatments did not have a no-roll control and the effects of rolling were best tested within a given treatment, comparison of chemical treatment effects were tested separately (one way ANOVA of ranked CFUs) for rolled and no-roll groups. The effects of rolling were analyzed by χ^2 analysis comparing the frequency distribution of total CFUs per egg within four categories: 0 CFUs, 1-100, 101-300, >300 CFUs; These tests were conducted separately for each combination of dilution, media, and treatment-dose. The percent hatch and deformity data were analyzed by the Kruskal-Wallis test in which combinations of chemical, dose, and rolling were combined into eight treatments (e.g., iodine 100 roll, iodine 500 no-roll, etc.).

Results and Discussion

Chemical treatment had no deleterious effects on the eggs. The chemical treatments had no significant effect on the survival of eyed rainbow trout eggs to hatch (92-96% across treatments), nor did they significantly increase deformity percentages (0.3-0.7%).

Chemical treatment reduced bacteria numbers, but differences among chemicals were few. Comparisons among chemical treatments for each dilution, media, and roll treatment indicated that each treatment significantly reduced CFUs relative to controls (Tables 2 and 3). For eggs that were not rolled, differences between the two iodine doses were not significant in undiluted samples for both media, and only significant for the ten-fold dilutions on TSA in which lower CFU numbers were observed in the 500 ppm iodine treatment (Table 2). For rolled eggs, iodine dose differences were not significant for any dilution or media. For EOT plates without dilution, CFUs were significantly lower after treatment with 100 ppm iodine than with 2000 ppm formalin or hydrogen peroxide. However, after ten-fold dilution, there was no significant difference among the four chemical treatments. For undiluted samples on TSA plates, formalin treated eggs had significantly higher CFUs than eggs treated with 500 ppm iodine or 2000 ppm hydrogen peroxide (Table 3). After dilution however, no significant differences were observed among the chemical treatments.

Egg rolling significantly reduced bacteria abundance in certain cases, especially for untreated eggs. For control eggs plated on EOT either with or without dilution there were significantly fewer CFUs in the rolled treatment (Table 4). For controls on TSA, there was no significant effect of egg rolling for undiluted samples or those diluted tenfold. The effect of rolling was not significant for eggs treated with iodine at either 100 or 500 ppm and plated on EOT. For TSA, there was a significant reduction in CFUs after rolling for eggs treated with 100 ppm iodine (no dilution), but after sample dilution, there was no significant difference in CFUs. For eggs treated with 500 ppm iodine and plated on TSA, there was no significant difference in CFUs between rolled and unrolled eggs at either dilution.

The effect of egg rolling appeared to be most effective for untreated eggs and was most noticeable on EOT plates, indicating that perhaps the effect works better for some species of bacteria than others that may be more adhesive. Since the EOT plates were targeted for isolation of *F. psychrophilum*, it appears that rolling reduced abundance of that bacterium. After chemical treatment, bacterial abundance was low enough that the effect of rolling was not detectable. The only exception was for eggs treated with 100 ppm iodine sampled without dilution and plated on TSA; enough bacteria survived this treatment that rolling had an additional effect on reducing CFUs. Given that rolling only occurred for 15 min, it would be interesting to do some follow-up studies to look at the bacteriological aspects of egg rolling continuously from the eyed egg stage to hatch. The rolling effects suggest the potential for removal of bacteria from the surface of eggs by physical means.

The chemical tests indicated that iodine is still the best option for disinfection. Also, there was little benefit of increasing the iodine dose from 100 to 500 ppm. Formalin or hydrogen peroxide at 2000 ppm reduced bacterial abundance, but still was inferior to iodine treatment in some cases. Each chemical treatment resulted in survival

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of bacteria after treatment, despite attempts to get better chemical contact using rolling. Further iodine tests are needed examining higher doses.

The data on the control eggs suggested wide variation in CFUs among individual eggs, with some samples that were still too numerous to count despite tenfold dilution. Further tests should carry dilutions further with the controls to better estimate total bacterial numbers for more accurate calculation of reduction rates.

Egg disinfection has two purposes. One is to reduce the overall abundance of bacteria that may affect egg respiration and survival (Barnes et al. 1999; Barnes et al. 2003; Barnes et al. 2005). The other is to reduce or eliminate pathogens that may affect egg and fry survival and compromise the health certification of a hatchery. The results of this study indicate that current practices of formalin prophylactic treatment and treatment with iodine achieve the first objective, but we are not succeeding in complete elimination of external bacteria. Further work is needed to identify more effective doses, mechanical strategies, or novel approaches such as the use of non-pathogenic bacteria that can compete with pathogenic species.

Eric Wagner

Table 2. Mean rank of colony forming units on two media types (enriched Ordahl's with tobramycin or trypticase soy agar) at two dilutions (0, or 10-fold) for rainbow trout eggs that were *not rolled* in the egg jar during chemical treatment for 15 min. Means followed by a common letter in a row are not significantly different.

Dilution Media	Control	Iodine 100 ppm	Iodine 500 ppm
0			
EOT	528.0 a	161.3 b	186.4 b
TSA	488.6 a	151.4 b	188.6 b
10			
EOT	483.4 a	178.6 b	170.3 b
TSA	505.6 a	237.0 c	151.4 b

Table 3. Mean rank of colony forming units on two media types (enriched Ordahl's with tobramycin or trypticase soy agar) at two dilutions (0, or 10-fold) for rainbow trout eggs that were *rolled* in the egg jar during chemical treatment for 15 min. Means followed by a common letter in a row are not significantly different.

Dilution Media	Control	Iodine 100 ppm	Iodine 500 ppm	Formalin 2000 ppm	Hydrogen Peroxide 2000 ppm
0					
EOT	492.0 a	196.2 b	236.7 bc	288.4 c	261.0 c
TSA	480.9 a	259.9 bc	213.5 c	318.3 b	241.8 c
10					
EOT	435.9 a	183.8 b	197.0 b	239.2 b	223.4 b
TSA	478.9 a	196.1 b	193.7 b	228.5 b	231.5 b

(Continued on page 11)

Table 4. Frequency distribution of colony forming units (CFUs) for rainbow trout eggs that were rolled or not rolled during treatment with iodine at either 100 or 500 ppm or with no chemical (control). Eggs were vortexed in a diluent that was plated on two media types (enriched Ordahl's with tobramycin or trypticase soy agar) without dilution (0) or after tenfold dilution (10). Probability values for chi-square maximum likelihood tests are shown for the comparison between rolled and unrolled treatments.

Dilution Media Treatment	0 CFUs	1 to 100 CFUs	101-300 CFUs	>300 CFUs	Chi-square probability
0					
EOT					
Control					
Rolled	0	4	7	7	0.002
Unrolled	0	0	2	16	
Iodine-100 ppm					
Rolled	13	5	0	0	0.200
Unrolled	16	2	0	0	
Iodine-500 ppm					
Rolled	9	9	0	0	0.080
Unrolled	14	4	0	0	
TSA					
Control					
Rolled	0	7	2	9	0.226
Unrolled	0	7	0	11	
Iodine-100 ppm					
Rolled	10	7	0	1	0.016
Unrolled	17	1	0	0	
Iodine-500 ppm					
Rolled	11	7	0	0	0.275
Unrolled	14	4	0	0	
10					
EOT					
Control					
Rolled	0	16	2	0	<0.001
Unrolled	0	3	8	7	
Iodine-100 ppm					
Rolled	14	4	0	0	0.178
Unrolled	15	3	0	0	
Iodine-500 ppm					
Rolled	13	5	0	0	0.200
Unrolled	16	2	0	0	
TSA					
Control					
Rolled	0	6	7	5	0.026
Unrolled	0	1	5	12	
Iodine-100 ppm					
Rolled	13	5	0	0	0.296
Unrolled	10	8	0	0	
Iodine-500 ppm					
Rolled	13	5	0	0	0.063
Unrolled	17	1	0	0	

Effect of Density on Growth and Survival of Larval Least Chub

As part of the recovery effort for the rare cyprinid *Iotichthys phlegethontis*, there was a need identified for developing techniques for producing least chub for restocking efforts. Crawford (1979) and Baugh (1980) demonstrated that adult least chub would spawn in aquaria with vegetation substrates, but fry rearing strategies were not researched. In 2002, a refuge population of least chub was established at the Utah Division of Wildlife Resources Fisheries Experiment Station, Logan, Utah, using fish from Mona Springs, near Mona, Utah. These fish, and their progeny, were used to conduct several experiments aimed at developing aquaculture techniques for rearing least chub. In this article, we evaluated the effects of rearing density on growth of fry.

Methods

Rearing density of least chub fry was evaluated for its effects on growth and survival over an 8 week period. To achieve sufficient numbers of fry, replicates were conducted over time using different cohorts. On 20 June 2005, 10, 50, and 100 fry from cohorts harvested 9 and 16 May 2005 were allocated to three plastic tanks (32 x18 cm) for the first replicate. The resulting fish densities were 2.27, 11.36, and 22.7 fish/L (2,270, 11,360 and 22,700 fish/m^3). The fish for the second replicate were from cohorts harvested 26 and 31 May 2005 and 2, 3, and 6 June 2005. The third replicate was initiated 22 July 2005 with fry that were harvested 13, 16, and 20 June 2005. So, for each replicate, the fish were 5-6 weeks old at the start of the test. Care was taken to proportionately allocate fish from different harvest dates to the different density treatments. Fish were fed brine shrimp nauplii (*Artemia franciscana*) frozen in ice cubes, 4 times/day. The low density treatment received 0.5 mL (8.8 mg dry weight), the medium treatment got 2.5 mL (44.4 mg), and the high density group received 5 mL (88.8 mg) brine shrimp at each feeding. Tank water volume was 4.4 L and flows of hatchery well water to the tanks ranged from 78 to 106 mL/min. Water temperature was 18.6°C.

Water quality was measured in the high-density treatment to verify that water quality was not limiting growth or survival. DO was measured with a meter (YSI, Yellow Springs, Ohio) calibrated the day of measurement. Total alkalinity was measured with a commercial kit (Hach Co., Loveland, CO). Total ammonia-N was measured using the Nesslerization method and a series of standards (APHA et al. 1989).

Digital pictures were taken of the fish in a shallow pan of water with a ruler at the beginning and end of the study; total lengths were derived from the digital images on the computer. Final weights were measured as a total of all fish in each tank, and then divided by the number of fish to estimate the mean weight per fish.

Results and Discussion

Initial lengths were significantly different among replicates ($P < 0.001$), but not among density treatments ($P = 0.897$; Table 1). Final mean lengths ranged from 17.9 to 18.3 mm and did not significantly differ among density treatments ($P = 0.391$) or replicates ($P = 0.108$; Table 3). Mortality among replicate tanks ranged from 0 to 6% and did not significantly differ among density treatments ($P = 0.147$). Mean final weights per fish ranged from 48 to 67 mg among replicate tanks and similarly did not significantly differ among densities ($P = 0.339$).

Water quality data indicated that water quality was not a problem during the study. Total ammonia-nitrogen was barely above detectable limits. Even at the highest densities, dissolved oxygen was at or above 6.5 mg/L. Total alkalinity was 205 to 222 mg/L.

Table 1. Mean ($n = 3 \pm$ S.E.) growth and survival of least chub held at three different densities for 8 weeks.

Treatment	Initial length (mm)	Final length (mm)	Final weight (mg)	Mortality (%)
10	9.1 ± 0.25	18.3 ± 0.26	58.7 ± 4.91	0.0 ± 0.0
50	9.1 ± 0.12	17.9 ± 0.13	51.0 ± 2.62	2.0 ± 2.0
100	9.1 ± 0.08	18.2 ± 0.11	53.6 ± 2.08	3.3 ± 1.2

(Continued from page 12)

A density of up to 22,700 fish/m^3 in this study did not significantly affect growth and survival of least chub. Common carp fry are generally stocked in fry ponds at 200-400/m^2 (rarely up to 600/ m^2; Horváth et al. 1992). Hepher and Pruginin (1981) noted that for intensive culture of common carp, up to 50,000 fry/m^2 could be reared in hatchery tanks until they reach 2 g. Fry densities of golden shiners in grow-out ponds were recommended to be 37-62/m^2, whereas fathead minnow (*Pimephales promelas*) densities were 25-75/m^2 (Stickney 1979). Our observations of least chub fry and adults indicate that they are a schooling species in which high densities are a behavioral norm, so the lack of any effect on growth was not surprising. Densities at which water quality may be compromised should be avoided. The tolerance of least chub to extremes in water quality has yet to be fully understood. Research on the critical thermal maximum has been conducted, and has established 34 to 35°C as the upper incipient lethal temperature (Crawford 1978). Measurements of least chub habitat indicate they are tolerant of a wide range of conditions (Perkins et al. 1998) and their small size would aid in tolerance to hypoxia (Robb and Abrahams 2003).

In summary, the data in these studies indicate that densities of up to 22,700 fish/ m^3 are not detrimental to growth of least chub fry.

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