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SPAWNING SUCCESS IN LEAST CHUB (*IOTICHTHYS PHLEGETHONTIS*) PAIRED AT DIFFERENT MALE TO FEMALE RATIOS AND DENSITIES

INSIDE THIS ISSUE:

Least chub reproduction occurs between April and August, depending on environmental conditions (Sigler and Sigler 1996). Least chub are polyandrous broadcast spawners, releasing demersal and adhesive eggs over vegetation, primarily algae (Crawford 1979). Females produce only a few eggs at a time, but production continues through the spawning period. Thus, least chub are a partial and intermittent spawner.

Least chub (*Iotichthys phlegethontis*) are considered a sensitive species in Utah, at risk of becoming threatened or endangered. The multi-agency Conservation Agreement (Perkins et al. 1998) for least chub addresses the need for expanding least chub distribution through introduction or reintroduction; least chub used for these activities would be raised at hatchery facilities. Current hatchery populations at Wahweap State Fish Hatchery and the Fisheries Experiment Station (FES) are maintained via extensive aquaculture techniques. In efforts to establish intensive culture techniques, a study was conducted at FES to determine the effects of different male to female ratios and fish densities on spawning success of least chub. The results of this study will be used to develop intensive culture techniques for propogation of least chub for stocking and for research.

Methods

Least chub were paired into 75 L tanks at two sex ratios (1:1 and 2:1, male:female) and two densities of females (low and high) in a 2 x 2 factorial design. The four treatments of 1:1 (male:female), 5:5, 2:1, and 10:5 were evaluated in triplicate. Fish were transferred to 12 test aquaria on 2 August 2004 from a wild broodstock from Mona Springs, Utah, maintained at FES. Aquaria were supplied with hatchery well water (18.5°C) in a flow-through system. A full spectrum UV light (two 4-ft bulbs) was on a timer to deliver a 14 h: 10 h (light: dark) photoperiod. Fish were fed a commercial flake diet (TetraMin) at 3-5% body mass.

Spawning material was provided in the form of unbraided nylon rope, hung in 3 strands from a piece of PVC pipe wired to the top of the aquarium. Once per week starting 10 d after aquaria stocked, the rope was carefully removed from the aquaria and transferred to clear plastic containers. The aquaria were siphoned clean to remove waste feed and feces, and new spawning substrate was added. Once removed, the spawning substrate was incubated for six days in a hatchery trough with water flowing around the containers to maintain temperature (18.1˚C). This provided ample time for eggs to hatch; eggs hatch in 5 d at 17.8°C (Crawford 1979). After the incubation period, fry were harvested from spawning substrate and enumerated. Spawning substrate was disinfected and dried for reuse in the spawning tanks.

After 21 weeks, the total number of fry produced for each tank was determined. Differences in total fry production between treatments were compared using a one-way analysis of variance (ANOVA). Total fry production was also compared separately between the two ratios and between the two densities using a two-tailed *t*-test. To determine how total fry production was affected by female size, total fry production was plotted against female size (g). Data were analyzed using SigmaStat (SPSS 1997).

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Results

Least chub in the high density tanks spawned within the first 10 days after being stocked into the tanks. Spawning did not occur in a low density tanks until the third week. Spawning behavior was observed in some tanks. Males would chase females, poking at their vents. Receptive females would swim into the rope, followed by the males; after a short period (2 – 5 s), fish would swim out of the rope. In high density tanks, unreceptive females and incessant males could be seen feeding on eggs.

Over the course of the study, which concluded 29 December 2004, a total of 3570 fry were produced. Fry production was highest in September and October and declined in late winter (Figure 1). Each treatment had some production, although one tank in the 1:1 treatment did not produce any fry. The most fry produced in one week was 256 fry by a tank in the 1:1 treatment; the most total fry produced was 1,084 fry by a tank in the 5:5 treatment.

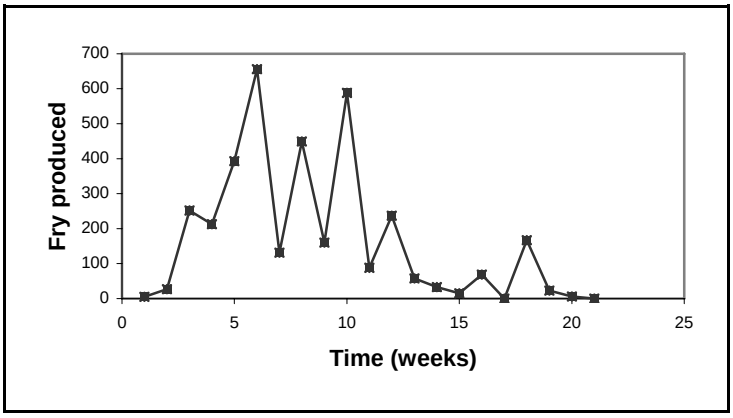

Figure 1. Total weekly least chub fry production for all four spawning ratio treatments.

There was no significant difference among treatments in total fry production ($F_{3,11} = 1.196$, $p = 0.371$; Figure 2) .Tanks with a 1:1 ratio produced more total fry than tanks with a 2:1 ratio (means of 443 and 153 fry, respectively); however, the difference in production was not significant ($T10 = 1.612$, $p = 0.138$). Also, tanks with more females (5 versus 1) had higher total fry production (means of 397 and 198 fry, respectively), but again the difference in production was not significant ($T10 = 1.041$, $p = 0.322$).

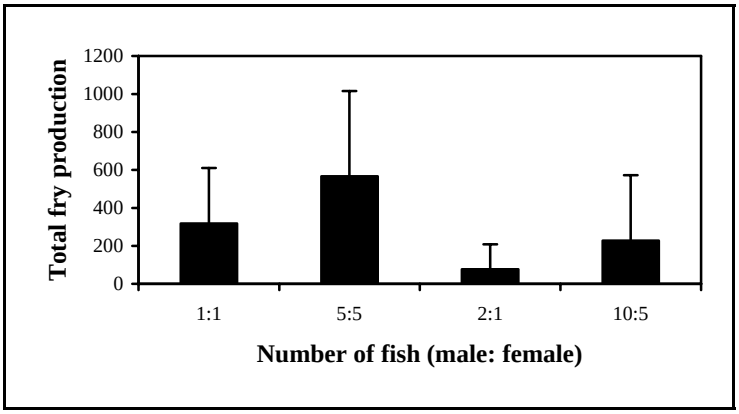

Figure 2. Mean (n = 3) total least chub fry production during a 21 week period for four treatments comparing different ratios (1:1 and 2:1, male to female) and female densities (1 and 5 females).

Fry production appeared to be affected by female size (Figure 3). Tanks that had a mean female weight between 1.3 and 2.2 g had the highest total fry production, while production declined at either end of this range.

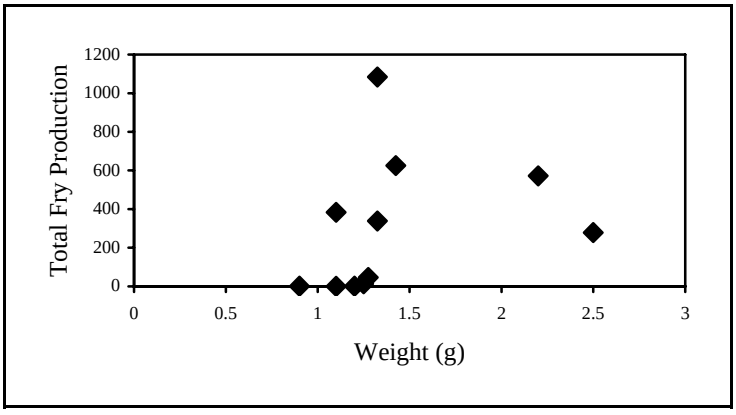

Figure 3. Total least chub fry production as a function of female body size (g).

Discussion

The finding of no significance in total fry production between the two ratios and the two densities indicates that least chub should be paired at a 1:1 male to female ratio at low densities to maximize production. Pairing least chub at a skewed ratio with more males than females only resulted in more fish to maintain for no additional return. Likewise, pairing least chub at higher densities required more maintenance to keep tanks clean and increased the likelihood for egg predation. Densities in between those used in this study may result in better fry production by

keeping egg predation low while increasing the number of females for spawning.

Crawford (1979) reported a 2:1 female to male ratio of adult least chub a Leland Harris Spring complex. Future studies will compare fry production between a 1:1 and a 2:1 female to male ratio. Females will be selected based on body size to ensure similar sized females in each tank. Spawning substrate transfer frequency will also be tested to determine if egg predation can be decreased by removing substrate more frequently.
Eric Billman and Eric Wagner

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temperature will be compared to determine the temperature range that will optimize all three variables.

Eric Billman

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Construction workers begin the site construction excavation for the new warmwater recycle hatchery at the Fisheries Experiment Station. The site is adjacent to the existing June sucker facility. Completion is scheduled for March 2006

Bacterial Loading in Milt and Ovarian Fluid of Vaccinated and Unvaccinated Rainbow Trout

Coldwater disease (a.k.a., rainbow trout fry syndrome) is a significant disease of fishes, caused by the bacterium *Flavobacterium psychrophilum*. The disease has been a problem in Utah hatcheries for many years and appears to inducing clinical signs at higher temperatures than in the past. Attempts at vaccination in the past have focused on bath vaccination of the fingerlings at three different hatcheries (see Ichthyogram 15 (1/2):4-7). These trials were unsuccessful, so we decided to address the problem at its source, the broodstock. The bacterium is known to pass on from the mother to the egg (Taylor 2004). If bacteria could be reduced or eliminated from the source, transfer of the bacterium to the production hatcheries might be reduced or eliminated.

A bacterin was commercially prepared from *F. psychrophilum* bacteria isolated from fish at Glenwood State Fish Hatchery, Glenwood, UT. This was diluted 1:2 with sterile phosphate buffered saline for injection. A total of 40 males and 40 females of 5-year-old Sand Creek rainbow trout were injected intraperitoneally with 0.2 ml of bacterin 4-6 weeks prior to the first egg take. Of these, milt or ovarian fluid was collected from 13 males and 13 females as aseptically as possible. In addition, fluids were taken from 15 unvaccinated females and 4 unvaccinated males. Sampling was distributed across 5 egg takes, 12-14 days apart from September 1 to October 26, 2004.

This fluid (100 ul) was plated onto enriched Ordahl's (EO) or EO plus tobramycin (EOT), a selective antibiotic used to reduce growth of competing bacteria (Kumagai et al. 2004). Three different dilutions of sexual fluid were made with sterile phosphate buffered saline: 0, 10-fold, and 100-fold. A strip of laboratory film was wrapped around the plate rim after inoculation to limit contamination. Every fish did not receive each media or dilution treatment, so data are not available for all combinations of media, dilution, and gender. The plates were observed at periodic intervals after inoculation and any colony forming unit (CFU) counted. Colony color and morphology was also noted.

Statistical analysis

The cumulative count after 2 weeks of yellow colonies (putative *F.p.*) and of total CFUs was used for statistical analysis. For plates with too many bacterial colonies to count, an arbitrary value of 10,000 was substituted in the data file for analysis. Tests were run separately for each media and dilution using SPSS software. Normality tests using the Kolmogorov-Smirnov test indicated that the data were not normally distributed. Hence, data were rank transformed prior to analysis with a general linear model with sex, vaccination, and the sex-vaccination interaction as fixed factors. For testing of prevalence, the data were tested with a hierarchical log-linear model to determine significant variables in the model, separately for each media and dilution. Since sex and the interaction of sex and vaccination were significant, separate chi-square tests or Fisher's Exact Tests (prevalence by vaccination) were conducted for each level of media, dilution, and sex. In addition to the prevalence data, the CFUs were split into three categories: 0, 1 to 9,999, or 10,000 CFUs for comparison of the frequencies in each category using chi-square analysis for each combination of media, dilution, and gender.

Results

The general linear model indicated that there were significant differences in total CFUs between vaccination treatments and between male and female fish. The effect was dependent on media and dilution. For example, on EO, significant differences were observed for sex at dilutions of 0 or 10, but not 100. Females generally had higher total CFUs than males. For yellow CFUs, there were no significant differences between sexes at any dilution for EO cultures. On EOT, there were no significant differences between male and female total CFUs or yellow CFUs at any dilution.

On EO plates, vaccinated fish had significantly higher numbers of total CFUs at no dilution ($p = 0.039$), but

did not differ among the 10 or 100-fold dilution treatments. On EOT plates, total CFUs were significantly higher in the vaccination group at 0 and 10-fold dilutions, but not at 100. Analysis of the prevalence data indicated that, for EO plates, total CFUs or yellow CFUs did not differ significantly between vaccination treatments for any dilution level with the exception of yellow CFUs, which were significantly higher in vaccinated females in the 10-fold dilution (Table 1). For EOT plates, there were generally no significant differences between vaccinated and unvaccinated fish in total CFUs, though there were significantly more yellow CFUs for vaccinated females in the 0 and 10-fold dilution treatment.

Table 1. Summary of the prevalence of colony-forming units (percentage of plates with CFUs of all types or just yellow CFUs) for samples taken from vaccinated or unvaccinated 5-year-old Sand Creek strain rainbow trout. Abbreviations: EO = enriched Ordahl's, EOT = enriched Ordahl's with tobramycin, F = female, M = male, *n* = sample size, CFU = colony-forming unit. An asterisk indicates a significant difference between vaccinated and unvaccinated treatments within a given media, dilution, and sex.

Media	Dilution	Vaccination	Sex (*n*)	Total CFUs (%)	Yellow CFUs (%)
EO	0	+	M(8)	62.5	37.5
		-	F(8)	100.0	37.5
		+	F(7)	100.0	57.1
	10	-	M(4)	0.0	0.0
		+	M (13)	38.5	23.1
		-	F (15)	66.7	13.3
		+	F (13)	69.2	61.5*
	100	-	M (4)	0.0	0.0
		+	M (5)	20.0	20.0
		-	F (7)	42.9	14.3
		+	F (6)	50.0	33.3
EOT	0	-	F (8)	37.5	25.0
		+	F (7)	85.7	85.7*
	10	-	M (4)	0.0	0.0
		+	M (13)	46.2	15.4
		-	F (15)	20.0	0.0
		+	F (13)	53.8	46.2*
	100	-	M (4)	25.0	0.0
		+	M (5)	0.0	0.0
		-	F (7)	14.3	14.3
		+	F (6)	0.0	0.0

If the data were split into three categories—0, 1 to 9,999, and 10,000 (i.e., too numerous to count), a similar pattern of significant differences were observed in which the vaccinated females had more yellow CFUs at the 0 and 10-fold dilutions. For total CFUs, only the female sample with no dilution on EO media was significantly different. As with the other samples, vaccinated fish had higher CFUs that unvaccinated fish. Males did not significantly differ in the load of bacteria (total CFU) between vaccinate groups for either media at any level of dilution.

Discussion

At dilutions of 100, the percentage of plates with CFUs decreased substantially, so these dilutions were not the best for comparing the vaccination treatments. The results for the lower dilutions indicated that vaccination did not reduce the number of yellow colonies, but rather increased it for some fish, especially females. Statistically, total CFUs were unaffected by vaccination treatment among males, but larger sample sizes might show similar significant increases in total CFUs in vaccinated fish. Since the vaccination process uses a needle to break the skin-mucous barrier, it is not too surprising to see higher numbers of CFUs in this treatment. The efficacy of the vaccination against coldwater disease was not tested directly in this evaluation, but the indirect results indicate that vaccination does result in an increase in CFUs in milt and ovarian fluid of peritoneally-injected fish. The duration of this effect is unknown and should be the subject of further study, as well as the efficacy of the vaccine against coldwater disease.

Eric Wagner

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EFFECTS OF TEMPERATURE ON GROWTH OF LEAST CHUB FRY

Least chub are currently found in warm springs areas in Utah (Sigler and Sigler 1996). Least chub have been listed in the state as a species of concern as their numbers and distribution have dwindled with increased anthropogenic effects, especially the introduction of non-native species (Perkins et al. 1998). Current conservation efforts include developing hatchery facilities, establishing refuge populations, and expanding populations within their historical range. Understanding thermal requirements of this species will help establish protocols for rearing facilities as well as selecting habitats that have or will most likely support least chub populations.

Hatchery populations of least chub exist at Wahweap State Fish Hatchery, near Big Water, UT and at the Fisheries Experiment Station (FES), Logan, UT. These populations serve as brood stocks, and are maintained via extensive aquaculture techniques. Currently at FES, intensive aquaculture techniques are being developed. As part of a series of studies, a temperature study was conducted using least chub fry to determine the temperature range in which fry growth can be optimized.

Methods

The temperature study was conducted at FES. Least chub young of year (YOY) were produced in fall 2004 from a wild broodstock from Mona Springs, Utah, maintained at FES. After hatching, YOY were held in 16.5°C well water until testing. Least chub ranged in age from 64 – 106 d old at the start of the study.

Aquaria for the study were set up in a flow through system. Cold well water (14°C) was mixed with heated warm well water (16.5°C heated with two 6 kw, 240 v and one 1kw, 240 v water heaters) to provide test temperatures between 14 – 27°C. Water from each source was passed through de-gassing columns and mixed in separate head boxes to achieve test temperatures of 14, 17, 21, 24, and 27°C. Each head box supplied water to three 38 L aquaria at a rate of 0.61 L/min. All aquaria and connecting pipes were covered with insulation to minimize temperature fluctuations. A full spectrum UV light (two 4-ft bulbs) provided a 14 h: 10 h (light:dark) photoperiod.

Twenty-three least chub YOY were randomly selected and placed into each aquarium on 2 December 2004. Mean total length was estimated for all test fish from 115 least chub; a digital image was taken from above and total lengths determined using imaging software (Adobe Photoshop 5.5). Mean weight was estimated from a length-weight regression from 11 mortalities ($r^2 = 0.9763$, $P < 0.0001$). Fish averaged 15.5 mm total length and 0.032 g. After least chub were placed into aquaria, temperatures were raised or lowered to treatment temperatures within 1 hr. Fish were held at test temperatures for 112 days.

Least chub were fed a mixed diet of a ground commercial flake feed (TetraMin) and frozen brine shrimp nauplii. Initial diet (Day 1 – Day 84) consisted of 84 mg flake feed and 88 mg brine shrimp per day. After Day 84, the amount of brine shrimp was increased to 132 mg per day. All tanks were fed the same amount of feed.

Tanks were cleaned twice weekly to remove excess feed. Mortalities were removed daily, and total length and weight measured. Water temperature in one tank from each treatment (representative tank changed daily) was measured daily. Water temperature in one tank from each treatment was monitored with a Hobo® Water Temp Pro temperature logger, which recorded temperature once every three hours. Temperatures were downloaded weekly, and the data logger rotated to the next tank within the treatment.

Lengths and weights of least chub in each tank were measured at the end of the experiment (112 days). Lengths were measured using digital images as previously described. Weights were measured as a total of all fish in each

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tank, and then divided by the number of fish to estimate the mean weight. Absolute growth rate was calculated according to the formula $G = (Y_t - Y_i)/t$, where Y_t is the mean weight at time t, Y_i is the initial mean weight, and t is the number of days (Ricker 1979). Differences in growth rates between temperatures were compared using a one-way analysis of variance (ANOVA) in SigmaStat (SPSS 1997). Growth was analyzed by fitting the data to a second-order polynomial regression using SigmaPlot (SPSS 2001; Selong et al. 2001).

Table 1: Mean temperatures for each treatment over the duration of the study.

Temperature	Survival (%)	Standard deviation
14.15	99	0.025
17.27	83	0.115
21.11	91	0.087
24.28	96	0.043
27.33	86	0.100

Table 2: Mean survival of least chub within each treatment.

Target temperature	Actual Temperature	Standard deviation
14°C	14.15	0.100
17°C	17.27	0.340
21°C	21.11	0.499
24°C	24.28	0.525
27°C	27.33	0.695

Results

Temperatures in the test tanks were maintained throughout the study with minimal fluctuations (Table 1). Daily fluctuations were less than 1°C. The survival of least chub ranged between 83% at 17°C and 99% at 14°C (Table 2).

The highest growth rate was achieved in the 21°C treatment tanks and the lowest growth in the 14°C tanks. The growth of least chub varied significantly between all temperatures except at 17°C and 27°C ($F_{4,14} = 285.22$, $P < 0.001$). The peak growth estimated by the second-order polynomial regression occurred at 22.3°C (Figure 1). The 95% confidence interval of the peak growth temperature would predict maximum growth between 20.7 – 24.4°C. Least chub within this temperature range (21 and 24°C) were the most active, swimming freely throughout the tanks. At higher or lower temperatures, least chub remained predominantly near or in contact with the bottom of the tanks. The upper and lower thermal limits as predicted from the regression line (intersection of regression line with the x-axis) were 32.2°C and 12.5°C, respectively.

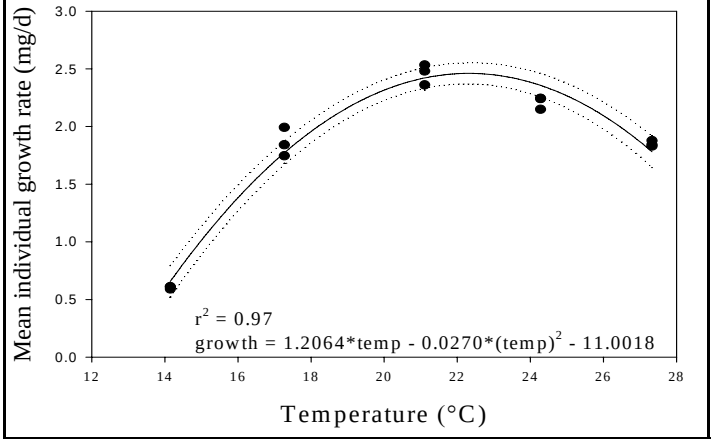

Figure 1: Growth of least chub in relation to temperature. Each circle represents the mean individual weight gain for each tank. Dotted lines indicate the 95% confidence interval of the regression line.

Discussion

Maximum growth of least chub fry in this study occurred at higher temperatures than the annual temperature variation (12 – 16°C) of the springs found at Mona Springs. This adaptation of higher thermal requirements of fry reflects the spawning behavior shown by the adults. Least chub adults occupy the pool habitats around the springs. When spawning, they swim into warm, shallow, marsh-like habitat, and then return to cooler habitat near springs (Perkins et al. 1998). Therefore, fry would hatch in these warmer, shallow habitats in which temperatures can fluctuate between 15 – 30°C.

Rearing facilities for least chub fry should utilize water temperatures between 20.7 – 24.4°C to optimize growth of the fry. The effects of temperature on fry at earlier stages in development are unknown. Future studies will determine fry production of adults at the same temperatures; survival and growth of fry produced at each

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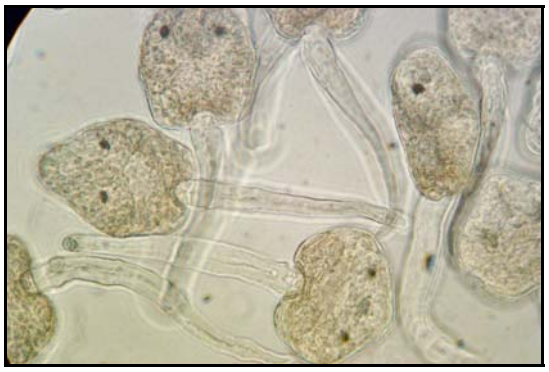
USE OF A SAND FILTRATION RECYCLE SYSTEM TO PREVENT INFECTION OF JUNE SUCKERS, CHASMISTES LIORUS, BY THE DIGENETIC TREMATODE CENTROCESTUS FORMOSANUS: FAMILY HETEROPHYIDAE



The proposed building of a new June sucker hatchery at Goshen or Gandy warm springs is in question due to the presence of *Centrocestus formosanus*, a digenetic trematode that parasitizes warm water fish. *Centrocestus formosanus* uses *Melanoides tuberculata* as its first intermediate host, an invasive mollusk now prevalent at both springs (Rader et al., 2003). Cercariae (the life stage that infects fish) are released from the snail and then encyst in the gills of fish, often causing lethargy and respiratory difficulties, and may potentially lead to death due to suffocation (Balasuriya, 1988) (Fig. 1).

Several experiments at the Fisheries Experiment Station have been implemented in an attempt to eliminate this parasite and its effects on warm water fish. An experiment was conducted using high doses of praziquantel (5 mg/L x 6 hours and 2.5 mg/L x 12 hr) to kill metacercariae encysted in the gills of naturally infected *Gambusia sp* found at Fish Springs National Refuge. Unfortunately the praziquantel had little to no effect on the cysts (Harvey, 2005). Another experiment looked at the phototaxis behavior of the cercariae. The cercariae proved to be phototaxis positive, a behavior we could use to concentrate cercariae in the water column and apply a mechanical or chemical treatment to kill them (Harvey, 2004b). Ultraviolet light was tested as a mechanical treatment to kill cercariae at different time intervals. The UV light killed cercariae but only after an unrealistic amount of time (Harvey, 2004a). Previous studies by Arndt and Wagner (2004) showed that sand filtration could eliminate triactinomyxons of *Myxobolus cerebralis* from infected water (the parasite that causes whirling disease). The objective of the following study was to test sand filtration as a means of eradicating cercariae from infected water.

Materials and Methods

The sand filtration system incorporated three 20-gallon aquaria on a recycle system. The sand filter portion consisted of three tubes of 6 inch diameter PVC pipe that were capped on the bottom end, with 4 inches of gravel on the bottom, topped with 7 inches of sand, 250-300 µm in size. A cross of PVC collection tubes in the gravel collected filtered water that flowed to the aquaria and was controlled by a valve. The filter was placed above the tanks and connected to a common head box using ½ inch PVC pipe (Fig. 2). A float valve was connected above the sand to stop the flow of water if the sand filter backed up. With the exception of the sand size, the design of the sand filter system was as seen in Arndt and Wagner (2004).

Ninety June suckers approximately 5 cm in length were placed into 9 tanks (10 fish /tank) one week before starting the experiment to allow for acclimation. The front of each tank was covered with black plastic to prevent disturbing the fish. Fish were fed a razorback diet twice a day and the water was kept at an average of 17.2°C. Three treatments were used in triplicate for this experiment: a positive control, negative control, and the sand filters. The positive control and sand filter treatments were exposed to cercariae while the negative control was not.

Cercariae for each treatment were freshly harvested the day of exposure from an aerated 10 gallon tank of 16

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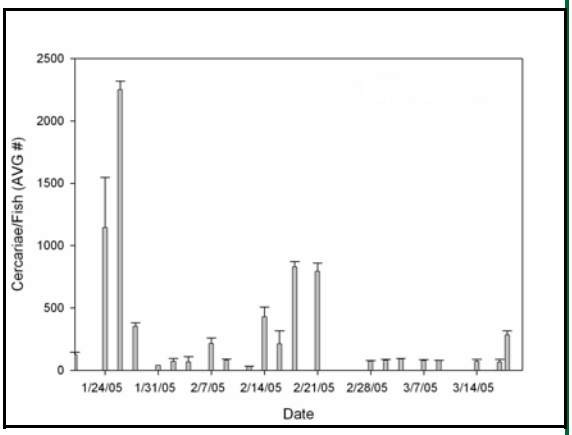
infected snails. Four gallons of water was siphoned out of the tank after it had been agitated thoroughly. Three 300 mL samples were taken from the 4 gallons of water, stained with 20 µL flourescein diacetate (FDA) stock solution (100µL FDA diluted in 8 mL deionized water), filtered through an 8 µm Nuclepore filter, and counted under a fluorescence microscope. The three-sample average was extrapolated out to estimate the total cercariae in each gallon of water. Two gallons of infected water were dripped into the head box of the positive control and the sand filter treatment over a period of approximately 45 minutes. This procedure was repeated three times per week for two months.

Biweekly water exchanges were implemented for each system. The sand filter treatment also required a weekly back flush to clean the extra debris that had accumulated in the filter. The back flush pumped fresh water up and out of the pipe into a waste bucket. The protocol was followed as described in Arndt and Wagner (2004).

Four fish from each tank were harvested and placed in 10% buffered formalin at the end of two months. One week later, the rest of the fish were harvested and pickled in formalin. Each individual gill from both sides of the head was cut out and placed on a slide for wet mount analysis. The gills were then placed back into 70% alcohol for possible histology.

Results

The number of cercariae harvested for each exposure varied significantly (Fig. 3). The most cercariae harvested were 2250/fish on January 20th, with the lowest harvest at 40/fish on January 31st. The two-month average was 305 cercariae/fish. The total cumulative dose of cercariae/fish was 7,614. Although one fish from the positive control may have had one cyst on the dorsal gill, the remaining samples showed no sign of metacercariae. Some gills were difficult to cut due to the small size of the fish, thus a small portion of the gill was lost. Due to the negative results, histology was not conducted.



Discussion

The intention of this study was to expose the June suckers to 1000 cercariae/fish for each exposure. However, the cercariae harvest from the 16 infected snails was inconsistent. In previous studies, shedding of cercariae from infected *M. tuberculata* significantly varied between snails as well as within snails. On a given day, one infected snail would shed 56 cercariae/ 3.4 ml in 2 hrs one day and 1733 cercariae/3.4 ml in 2 hrs on the next. (unpublished data, FES).

The results from the sand filter treatments and positive controls were 0% infected fish. Using the same sand filtration design, Arndt and Wagner (2004) found 98.3% fish infected with whirling disease from the positive control. Therefore, reasons beyond the experimental design should explain the lack of infected fish. The coke rings used to degas the water in the 6 inch PVC pipe of the controls may have caused the cercariae to lose their tails, eliminating the possibility of infection. Without their tails they are incapable of motility and penetration into gills of a fish. Another reason may be due to their tendency to become motionless in moving water. Velez-Hernaández et al. (1998) found cercariae to freeze in response to water current, perhaps in hopes to be siphoned through the fish's gills. The positive phototaxis behavior of *C. formosanus* may have also contributed to the 0% infection rate in the positive control. The June suckers in this experiment tended to aggregate on the bottom of the tank where a negative phototaxis behavior would be advantageous to a parasite. Benthic fish would not be infected if the cercariae remained near the surface. This would also increase the possibility of cercariae being sucked through the net down the standpipe drain and sent through the pump, likely resulting in death.

Cercariae of *C. formosanus* are much larger in size than TAMs, 362 x 197 µm vs. 146 x 12 µm, respectively (Arndt

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and Wagner, 2004, and Chen,1942). Therefore the 250-300 µm sand should have theoretically filtered out the parasite. Sand filtration may have worked, but we are unable make any conclusions due to 0% infection rate in the positive control.

Additional experiments are planned to determine if sand filtration will work as a means of eradicating *C. formosanus*. A smaller scale experiment will be conducted to test if the cercariae are making it through the sand filter PVC and the coke rings PVC from the positive control. Viability will be tested if they are found in the filtered water.

Melissa Harvey

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INITIAL LOOK AT BACTERIAL LOAD OF EGAN BROOD STATION WATER SAMPLES

Coldwater disease is becoming a chronic problem within several state hatcheries in Utah. To get a better understanding of the dynamics of the disease at the Egan hatchery, Utah's brood station, water samples were taken in early May from various locations within the hatchery. These water samples were then analyzed in two different ways. The first was to filter the water samples to get an actual bacteria count from a given location. The second method was to culture sub-samples of the water on plate media to identify the resultant cultures as near as was possible.

Water for both of these methods was collected at the artesian spring source for the hatchery (spring), at a raceway head (head), midway down the length of the raceway (mid), and at the tail of the raceway below all fish compartments (tail). Triplicate samples were collected into 50 mL sterile, plastic centrifuge tubes. From this sample, a 9 mL sub-sample was taken and injected into a sterile 10 mL tube containing 1 mL of a 5% buffered gluteraldehyde solution. This preserved solution was subsequently used for the direct count procedure, and the remaining sample in the 50 mL tube was used to inoculate the media plates.

Water Filtration

Water samples were filtered according to Standard Methods 9216 Direct Total Microbial Count (APHA 1989). This methodology involved collecting and preserving a water sample, filtering of the water through a 0.2 µm membrane filter, staining of the filter with a fluorescing stain (acridine orange, 0.1%), and viewing and enumeration via an epifluorescent microscope. Modifications of this methodology included the filtration of a 3 mL sample water rather than the specified 1 mL. For enumeration, 100 fields per slide were counted, and these values were used to derive total bacteria per water volume. Also, to improve microscope resolution, a counter-

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staining step was included which involved delivering 1 mL solution of Eriochrome Black T after the PBS rinse (Elliot and Barila 1987). We also characterized the bacteria into three categories based upon morphology according to the following categories: rods, cocci strands, and individual cocci.

The results from the filtration work indicate some potential trends, however no significant differences were found with respect to location and counts of morphological type or total counts. For rod-shaped bacteria, cocci strands, and the total counts, there was a general, increasing trend that corresponded with the flow of water from the spring source, down through the fish within the culture system. Samples from the raceway tail displayed fewer bacteria than the mid raceway section; however it should be noted again that none of these differences were significant. All of these values are less than the value of 10^3 mL found by Cahill (1990) for water sampled from rivers and lakes, and one would expect that a spring-fed water source for a hatchery would contain fewer bacteria than those in the open environment.

Table 1. Bacterial counts based on bacterium morphology. From the filtration of 3 mL water samples extrapolated to total bacteria per 1.0 L

Time post-inoculation		Spring	Raceway Head	Raceway Mid	Raceway Tail
48 h	EO	0.6	0	0	14
	EOT	0	0	0	0.6
72 h	EO	0	0	17	25
	EOT	0	0	5	38
96 h	EO	0	0.3	14	10
	EOT	0	0	2	11
120 h	EO	1	0.3	3	13
	EOT	0	0.3	19	15
168 h	EO	0.3	0.6	10	8
	EOT	0	0.6	7	5

Plate Cultures

From the initial water samples, a 100 µl water sample was dispensed and spread onto two sets of culture plates, one with enriched Ordahl's (EO) media, and the other with enriched Ordahl's plus tobramycin (EOT). The addition of the selective antibiotic tobramycin would help the selective growth of *Flavobacterium psychrophilum*, the coldwater disease bacterium (Kumagai et al. 2004). The inoculated plates were then placed into a 15º C incubator and checked every 24 h for the first several days to document the formation of colony growth. A triplicate set of un-inoculated plates with both media types was also included as contamination controls.

No culture growth was observed until 48 h post-inoculation (Table 2) when two colonies each were found on single plates from the EO-Spring and EOT-Tail samples, and an average of 14 colonies on EO-tail plates. After 72 h significant growth was found on Tail plates of both media types. A similar but smaller pattern was found on both EO and EOT plates from the Mid sample of the same time. For the most part, this pattern was maintained through to 168 h except colonies began to appear within spring and raceway head samples. It is worth noting that no growth occurred on EOT plates inoculated with spring water as its source. None of the contamination control plates exhibited growth either.

Table 2. Average (*N* = 3) new bacterial colonies counted for the first 168 (7 d) post-inoculation from water samples spread unto plates containing enriched Ordahl's and Ordahl's plus tobramycin

Location	Rod	Round	Cocci Strands	Total Count
Spring	418 ± 54	156 ± 134	21 ± 15	595 ± 173
Head	581 ± 673	259 ± 49	63 ± 25	903 ± 648
Mid	959 ± 1237	103 ± 84	565 ± 317	1626 ± 837
Tail	331 ± 114	128 ± 5	490 ± 124	950 ± 243

The day after the final colony counts were made, secondary cultures were taken from cultures of interest. Clear, whitish colonies were plated onto TSA media, and yellowish ones onto EOT. From these plates subsequent analysis via gram staining and API testing was conducted. Gram staining was conducted on colonies, which tended to be yellow, representing each of the tail replicates. Two of the replicates revealed gram-negative rods, whose shape was similar to those

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viewed during the direct-count procedure. Within two replicates were long, thin, almost filamentous rods that were also gram-negative.

API testing was used to further characterize the phylogeny of the bacteria. We specifically used the API® 20E kit (bioMérieux SA), which is designed to identify gram-negative bacilli by looking at the byproduct or reaction of 23 different biochemical tests. Four distinctive cultures were tested via the API system; one was derived from a colony of white cells cultured on EO, and the second was comprised of clear/opaque cells also grown on EO. The final two were yellow-pigmented colonies cultured on EOT. All four of these cultures were initiated from raceway tail water samples. For the first culture, the API tests indicated it was highly likely to be a Psuedomonad (*P. aeruginosa, P. fluorescens, or P. putida*), but it was also possibly *Chrombacter violacuem*. Neither of the two yellow-pigmented colonies displayed enough distinguishing characteristics to be keyed out at any level. The first of the two was positively reactive for N_2, and the second was weakly positive for ONPG hydrolysis. These results reveal the limitations of the API system with respect to *F. psychrophilum* identification. The API® 20E was designed to function at 35-37º C, while *F. psychrophilum,* a pathogen of a cold-blooded animal, is typically cultured at 15º C. As a compromise, we incubated our API tests at room temperature, approximately 24º C. From the API tests it is safe to say that the two yellow-pigmented colonies were possibly *F. psychrophilum.* Two yellow colony cultures were analyzed by PCR at the Utah State University Veterinary Diagnostic Lab. One was positive and the other negative for *F. psychrophilum.*

Overall, this look at the water supply of the Egan brood hatchery, and the bacteria within the water as it flows through a single raceway were valuable. It was encouraging to see that total bacteria counts throughout the whole system, but especially within the spring, were well below that of more open systems (Cahill 1990). It was also encouraging to see that no yellow-pigmented colonies were cultured from the spring source on EOT medium. This may loosely suggest that *F. psychrophilum* was not present. However, as has been observed in the past, *F. psychrophilum* is present in the broodstock. If the broodstock can be liberated of the bacterium, it appears that the water supply would help keep them free of the bacterium.

Because there appears to be a circumstantially higher rate of cold-water disease among heat-shocked triploid rainbow trout produced from Egan, a more detailed look at location of bacterial pathogens is required. This coming fall we will take a closer look at bacteria presence and numbers within the water delivery system, and also within the equipment used for the heat shocking of eggs. Additional cultures may be initiated from ovarian and milt samples.

Ronney Arndt

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