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Comparison of the Bacterial Load Between Diploid and Triploid Rainbow Trout Eggs From Either Vaccinated (*Flavobacterium psychrophilum*) or Un-vaccinated Parents

INSIDE THIS ISSUE:

Concerns about the higher levels of mortality among triploid lots of rainbow trout produced in the Utah hatchery system have led to additional research in possible causes. Controlled studies of different strains within the Utah hatchery system have shown good survival in studies conducted at the Fisheries Experiment Station, Logan, Utah (Wagner et al., unpublished data). The poor performance at other hatcheries suggests that factors other than triploidy per se are involved and possibly inter-related with ploidy status. Some of the differences posited include temperature, some interaction with coldwater disease, or additional bacteria that might be amplified as a result of the heat shock treatment used to induce triploidy.

Coldwater disease or rainbow trout fry syndrome is a bacterial disease caused by *Flavobacterium psychrophilum*. The bacterium can be vertically transmitted (Taylor 2004), passing from parent to egg. The bacterium is present at the Egan State Hatchery, Bicknell, Utah, where eggs are heat shocked and disseminated to other state hatcheries. Coldwater disease has been an increasing problem at several hatcheries, so some vaccination efforts have been initiated using an autogenous bacterin. Initial efforts have indicated that bath immersions of juveniles were not successful (Wagner and Thompson 2004). Subsequent vaccination efforts were made by injection of the bacterin into 2-year-old rainbow trout brood stock and into a small number of 5-year-old males and females of the Sand Creek strain. Of interest in this study was the bacterial load of eggs from vaccinated fish and how that load differed between diploid and triploid eggs and from eggs from fish that were not vaccinated.

Methods

Fertilized eggs from vaccinated 5-year-old parents and from un-vaccinated parents were each split into two groups. One group was heat shocked to induce triploidy (26.5 C at 20 min after fertilization for 20 min) whereas the other group was not treated. This resulted in four treatment groups: vaccinated diploid, unvaccinated diploid, vaccinated triploid, and unvaccinated triploid. Upon reaching the Fisheries Experiment Station, Logan, Utah, 6 to 8 h after fertilization, the eggs were treated with 100 ppm of povidone iodine for 15 min. The eggs were subsequently treated daily with 1667 ppm formalin for 15 min within incubation trays.

Upon reaching the eyed egg stage, 60 eggs from each treatment were treated with 100 ppm povidone iodine for 15 min. Eggs were subsequently rinsed in sterile water. A fresh batch of iodine was used for each vaccinate group. Each egg was first rolled onto Enriched Ordahl's growth agar (EO; 5 g tryptone, 0.5 g yeast extract, 0.2 g beef extract, 0.2 g sodium acetate, 9 g agar, distilled water to 1 L; Anacker and Ordahl 1959; Toranzo and Barja 1993) to determine the quantity of external bacteria remaining on the egg surface. The egg was rolled across the center of the petri dish 3 times, taking a new path each crossing. Next, the egg was transferred to 1.5 mL microcentrifuge tube, homogenized with a sterile pestle, and 0.5 mL of sterile phosphate-buffered saline was added. This mixture was mixed well with a vortexer and 100 uL of this was spread across a separate petri dish (EO) using a sterile plastic spreader and dish spinner. Three additional eggs, which had not been disinfected with iodine, were similarly sampled for each treatment. Media controls were included as well

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(n = 3), as well as three additional plates streaked only with the sterile PBS. All plates were wrapped with laboratory film to prevent any subsequent contamination. All the inoculated plates were maintained in a single incubator at 15 C. The plates were checked for growth at 48 h, 5 d, 9 d, and 14 d, recording the total number of colony forming units (CFUs) and noting colony color and morphology. On the last day, a summary was made of each plate noting colony type and number, subtracting out false colonies.

The basic colony types observed were sampled for gram staining. Yellow colonies, which could potentially be *F. psychrophilum*, were sent to Utah State University's Veterinary Diagnostic Lab for PCR testing. The other colony types were characterized using standard bacteriological tests.

Statistical Analysis

For plates in which the colonies were too numerous to count, an arbitrary figure of 10,000 was used for statistical analysis. Total CFUs were recoded into three categories: 0 (negative plates with no bacterial growth), 1 to 300 CFUs, or >300 colonies. These were analyzed with chi-square analysis and the maximum likelihood probability was used to determine significance for each test. Separate tests were conducted to compare differences between diploid and triploid samples for each vaccinate/non vaccinate treatment separately for external and internal samples. Similarly, differences between vaccinate and non vaccinate groups were tested separately for each level of ploidy and location (internal or external). For significant differences among the three CFU code levels ($P \leq 0.050$), partial tables were constructed to determine significant differences for the subsets within that level. CFUs were subdivided into counts for three colony types: fungus, orange and pink, and yellow. Differences between vaccinated and unvaccinated groups were tested for each colony type with the Mann-Whitney U test separately for each ploidy level and location. Similarly, differences between diploid and triploid CFUs for each colony type were tested separately for each vaccination group and location.

Results

Unexposed media plates did not display any bacterial growth, indicating the media was not contaminated. Similarly, only one CFU was found on one of three plates inoculated with sterile PBS. For eggs that were not disinfected, bacterial growth was too numerous to count for both triploid and diploid groups from both vaccination groups.

The percent eyeup and hatch could not be statistically compared among treatments, but values were lower for triploid groups (eyeup of 68.2% vaccinated, 68.8% unvaccinated; hatch as a percent of eyed eggs: 74.1% vaccinated, 74.6% unvaccinated) than for diploid groups (eyeup: 82.8% vaccinated, 91.9 unvaccinated; hatch rate: 91.7% vaccinated, 93.3% unvaccinated).

Table 1. Differences between diploid and triploid rainbow trout eggs in the percentage of total CFUs within 3 categories. Data were analyzed separately for each location (external/internal) and *Flavobacterium psychrophilum* vaccination treatment. An asterisk indicates a significant difference within a given treatment group (chi-square test, $P < 0.05$).

Treatment group	Negative CFU	1 to 300 CFU	>300 CFU
Internal, vaccinated*			
Diploid	56.7	21.7	21.7
Triploid	10.0	13.3	76.7
Internal, unvaccinated			
Diploid	43.3	30.0	26.7
Triploid	38.3	36.7	25.0
External, vaccinated*			
Diploid	50.0	41.7	8.3
Triploid	0.0	1.7	98.3
External, unvaccinated			
Diploid	73.3	23.3	3.3
Triploid	61.7	35.0	3.3

There were significant differences in the total CFU distribution between diploid and triploid groups, though it was not consistent across vaccination groups (Table 1). Only in the vaccinated groups were CFUs significantly higher in the triploid eggs than diploid eggs. There were also significant differences between vaccinated and unvaccinated groups for internal samples for triploids, and external samples for both diploids and triploids (Table 2). In each case, vaccinated groups had higher CFUs than unvaccinated groups. Overall prevalence rates for all bacteria types combined ranged from 26.7 to 100% for external samples and from 43.3 to 90% for internal samples.

Table 2. Differences between *F. psychrophilum* vaccinated and unvaccinated groups in the percentage of total CFUs within three categories. Data were analyzed separately for each level of ploidy and location (external/internal). An asterisk indicates a significant difference within a given treatment group (chi-square test, $P < 0.05$).

Treatment group	Negative CFU	1 to 300 CFU	>300 CFU
Internal, diploid			
Vaccinated	56.7	21.7	21.7
Unvaccinated	43.3	30.0	26.7
Internal, triploid*			
Vaccinated	10.0	13.3	76.7
Unvaccinated	38.3	36.7	25.0
External, diploid*			
Vaccinated	50.0	41.7	8.3
Unvaccinated	73.3	23.3	3.3
External, triploid*			
Vaccinated	0.0	1.7	98.3
Unvaccinated	61.7	35.0	3.3

Table 3. The mean CFUs (±SD) and prevalence (% of positive plates) for each of three different colony types. An asterisk indicates a significant difference between ploidy type within a given treatment group and colony type ($P < 0.05$).

	Fungus CFUs ±SD	%	Orange and pink CFUs ±SD	%	Yellow CFUs ±SD	%
Internal, vaccinated						
Diploid	0.05 ± 0.22	5.0	0.03 ± 0.18	3.3	0.10 ± 0.54	5.0
Triploid	0.02 ± 0.13	1.7	0.20 ± 1.42	3.3	0.02 ± 0.13	1.7
Internal, unvaccinated			m			
Diploid	0.07 ± 0.25*	6.7	0.05 ± 0.22	5.0	166.72 ±1290.99	6.7
Triploid	0.00 ± 0.00	0.0	0.03 ± 0.18	3.3	0.32 ± 1.95	6.7
External, vaccinated						
Diploid	0.10 ± 0.35*	8.3	166.73 ±1290.99*	8.3	0.07 ± 0.31	5.0
Triploid	0.00 ± 0.00	0.0	0.00 ± 0.00	0.0	0.00 ± 0.00	0.0
External, unvaccinated						
Diploid	0.07 ± 0.25	6.7	0.07 ± 0.25	6.7	0.03 ± 0.18	3.3
Triploid	0.18 ± 0.70	11.7	0.05 ± 0.22	5.0	0.00 ± 0.00	0.0

Prevalence rates for pink and orange (combined total), yellow, or fungal CFUs were much lower (Table 3). The prevalence of fungal CFUs ranged from 0 to 5% among internal samples and 0 to 11.7% among external samples. There were significant differences between vaccination groups only for the external sample of the triploids (unvaccinated > vaccinated, $P = 0.007$). Diploids had significantly higher fungal CFU counts in the

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external vaccinated group and the internal non-vaccinated group ($P < 0.044$; Table 3). The prevalence of orange and pink CFUs ranged from 3.3 to 5.0% among internal sample groups and from 0 to 8.3% among external sample groups. Significant differences between ploidy types were only observed for the external, vaccinated group ($P = 0.023$). In this group, one plate was too numerous to count and artificially biased the average count in Table 3. Since a non-parametric test was used for statistical analysis, this should not have influenced the analysis. No differences in orange and pink colony numbers were observed between vaccination groups. A gram stain of one orange colony indicated these were ovoid, gram-negative cells, 2.8-3.6 um long by 2 um wide. Another orange colony was a mixture of gram-positive cocci 1 um in diameter and gram-negative rods, 2-2.8 um long. A pink colony had gram-negative cocci, 0.8 um in diameter.

For yellow colonies, no significant differences were observed between ploidy types or vaccination groups for any treatment group. The prevalence of yellow CFUs ranged from 0 to 5% among external samples and 1.7 to 6.7% among internal samples. A yellow colony submitted for PCR analysis was positive for *F. psychrophilum*. Not all yellow colonies were tested, so not all of these can be assumed to be *F.p*. Indeed, a gram stain of one colony indicated it was composed of cocci, 0.8 mm in diameter, rather than the gram negative filamentous rods typical of *F.p.*

Other colony types observed included 1) an off-white, thin spreading colony composed of long rods 1.2-8 um long x 1.2 um wide, 2) an off-white, thick, lobed margin colony of gram negative rods 2.8 um long x 0.4 um wide, and 3) thin-spreading yellow-white colonies of gram negative rods, 2 um long. These colony types dominated the plates and likely suppressed growth of the slower growing yellow, pink, and orange colonies.

Discussion

Some of the plates had more condensation than others, likely a result of different temperatures during the pouring process. Coincidentally or not, these two groups (internal-vaccinated-triploid, external-vaccinated-triploid) with condensation had the highest level of bacterial growth. This may have biased the statistical analysis and interpretation of the data. Of the conclusions that can be drawn, the data reconfirmed the presence of *F. psychrophilum* in eggs from Egan. Another conclusion is that 100 ppm iodophor treatment for 15 min did not eliminate all the surface bacteria. Bacteria remaining on the exterior of the eggs compromised the interpretation of the 'internal' sampling, so internal CFUs were really a mix of internal and external sources. Diploid and triploid CFU loads were inconsistent, indicating the triploidy induction process per se was not influencing bacterial loads. Vaccination (intraperitoneal injection of 100 uL of bacterin) appeared to have resulted in significantly higher bacterial loads, though the water condensation issue clouds this interpretation.

Further work is needed to develop techniques for more effective disinfection of fish eggs. Use of antibiotics such tobramycin in EO media (Kumagai et al. 2004) would also be helpful in future work targeting *F. psychrophilum* more specifically.

by Eric Wagner

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Limiting Cutthroat Growth by Restricting Daily Feedings

In Utah, cutthroat trout eggs from brood lakes are typically collected in May through June. Given the natural growth of cutthroat in a hatchery, fish are not of stockable size by autumn, and must be held over the winter for stocking in the spring-summer. Unfortunately, during this over wintering period, fish may become larger than the hatchery managers desire, occupying valuable raceway space and consuming increasing quantities of feed. Research has shown when rainbow trout were fed rations of 2.0 down to 0.5% wet body mass per day, there was a corresponding drop in specific growth rate (SGR), and an increase in fin damage attributed to competition for limited food resources (Gregory and Wood, 1999). Robert R. Smith (1987) determined that by alternating between feeding and fasting periods of equal lengths, he was able to significantly reduce the growth of steelhead to the point he desired. To address this unique need of managers to hold back the growth of cutthroat, a study was formulated and conducted during fall-winter at the Fisheries Experiment Station to compare different feeding regimens aimed at reducing cutthroat growth.

Beginning August 31, 2004 cutthroat of the Bear Lake strain were stocked into small outdoor circular tanks (75 L) at a density of 200 fish per tank. The fish were all from a common lot of fish that had been reared together in a large outdoor raceway. All fish were hand-fed a standard commercial trout diet (Silver Cup, Nelson and Sons, Inc., Murray, Utah). Throughout the study fish feed ration was on a percent fish body weight (BW) basis. For the first 25 d, fish were fed 3.2% BW. For the following 61 d the ration ranged from 3.2% to 1.9% as the fish got larger. For the remainder of the trial the ration was set at 1.9-1.8%. The fish were fed according to the following six treatments: T1 = fed seven days/wk, T2 = 5 d on feed/2 d off, T3 = 1 d on/1 d off, T4 = 4 d on/4 d off, T5 = 7 d on/7 d off, T 6 = 4 d on/3 d off.

Throughout the study flows were set at 3.4 lpm (0.9 gpm). Well-water was supplied to each tank with supplemental oxygen injected via a low-head oxygenator (LHO) placed in a central head box. Water temperature remained constant at 13.0° C ± 0.2 with oxygen concentrations ranging from 5.4 – 7.2 mg/L (mean = 6.5 ± 0.5 mg/L). The fish in each tank were inventoried on a monthly basis by removing, weighing and counting approximately 30 - 50% of the fish. This data along with feeding records were used to calculate feed conversion ratios, FCR = total grams of feed/total grams of weight gain and specific growth rate, SGR = $[(\log_e \text{weight}_{\text{end study}} - \log_e \text{weight}_{\text{beginning}})/(\text{number of days})] \cdot 100$. Density indices were calculated: DI = weight/(volume · fish length), where weight is in pounds, volume in cubic feet, and length in inches. Density indices ranged from 0.10 – 0.35. On two occasions fish were thinned from treatments T1 and T2 so that density indexes would not pass 0.40, and on a subsequent occasion fish were thinned from all treatments for the same purpose.

At the conclusion of the study, Health Condition Profiles (HCP; Goede and Barton 1990; Goede 1991) were conducted on four fish from each tank (12 per treatment). Twenty fish are generally tested from each rearing unit, however as these cutthroat were in limited supply and were to be stocked following the study, only a small lethal sample for the HCP process were available. From the same twelve fish, maximum fin length measurements were made on all but adipose fins to determine relative fin index values according to Kindschi (1987). Mesenteric fats were scored as either: 0 = no visceral fats, 1 = approximately 25% of pyloric caeca covered with fat, 2 = approximately 50% covered, 3 = more than 50% covered, or 4 = caeca completely covered

Beginning 95 d after the study's start a spike in mortality occurred within one tank of treatment #1. On the first day of the episode, 14 fish died, seven fish the following day, and a total of 12 fish the following two days. Thereafter, mortalities stopped. In response, tissue samples were taken to determine the presence of bacteria or viruses, and nothing consequential was found. An analysis of water quality also revealed nothing. On the first day of the episode, the single tank experiencing mortalities was given a 1,000 ppm NaCl treatment followed by a 2,000 ppm treatment. Through the remainder of the study, mortalities were negligible among all treatments. Despite the mortality within this one tank, there were no significant differences between treatments. Mean

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mortality for treatments #2-5 was all 0.5% or less, and mean mortality for treatment #6 was 1.2%, and 7.3% for treatment #1.

Aside from the limited mortalities, overall fish performance was good. As might be expected, final fish weight was better for fish fed 7 d/week compared to all other treatments. Fish fed 5 days on feed/2 d off had better weight than T3-T6 (Table 1). Fish weights were similar for T1 and T2 for the entire length of the study until the final month when T1 fish surpassed T2. With a few exceptions treatments T3-T6 exhibited similar monthly weights throughout the study. Fish lengths followed a somewhat similar trend with the longest fish in T1 and T2 followed by T3 and T6, which had similar lengths, followed by T4 and T5 which had the smallest fish. Condition factors (K_{tl}) were not significantly different and averaged 0.87 ± 0.04. Daily growth, in terms of length increase per day (cm), showed highest growth among treatments T1 and lowest among T5, with the other four treatments exhibiting intermediate growth. Feed conversion ratios were best for T3-T6. The FCR exhibited by T2 fish was poorer than T3, but not different than T4-T6. The T1 fish showed the poorest FCR, 1.07, which was significantly poorer than all other treatments (Table 1). Mesenteric fat values as quantified by the HCP methodology were highest for T1, 2.6, T2, 2.4, and T6, 1.8. Fat levels were lowest for T3-T5, and averaged 1.6.

Table 1. Hatchery performance of cutthroat trout fed according to the following feeding regime treatments: 7 d/wk = T1, 5 d on feed/2 d off = T2, 1 d on/1 d off = T3, 4 d on/4 d off = T4, 7 d on/7 d off = T5, 4 d on/3 d off = T6. Means that have a different subscript letter are significantly different from each other ($P \leq 0.05$).

Treatment	Final weight (g/ fish)*	Final length (mm)	Condition factor (K_{tl})	Daily growth**	Feed conversion ratio	Fat index (HCP)
T1	38.2 ± 12.9 a	159.9 ± 17.2 a	0.89 ± 0.11	0.026 ± 0.001 a	1.07 ± 0.10 c	2.6 ± a
T2	28.7 ± 9.8 b	146.93 ± 16.1 a	0.87 ± 0.05	0.024 ± 0.001 ab	0.81 ± 0.03 b	2.4 ± ab
T3	16.4 ± 4.9 c	122.1 ± 11.5 bc	0.88 ± 0.05	0.017 ± 0.001 ab	0.68 ± 0.03 a	1.5 ± b
T4	13.1 ± 3.8 c	115.7 ± 10.8 c	0.82 ± 0.04	0.016 ± 0.001 ab	0.81 ± 0.02 ab	1.8 ± b
T5	15.1 ± 15.1 c	115.7 ± 12.3 c	0.94 ± 0.88	0.015 ± 0.000 b	0.77 ± 0.02 ab	1.5 ± b
T6	16.7 ± 4.4 c	125.0 ± 11.1 b	0.84 ± 0.06	0.017 ± 0.002 ab	0.77 ± 0.02 ab	1.8 ± ab

Although the sample size was small ($N = 12$), several treatments did exhibit significantly better fin condition than others. The dorsal fin was significantly longer for T1 compared to T3 and T6 fish, and fish within T2, T4, and T5 exhibited intermediate fin condition. The caudal fin was significantly longer for T5 compared to T2 fish, and fish within T1, T3, T4 and T5 exhibited intermediate fin condition. Both pelvic fins were significantly longer for T2 compared to T3 and T6 fish, with the other treatments exhibiting intermediate fin condition. These findings did not reveal any clear trend with respect to restricted feeding treatment.

In summing up the results, the fish grew as one might expect given the various feeding restrictions. It should be noted the best growth yet the poorest FCR was found among those fish fed seven days a week (T1). Throughout the study it was difficult to get the T1 fish to consume their daily feed ration. Data were not collected regarding this, but it was common to find uneaten feed particles on the bottoms of tanks within this treatment. This was also occasionally a problem within the five days on, two days off (T2) fish, but only to a limited extent. The best FCR was found among the one day on, one day off (T3) fish indicating they were utilizing their feed efficiently. Hatchery managers do want their fish to utilize feed efficiently, but they also want a high percentage of the fish to survive post-stocking. The higher fat levels found among the T1 and T2 fish would be a preferable scenario

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to the lower levels found among the other treatments. However, given the ability of trout to compensate in growth for earlier periods of fasting, it seems likely that cutthroat could be fed according to one of our feeding treatments, and then put on a normal ration for several weeks to build the fat reserves back up prior to stocking. Based on that likelihood, we recommend following any of the four more restrictive feeding treatments, T3 – T6 for holding cutthroat back when fish size, limited rearing space, and feed costs are of a concern. Something else to consider when limiting growth is the reduction of pollutants discharged in the effluent, especially phosphorus. By feeding less, there will be less nitrogen and phosphorus leaving the hatchery, and by feeding fish at a more efficient FCR, these pollutants will also be discharged at lower concentrations as well.

Ronney Arndt

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New Faces at FES

We're pleased to welcome a new fish health specialist at the Fisheries Experiment Station. **Wade Cavender**, a native of Ohio and a graduate of the University of Idaho, took over the position vacated by Patrick Goddard in March 2005.

Wade previously served as fish health specialist for the state of Arizona, working out of Pinetop. He completed his Masters degree in fish health at the University of Idaho, under the supervision of Dr. Ken Cain.

Wade was accompanied to Logan by his wife, Dr. Kimberly Cavender, who is a small animal veterinarian. They have two Labrador retrievers to help keep their lives from being boring. Wade is an avid hunter and fisherman, and we look forward to working with him.

Leaving FES is **Eriek Hansen**, who has worked several years here, taking care of the June sucker culture program. Eriek has accepted a graduate program at Utah State University under Dr. Phaedra Budy. He will be working on riparian enhancement on Spawn Creek, a tributary to the Logan River, as a means to mitigate the impact of whirling disease on native cutthroat. Congratulations to Eriek!

Effects of Praziquantel on Encysted Metacercariae in *Gambusia sp.* Naturally Infected with *Centrocestus formosanus* (Digenetic Trematode).

Introduction

The site for the proposed June sucker recovery hatchery at Goshen Warm Springs is currently inhabited with the mollusk *Melanoides tuberculata,* the obligate first intermediate host of the parasite *Centrocestus formosanus* (Rader et al.2003). The secondary intermediate host is a warm water fish. *Centrocestus formosanus* encyst in the lamella of the gills sometimes causing suffocation (Fig. 1). The metacercariae (stage of parasite encysted in the gills) of *C. formosanus* have been shown to parasitize the gills of over 40 different species of warm water fish (Salgado-Maldonado et al., 1995). Our studies (unpublished data) show that June suckers, *Chasmistes liorus,* are highly susceptible as well. Significant mortalities could occur if fish at the proposed June sucker recovery hatchery become infected with metacercariae.

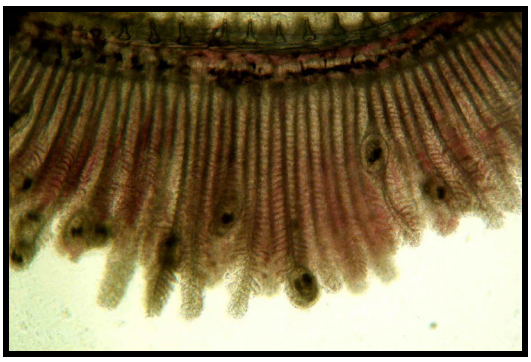

Figure 1. *Centrocestus formosanus* metacercariae encysted in the gills of *Gambusia affinis*.

A veterinary anthelmenthic drug called praziquantel has been used in treating fish infected with monogenetic trematodes and other types of infections (Noga, 1996). The objective of this study was to determine if praziquantel is effective for killing metacercaria of *C. formosanus* encysted in gill tissue of a host fish. The drug was tested at two different doses in *Gambusia affinis* naturally infected with 24 metacecariae per fish.

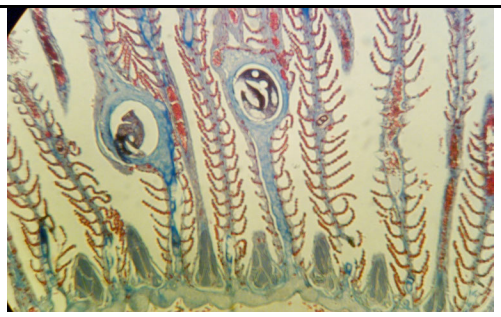

Figure 2. Masson's Trichrome Stain showing encysted metacercariae in sample harvest gambusia.

Materials and Methods

Naturally infected *Gambusia* sp. were harvested from Fish Springs, Utah and held in 20 gallon recycle systems for several weeks at 17°C. A wet mount of the gills from 4 fish was observed under the microscope for metacercaria before the experiment to verify natural infection. A histological cut was also examined using a Masson's trichrome stain (Fig 2).

Ten fish were placed into 4 L aerated water bath for each replicate of each treatment. Two different doses of praziquantal were used: 5 mg/L for 6 hours (hr), 2 ml solution/ 4 L, or 2.5 mg/L for 12 hr (1 ml solution/ 4 L)., In addition, a control group was exposed to 1 ml 70% ethanol in 4 L hatchery well water for 12 hr. After the allotted time per treatment, fish were placed into 20-gallon recycle systems and fed Tetramin twice a day until harvested.

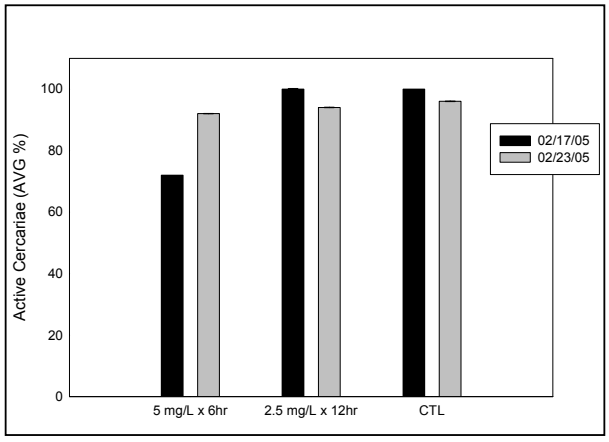

Figure 3. The percent of metacercariae cysts active after praziquantel treatments.

Five fish from each treatment were sampled on day 3 and day 7. A wet mount consisting of one gill from each fish was analyzed under the microscope. Each cyst was observed for at least 2 minutes watching for movement of metacercariae. If no movement was observed, the metacercaria was pronounced dead. Necrosis of the

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parasite was also looked at and recorded. The remaining gills were preserved in 10% neutral buffered formalin for possible histology.

Results
The gambusia sampled before the experiment were heavily infected. Praziquantel treatments at the observed doses had little effect on metacercariae encysted in the gills of *Gambusia* sp. On each sample day, 90% of the metacercariae were still active from the 5 mg/L x 6 hr treatment. On day 3, 100% of the metacercariae exposed to 2.5 mg/L for 12 hr were active, while 90% were active on sample day 7 (Fig. 3). Only one cyst from sample day 3, treatment 5 mg/L x 6 hr, showed visible signs of necrosis (Fig. 4). Because most of the metacercaria were active, histology was not conducted.

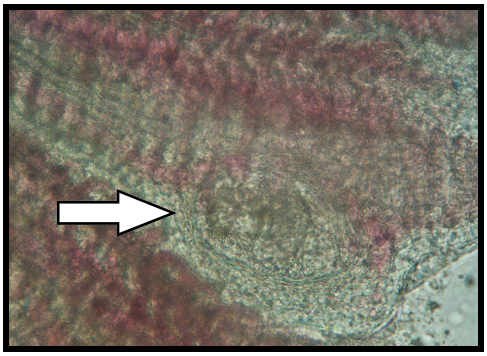

Figure 4. Necrosis of metacercariae from gambusia treated with praziquantel at a dose of 5 mg/L x 6 hr, harvested on day 3.

Discussion
Praziquantel is highly effective for several species of monogenean trematodes and tapeworms (Sharp et al., 2004, Mitchell, 2004). The doses used in this experiment were relatively high compared to the wide range of doses used throughout the literature (Noga, 1996). Increasing this would not be practical on a large hatchery system, thus using praziquantel is not an effective solution for encysted metacercariae of *C. formosanus*. An oral dose may be successful in treating the parasite, but would most likely not be feasible on a hatchery scale (Noga, 1996). Further studies are being conducted to eradicate the cercarial stage of this parasite before the fish become infected.

By Mellisa Harvey

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Myxobolus cerebralis Discovered at Springville State Hatchery

Fish Pathologists with the Utah Division of Wildlife Resources have confirmed the discovery of the whirling disease parasite, *Myxobolus cerebralis* (MC) at the Springville State Fish Hatchery. The finding was made during the course of the annual fish health inspection. Initial testing by pepsin-trypsin digest of catchable-sized rainbow trout showed 2/60 fish with myxospores consistent with MC. Immediate retesting again showed the presence of spores in 5/60 fish, and subsequent polymerase chain reaction (PCR) testing of the digest residue confirmed the infection as MC.

The finding was shocking, but not totally unexpected, given the proximity of contaminated Provo River and Hobble Creek. Contaminated creek water had apparently been used for irrigation in adjacent hayfields prior to the discovery. The main spring sources of water for the hatchery are collected in a large open spring pond, which is home to large numbers of waterfowl at certain times of the year.

The confirmed finding resulted in an immediate cessation of all stocking from the hatchery, since MC is regulated as a prohibited pathogen, according to Utah's fish health rule. The Utah Fish Health Policy Board was notified and an emergency response team was convened to assess the situation and come up with a plan of action.

The team labored to produce a plan to salvage any fish, while continuing to protect the health of wild salmonids. Initially, a variance plan was formulated to stock a limited number of the catchable fish into selected community fishery ponds within a short period. The ponds were selected according to a very strict criteria. At the time of presentation, however, there were lingering questions about the suitability of these ponds and the variance proposal was withdrawn.

The situation was brightened slightly by the subsequent discovery that smaller fish, housed in two hatchery buildings which are supplied by separate, contained springs, tested negative for the parasite. Accordingly, another variance was presented to the Fish Health Policy Board to allow the stocking of these negative fish (approximately 270,000) into Otter Creek and Piute Reservoirs in southern Utah. Both of these reservoirs are put and take and already positive for the parasite. This variance was unanimously approved by the Board.

Plans for the remainder of the catchable-sized fish include cleaning and dressing of as many as possible by Dedicated Hunter volunteers for public distribution. The remainder will be composted/buried at the local landfill.

The response team is also drawing up plans for the continued operation of the hatchery, utilizing the pathogen-free water from the clean springs. Ultraviolet disinfection systems are being considered as a means to insure the future security of the water supplies. Finalized plans will be presented before the Board at a special meeting held at Springville in July.

Springville is the third state hatchery to test positive for the parasite. In 2000, Midway Hatchery was confirmed positive, and Mammoth Creek Hatchery tested positive in 2003. In both cases, irrigation water contaminating the springs were determined to be the source of the infection.

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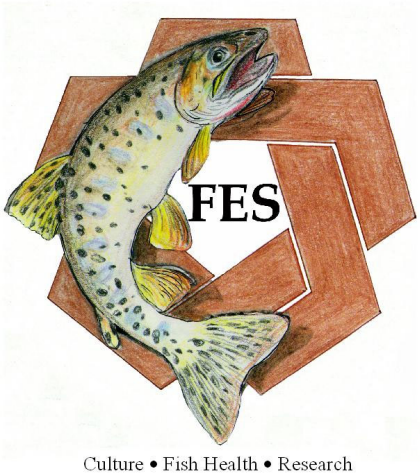

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