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Pilot Project At Midway Hatchery To Evaluate Several Whirling Disease Filtration Technologies

INSIDE THIS ISSUE:

For the past three years we have been investigating the potential of sand filtration as a means of filtering out triactinomyxons (TAMs), the water-borne, fish-infectors of whirling disease (*Myxobolus cerebralis*). The initial test indicated that when using the appropriate-sized sand (particles of >180 µm), 99% of infection could be prevented (Arndt and Wagner 2003). Because faulty back flushing was seen as the weak link in that test, the follow-up test concentrated on improving the back flush procedure. Sand filters must be periodically back flushed to remove trapped debris. So, for our second attempt (Arndt and Wagner 2004), an experiment was conducted to improve back flush procedures by: 1) *extending backflush*, in which the duration of backflushing was increased to theoretically flush out more TAMs, 2) *diverting return flow*, in which water that was headed for the tanks with fish, immediately after backflushing, was diverted for 5 min to "re-seat" the sand in the filter, and 3) *slow sand filtration*, in which water was passed through a larger filter at a slower rate and no backflushing was conducted. The results from that test revealed that the only fish that were 100% clean of whirling disease, were within the diverted back flush treatment. A third experiment was conducted to determine if cheap, readily available sandblast sand would serve as an adequate filter medium (Ichthyogram, June 2004, Vol. 15, Number 1/2). The two sands used, #4010 and #4060, both effectively trapped the water-borne TAMs, but sand loss was a recurring problem with filters containing the #4010 sand. Those results, combined with some follow-up research with large, commercial filters (Ichthyogram, October 2004, Vol. 15, Number 3), determined a multi-layered sand bed consisting of #4010 and #4060 would give good flow characteristics that minimized sand loss, yet gave the protection needed to eliminate TAMs.

As a final test of the efficacy of sand filtration in removing TAMs from contaminated water supplies, we participated in a pilot project that took place the fall and winter of 2004 at the Midway State Hatchery. The aim of this project was to solicit interest from commercial filtration providers who felt their technology would remove TAMs from contaminated water. After numerous proposals had been reviewed, two types of technology were selected for testing, along with the sand filter we had developed. The first system, provided by PRAqua Technologies Ltd. (Nanaimo, British Columbia, Canada), consisted of a drum filter containing 21-µm mesh followed by a UV disinfection unit, which was supplied by Trojan Technologies Inc (London, Ontario, Canada). The drum filter was meant to pre-filter the incoming water prior to passing through the UV unit. The Trojan UV filter was set to deliver a constant dosage of 120,000 mWs/cm^2. The second system was provided by Filtronics Inc. (Anaheim, California, USA), and consisted of a media filter analogous to a sand filter, which contained their proprietary mixture of Electromedia®. This filter was also followed by a model multi-bulb UV unit supplied by Aquionics (Erlanger, Kentucky, USA) set to deliver dosages of 120,000 mWs/cm^2. Two raceways of fish were assigned to each of these filtration systems, the first raceway of each respective system received water that had been mechanically filtered; for PRAqua this meant the drum filter, and for Filtronics, this meant their media filter. The second raceways received water that had also been filtered by the respective U.V. units after passing through the filter media. This system would allow for analysis of the efficacy of the individual filtration components.

The final system tested was the sand filter, which we had developed at the FES, and consisted of a 91 cm diameter Baker-Hydro sand filter. The composition of media within the filter from bottom to top was as

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follows: 10 cm pea gravel covering the laterals, 5 cm #4095 sand, 10 cm #4060 sand, 10 cm #4010 sand. The effective size (ES) for each is as follows: #4010 = 250 µm, #4060 = 300 µm, #4095 = 800 µm. During normal operations water was pumped to the sand filter from the springhead box at a rate of 127 lpm (34 gpm). Water from the filter was then piped to a 1,900 L reservoir that supplied water to the rearing tank. The sand filter was backflushed twice weekly at a flow of 115 lpm (31 gpm) for a duration of five minutes. This was followed by a five-minute purge flow of 146 lpm (39 gpm). The purge flow consisted of normal operational flow through the filter, but the filtered water was discharged into a waste system. During this and the back flush period, the filtered-water reservoir was used to provide regular flow to the fish tank. The average post-purge filter flow was 131 lpm (35 gpm). Filtered water from this system was fed to an individual raceway containing fish. Two control raceways were also included in the overall test. The spring-control was fed unfiltered water, which was known to be contaminated with TAMs (Arndt and Wagner 2003), and the second was fed the same water, but was weekly spiked with TAMs, the process of which will shortly be discussed.

Fish for this test were obtained as eyed eggs from the J. Perry Egan State Brood Hatchery (Bicknell, Utah), and were of the Sand Creek strain of rainbow trout. At the Midway Hatchery, the eyed eggs were placed into individual incubation trays that fit inside the various test raceways. As the eggs hatched they were released from the incubation trays into the raceways. The TAM exposures started approximately four weeks after the fry had hatched. The PRAqua and Filtronics systems started with approximately 1,200 fish each, the FES and filter had 700 fish, and the spiked-control had 225.

For the three filtration systems and the spiked-control there was an injection point on the main water line that fed into each system. It was at this point that TAMs were injected into the system, via a peristaltic pump, on a weekly basis for ten weeks during November 2004 through January 2005. For the injections, a known quantity of TAMs (Table 1) was diluted into a 1.0 L graduated cylinder containing 500 mL of U.V. filtered water. This solution was then pumped directly into the given filter system's water delivery pipe. The quantity of water in the cylinder was closely monitored, and when it was just emptied, 200 mL were additionally added to ensure the TAMs were all pumped into the systems. The TAMs were collected freshly the morning of each exposure from mixed oligochaete worm cultures that were kept at the FES. The TAM cultures were maintained according to Arndt et al. (2002), and the TAMs were harvested and enumerated according to Arndt and Wagner (2003, 2004).

Table 1. Midway WD Filter Project – Summary of Data

System	Initial fish numbers	Projected TAMs/fish	End of Study fish numbers	Actual TAMs/fish	Weekly Mean TAM Dose
PRAqua	1194	205	1083	227	245,410
Filtronics	1268	205	1000	260	260,283
FES sand filter	706	205	650	223	145,015
Control - spiked	225	205	190	243	46,107

At the conclusion of the study, January 24, 2005, 50 fish from each rearing unit were euthanized, lengths and weights were measured, and a deformity index, designed to quantify cranial and skeletal deformities, was conducted. The presence or absence of deformities such as vertebral, mandibular, cranial, or opercular deformities was noted and quantified for each treatment. Such deformities are typical clinical signs of whirling disease. Fish without deformities were categorized as normal. The whole fish were then sent to an independent laboratory (Pisces-Molecular LLC, Boulder, CO) for assaying of *M. cerebralis* via PCR.

The pilot project conducted at the Midway Hatchery clearly indicated which systems were effective at containing the water-borne TAMs. Fish that were reared in unfiltered spring water, the same water that was supplied to all filtration systems, exhibited *M. cerebralis* infection within 65% of fish tested. Spiked-control fish were 100% infected. Fish reared in water that had passed through only the PRAqua drum filter were 98% infected, while those reared in water that had passed through the drum filter then the Trojan U.V.filter were 0% infected. Fish reared in water that had passed through only the Filtronics media filter were 0% infected, and those reared in water that had passed through the media filter and the Aquionics U.V. filter were also 0% infected. And finally, the fish reared in filtered water from the FES-developed sand filter exhibited 8% infection among the subsampled-fish. Fish survival was relatively good throughout the test, although low dissolved oxygen levels (< 6 mg/L) within the Filtronics raceway near the beginning of the test may have contributed to slightly higher mortality among that group. The final fish weights on a gram per fish basis were as follows: spiked control = 0.8 g, spring control = 0.8 g, Filtronics post media = 1.4 g, Filtronics post U.V. = 0.6 g, PRAqua post drum = 0.7 g, Filtronics post U.V. = 0.6 g, FES sand = 0.9 g.

The comparison of the FES sand filter at the Midway Hatchery, against two commercially available filtration systems, allowed the side by side comparison necessary for confidence in employing a new technology as well as the testing of our laboratory-derived sand filter at near hatchery-scale water flows. The FES sand filter was only 92% effective at preventing infection compared to 100% for the complete systems provided by both Filtronics and PRAqua. Because the Filtronics media filter was 100% effective at removing TAMs, their U.V. system was not realistically tested because any incoming TAMs would have been removed by the filter before the had the chance to be sterilized by the U.V. unit. The PRAqua drum filter was only 2% effective at removing TAMs, but their U.V. filter (Trojan), was 100% effective. The drum filter, with its 21 µm opening mesh size was not designed as a "TAM catcher", rather as a prefilter to the U.V. unit, which purpose it seemed to serve. It has been demonstrated that filters made of 20 µm mesh are not 100% effective at catching TAMs (Thompson and Nehring 2000), and that when their grappling-hook-shaped body is folded together, they may be only 12 µm wide (El-Matbouli and Hoffmann 1998). Whether this comparative evaluation was a true test of the drum filter's capacity to pre-filter water cannot be ascertained. It should be noted that the spring water provided to the filter systems was almost, without exception, clear, without an appreciable load of suspended particles. A real test of the drum filter as a prefilter during variable water quality events may be of interest.

Both of the controls verified that TAMs were in Midway's spring-fed water supply. The spiked-control demonstrated that the injection of TAMs into the water lines was successful and that the three filter systems experienced a similar load. The spring-control demonstrated there was a constant background level of TAMs within the spring and that the filter systems were being challenged on a 24 h basis rather than only by the weekly planned exposures. Overall both Filtronics and PRAqua systems performed adequately at removing TAMs, and it seems highly likely, that when coupled with a U.V. filter, the FES sand filter would serve a useful purpose as a prefilter similar to the PRAqua drum filter.

Ronney Arndt

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Effects of Sex Ratio and Spawning Substrate Transfer Frequency on Spawning Success of Least Chub

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 Waheap State Fish Hatchery and the Fisheries Experiment Station (FES) are maintained via extensive aquaculture techniques.

The objective of the current research project was to establish intensive culture techniques for least chub. Previous research indicated that least chub should be paired in low densities and should not be paired with more males than females (Billman and Wagner 2005). This report summarizes the results of a study that examined the effects of different female to male ratios and spawning substrate transfer frequencies on spawning success of least chub.

Methods

The spawning study was conducted at the Utah Division of Wildlife Resources' Fisheries Experiment Station (FES) in Logan, Utah. Least chub adults were obtained from a wild broodstock from Mona Springs, Utah, maintained at FES. Test fish were distributed into twelve 75-l aquaria in a laboratory setting. Aquaria were supplied with 1.89 l/min hatchery well water in a flow-through system. A full spectrum fluorescent light (two 4-ft bulbs) was on a timer to deliver a 14 h: 10 h (light:dark) photoperiod. 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 each aquarium.

Least chub were paired into aquaria at two sex ratios (1:1 and 1:2, male to female) but at equal densities of six fish. Fish were transferred to test aquaria (six per ratio) on 28 March 2005. Least chub were selected such that all individuals of each sex were similar in size. Water temperature was maintained at 18°C for the duration of the study. Fish were fed a commercial flake diet (TetraMin®) at 2% body mass. Mortalities were replaced by similarly sized least chub to maintain the sex ratio and density of fish in each tank.

Spawning substrate was added into the aquaria eight days following the introduction of the fish. After one week, substrate was removed on two schedules. For half of the tanks in each ratio treatment, spawning substrate was removed twice per week (Tuesday and Friday), while spawning substrate was removed once per week for the remaining tanks. The spawning substrate was transferred to a plastic container with well water and incubated in a trough where water at 18°C bathed the outside of the containers. Aquaria were siphoned cleaned as described in the previous study. After the 6-day incubation period, fry were harvested from spawning substrate and enumerated. Spawning substrate was disinfected and dried for reuse in the spawning tanks when the alternate set of substrate ropes were removed. The study was terminated 20 weeks after the spawning substrate was first added to the tanks.

Differences in total fry production were compared using a two-way ANOVA with sex ratio and substrate transfer frequency as the two independent factors. Data were analyzed using the SPSS 13.0 program.

Results and Discussion

Least chub in all aquaria produced fry within 14 days following introduction into the aquaria. Fry were produced every week in ten aquaria; fry were not produced in two aquaria during two of the 20 weeks. Total weekly production ranged between 182 and 851 fry, with a mean weekly production of 446 fry (Figure 1). A total of 8,922 fry were produced over the course of the study. The most total fry produced in a tank was 1,555 in a tank with a 1:1 sex ratio with substrate transferred twice per week; the fewest total fry produced in a tank was 385 in a tank with a 2:1 sex ratio with substrate transferred twice per week.

Total fry production appeared to be higher in tanks with a 1:1 sex ratio (5,021 fry) compared to tanks with a 2:1

ratio (3,901 fry). Similarly, production appeared higher in tanks with a spawning substrate transfer of twice per week (5,174 fry) compared to tanks with once per week substrate transfer (3,748). Differences in production, however, were not significant between treatments ($P > 0.255$; Figure 2).

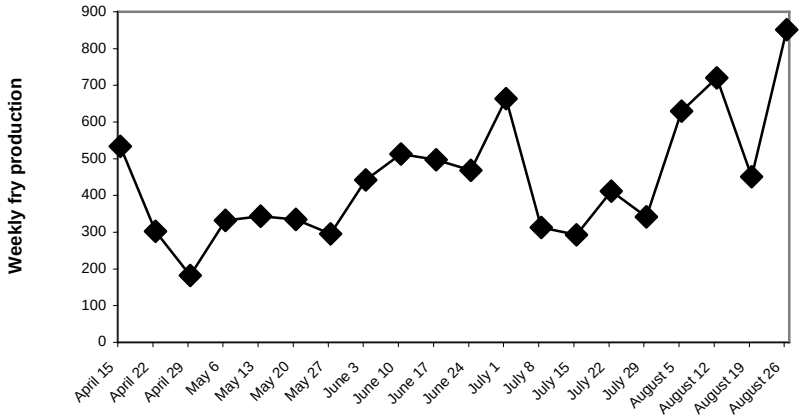

Figure 1. Total weekly fry produced by least chub adults in 12 aquaria over 20 weeks

Pairing least chub at a skewed ratio with more females than males did not increase production compared to an equal ratio; production was higher in tanks with an equal sex ratio, although differences were not significant. As with the previous spawning study (Billman and Wagner 2005), we recommend that adults should be paired at equal sex ratios. Different frequencies of spawning substrate transfer were compared to determine if removing substrate more frequently could decrease egg predation. Differences in production between tanks with the two transfer frequencies were not significant, indicating that one transfer per week is the better method because it requires less time. The twice per week transfer, however, may have an additional advantage compared to the once per week transfer. Least chub fry begin exogenous feeding 5 days after hatching (Ronney Arndt, unpublished data). In tanks with the once per week transfer, fry had already begun to hatch at time of transfer (an average of 6.5 of the 20 weeks), while tanks with a twice per week transfer only averaged 0.67 weeks with hatched fry. Substrates were incubated for 6 days before fry were harvested. Thus, fry from tanks with a once per week transfer could reach the developmental stage of exogenous feeding at least 1 day before being transferred to rearing tanks and initiated on rearing diets. This time without food could potentially affect the long-term survival of the least chub fry. Unless protocols for substrate incubation are changed, the twice per week transfer might prove to be superior not only for fry production but also for fry survival.

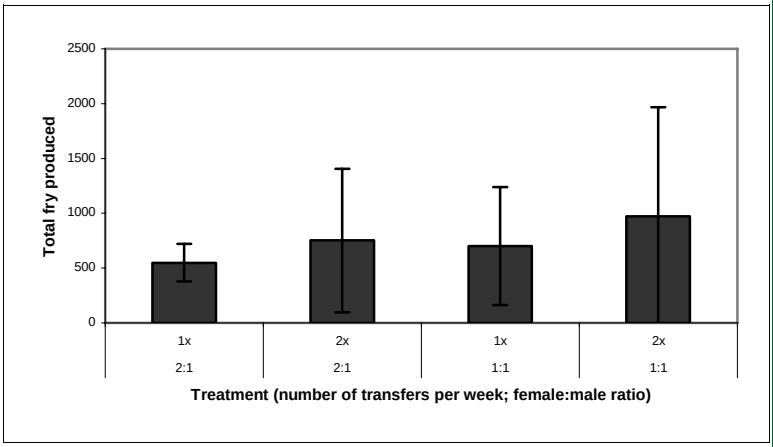

Figure 2. Mean ($n = 3$) total fry produced during the 20-week study by least chub adults in four treatments comparing two spawning substrate transfer frequencies (1x or one time per week versus 2x or two times per week) and two sex ratios (2:1 versus 1:1, female to male). Error bars indicate one standard deviation.

Results of this study can be used to establish intensive culture techniques for least chub. Further research should examine the use of intensive and extensive culture techniques to increase fry production, growth, and survival. For example, spawning substrates used in this study (intensive culture) could be used to inoculate rearing ponds for fry where growth and survival might be maximized (extensive culture).

Eric J. Billman and Eric J. Wagner

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Myxobolus cerebralis Found in Green and San Rafael River Drainages

Myxobolus cerebralis (*MC*) is the myxosporidian parasite that causes whirling disease in fish belonging to the family salmonidae. Since its original detection in Utah in 1991, the parasite has spread to a number of areas throughout the state including the Logan, Little Bear, South Fork, Ogden, Green, Weber, Provo, Beaver, Otter, Sevier and Fremont River drainages. As part of a statewide survey effort, the Fisheries Experiment Station (FES) technical service team, in conjunction with Division of Wildlife Resource biologists has most recently discovered the parasite in several new locations within the northeast and southeastern regions of the state.

In the northeast, the parasite has been found in fish sampled from Carter Creek, Beaver Creek and the North Fork of Sheep Creek. All of these tributaries are located in the Ashley National Forest just west of Flaming Gorge Reservoir (Figure 1). Fish were collected from these locations as part of the states whirling disease survey and were composed of cutthroat, rainbow, brown and brook trout. Following standard protocol, fish from this inspection were sent to FES for pathogen testing. During this investigation, spores morphologically consistent with the parasite were detected using Pepsin Trypsin Digest (PTD). Spores were later confirmed through nested Polymerase Chain Reaction (PCR) analysis to be those of *MC*.

The parasite's presence in this region is concerning as these tributaries ultimately flow into Flaming Gorge Reservoir which in turn is connected to Utah's Green River system. Sheep Creek and Beaver Creek also flow within close proximity to Sheep Creek Lake, which contains a population of Colorado River cutthroat trout that are used for native trout supplementation. In response to these findings, additional survey locations are continually being identified to help determine the parasite's distribution and potential spread into uninfected areas. One such location is the mainstem of the Green River where fish were obtained in September of this year for pathogen testing. In addition, biologists with the Utah Division of Wildlife Resources and Wyoming Game and Fish have recently collected kokanee salmon from Flaming Gorge Reservoir for a general fish health assessment.

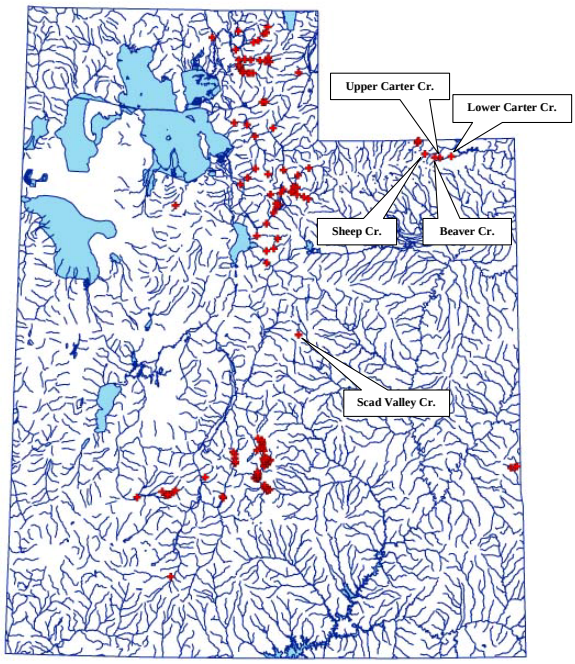

Figure 1. Map of Utah showing positive locations for the whirling disease parasite designated by red dots.

In southeastern Utah, *MC* was recently found in fish sampled from Scad Valley Creek. Scad Valley Creek is located in the Manti-La Sal National Forest just south of Huntington and Cleveland Reservoirs (Figure 1). Fish from Scad Valley were collected as part of a fish health inspection and were composed of Colorado cutthroat trout. As described in the previous investigation, fish from this location were sent to FES for pathogen testing. Spores from this analysis were presumptively identified through PTD and confirmed through nested PCR analysis to be those of *MC*.

The parasite's presence in this region is concerning as this tributary is within the San Rafael River drainage and is connected to an important native Colorado River cutthroat trout fishery located in Huntington Creek. Fish from this area were also being considered as a potential brood source for native trout supplementation. In accordance with Utah's fish health policy, Scad Valley Creek can still be utilized for native trout supplementation, but the parasite's presence in this area will limit the effort to the movement of fertilized eggs rather than live fish. Future efforts include sampling from Huntington Creek and surrounding tributaries to better identify the parasite distribution within the area.

How the parasite became transplanted to the above locations is unknown, but angler use, avian vectors and instream diversions may all have played a part in its dispersion. Although the parasite continues to spread, efforts such as the whirling disease survey continue to provide valuable information regarding the parasite's distribution throughout Utah. The information gathered from this survey is readily available to the public and regional biologists interested in management of the parasite.

Wade Cavender

Prey Selectivity of Least Chub and Mosquitofish Fed Mosquito Larvae and Two Other Prey Types

Least Chub *Iotichthys phlegethontis* are native to the Bonneville Basin, Utah where it was once widespread in rivers, streams, creeks, springs, ponds, marshes and swamps (Sigler and Sigler 1996). Least chub are considered to be at risk of becoming extinct, and are currently classified as a 'sensitive species'. One of the principal threats to their continued existence is the competition with non-native fish, especially the mosquitofish, *Gambusia affinis* (Wilson and Whiting 2003). The species has been introduced for mosquito control into many waters with little thought given to its impacts on native fauna. Given human health concerns for emerging pathogens such as West Nile Virus, the need for mosquito control remains high.

The objective of the current research effort was to determine if least chub would readily consume mosquito larvae in the presence of other prey items. If so, least chub could be stocked in lieu of mosquitofish within its historical range for mosquito control. Prior research indicated that least chub will consume mosquito larvae, though not to the extent that mosquitofish can (Wagner and Billman 2004). This report summarizes information generated in the set of trials in which least chub and mosquitofish were presented mosquito larvae and two other prey types.

Methods

Prey selectivity was tested in one and two fish groups in replicate trials between April and July 2005. Fish were presented three prey items: mosquito larvae, midge larvae (*Chironomidae sp*.), and *Daphnia magna*. Mosquito eggs were obtained from Carolina Biological Supply and hatched on station; mosquito larvae were in the 3rd and 4^{th} instar stage of development before use in the trials. Midge larvae were collected at FES in raceways that were not being used for culture purposes. *Daphnia* were obtained from Ward's Natural Science Establishment, Inc. (Rochester, NY), from which a culture was maintained at FES.

Least chub and western mosquitofish for the 1-fish trials were reused from the mosquito consumption test; for the 2-fish trials, new least chub and western mosquitofish were obtained from their sources. Least chub and mosquito fish were introduced into 19-L plastic containers, and starved for 24 hours. Aquaria were enclosed behind a curtain to minimize disturbance. After the acclimation period, equal numbers (20 each) of mosquito larvae, midge larvae, and *Daphnia* were poured into the containers, after which containers were stirred immediately to randomize distribution. Fish were allowed to feed for 2 hours in 1-fish trials and for 1 hour in 2-fish trials, at which time fish were removed and remaining prey items were enumerated. Temperatures ranged from 15.2 and 18.4°C for all trials. For 1-fish trials, we conducted 29 least chub trials and 23 western mosquitofish trials. Nine 2-fish trials were conducted for each species. Trials in which fish did not consume any prey items were not used in analyses. No-fish controls were used in each group of trials (9 for 1-fish trials and 2 for 2-fish trials) to evaluate counting and recapturing accuracy for each prey type. The average number of mosquito larvae, midge larvae, and *Daphnia* transferred into the containers and subsequently recaptured for all 11 controls was 20, 19.8, and 19.8, with coefficients of variation (CV = 100 ´ SD/mean) of 0.0, 2.0, and 2.0%, respectively, indicating that these methods were performed with minimal error.

Consumption rates (percent eaten) by least chub and western mosquitofish were compared for each prey type in the 1-fish and 2-fish trials using a t-test assuming equal variances at a significance level of 0.05. Percentages were arcsine transformed prior to analysis. SPSS version 7.0 (SPSS Inc. 1993) was used for analyses. Prey selectivity was estimated by calculating Chesson's (1983) coefficient of selectivity, where r_i is the proportion of food type i in the diet, p_i is the proportion of food type i in the environment, and m is the number of prey types available. For each set of trials, mean selection coefficient and 95% confidence intervals were compared with

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random feeding (1/*m*) to determine prey selectivity. We assumed positive selectivity if the 95% confidence intervals were above the random feeding line, neutral selectivity if the 95% confidence intervals overlapped the random feeding line, and negative selectivity if the 95% confidence intervals were below the random feeding line.

Results and Discussion

In 1-fish trials, 7 least chub and 1 western mosquitofish did not consume a prey item, and were not used in analyses; prey items were consumed in all 2-fish trials for both species. Least chub consumed on average 9.5% of the prey items in the 1-fish trials, while western mosquitofish consumed 23.2% on average. More prey were consumed by both species in the 2-fish trials (53.7 % and 89.6% by least chub and western mosquitofish, respectively), indicating that stress was reduced by pairing fish. Western mosquitofish consumed significantly more individuals of each prey type than did the least chub in both the 1-fish and 2-fish trials (all $P < 0.02$), the exception being *Daphnia* in the 1-fish trials ($T_{42} = 0.67$, $P = 0.254$).

Least chub and western mosquitofish demonstrated their opportunistic feeding characteristics in the selectivity trials. Both species neutrally selected all 3 prey types in the 1-fish and 2-fish trials (Figure 1). Western mosquitofish had higher selection coefficients for mosquito larvae, but we could not conclude that western mosquitofish were more positively selective for mosquito larvae than were least chub because of high variation.

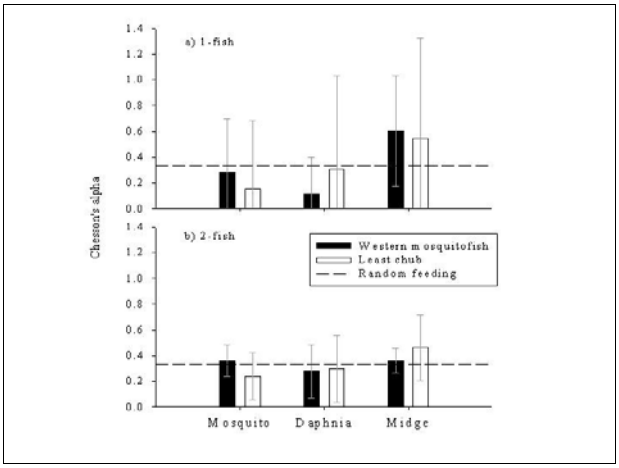

Figure 1. Mean prey selection by least chub and western mosquitofish for mosquito larvae, *Daphnia magna*, and midge larvae as determined by Chesson's selectivity coefficient. Positive selectivity was assumed when the 95% confidence intervals were above the random feeding line (dashed line), neutral selectivity when the 95% confidence intervals overlapped the random feeding line, and negative selectivity when the 95% confidence intervals were below the random feeding line.

Least chub showed that they would consume mosquito larvae in the presence of other prey. Both species neutrally selected mosquito larvae in the presence of other prey, indicating that both fish would consume mosquito larvae at rates relative to larval abundance. Western mosquitofish, however, consumed significantly more prey items in almost every trial, indicating that western mosquitofish would be more effective at mosquito control than least chub.

These studies demonstrated that in laboratory settings, least chub were not as efficient at mosquito consumption as western mosquitofish. These studies failed to present results with realism in regards to mosquito control under field conditions, where biotic factors (vegetation and alternative prey) reduce the efficacy of mosquitofish (Kramer et al. 1987, Nelson and Keenan 1992, Homski et al. 1994). Further studies should compare the efficacy of least chub and western mosquitofish for mosquito control in field settings. If least chub proved to be just as effective in mosquito control as western mosquitofish in field settings, indigenous least chub would appear advantageous over western mosquitofish. Least chub are not only native to Utah, but the fish is capable of surviving drought conditions and harsh winters of Utah, while western mosquitofish are poorly adapted for such conditions (Rees 1934, Nelson and Keenan 1992). Efforts should continue to find indigenous fish as substitutes for western mosquitofish in mosquito control to minimize the negative effects this fish has on native fauna where introduced.

By Eric J. Billman and Eric J. Wagner

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Effects of the Commercial Feed Additive *Ration Plus* on Hatchery Performance of Rainbow Trout

Ration Plus is a commercial feed additive composed principally of microbial metabolites derived from bacteria including *Lactobacillus acidophilus*. It is not a drug, nor does it contain any live microorganisms. It is prepared by Cytozyme, Salt Lake City, Utah, a Tea Tree Products company subsidiary. The brown liquid is stable at room temperature.

The product is commercially available as a feed additive for dogs and horses. According to the website www.rationplus.com, the additive 'provides a favorable environment for the growth and activity of the important and beneficial digestive microorganisms. The active ingredients concentrated in *Ration Plus* are bacterial byproducts produced through a unique multi-stage fermentation process. The end result is a highly stable concentrate that supplies a combination of nutrients to target specific beneficial bacteria found in the dog's digestive system. This helps to establish a healthy balance of populations in the digestive microflora.'

Bacteria are part of the natural flora of intestines of mammals, fish, and other organisms. Some bacteria, such as *Lactobacillus acidophilus* and other lactic acid bacteria, can inhibit the growth and activity of intestinal pathogens, produce vitamins, and help break down indigestible diet components (Hosono et al. 1997; Irianto and Austin 2002). These so-called probiotic bacteria have recently been of interest as alternatives to antibiotics for treatment of fish (Ringo and Gatesoupe 1998; Verschuere et al. 2000). Some studies have shown promising improvements in fish fed probiotics (Austin et al. 1992; Smith and Davey 1993; Carnevali et al. 2004). Probiotics can be defined as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance' (Fuller 1989).

The exact composition of *Ration Plus* is proprietary, so exactly how this product was supposed to affect fish was unknown, but we presume the mechanism is related to the probiotic phenomenon, but without feeding live organisms. In this study, the objective was to evaluate the feed additive product against a commercial control diet in rainbow trout.

Methods

On 16 March 2005, 10,000 rainbow trout fry of the TenSleep strain were put into each of six outdoor, concrete, plug-flow raceways (1.22 m x 11.58 m x 0.57 m deep). The eggs of this lot had been heat shocked (26 to 27°C) at 20 min after fertilization for 20 min to induce triploidy. A 30 fish sample analyzed by flow cytometry (Allen 1983) indicated that 100% were triploid. The study was initiated when the fish averaged 40 mm long and 0.74 g. Initially, in each raceway the fish were given 1.98 m^3 of rearing space (Piper Density Index = 0.14) and 95 L/min of hatchery well water at 16.3 ± 0.4°C (Piper Flow Index = 0.42). Three raceways were assigned to the additive treatment and three were used as controls.

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The treatment group was given a commercial feed (Silvercup steelhead) that was top-dressed with a mixture of *Ration Plus*® and fish oil. *Ration Plus* was added at 40 mL per liter of fish oil. The two ingredients were mixed thoroughly in a liquid blender at high speed. The mixture was dripped onto fish feed in a bucket mixer as a paddle blade of the mixer mixed the feed. The mixture was added at a rate of 40g/kg feed (4%). Control diets also received a top dressing of fish oil at the same rate, but without the additive. The feed was top-dressed in batches of 4.5 to 5.0 kg due to the volume limitation of the bucket mixer. The fish feed was mixed several times during the study (Experiment Day –2, 8, 25, 49, 70, 95) to insure freshness.

The feed was fed six times a day initially (5.4% of body weight), reducing the ration as uneaten feed was observed (day 18, to 4.4%; day 27, to 4.0%; day 28, to 3.7%; day 48, to 2.9%). On day 56, the feed frequency was reduced to 4 times/day. Other adjustments to flow and space were made during the study, applying these increases to both treatments alike. E.g., on day 32 and 70, the rearing volume was increased to 4.50 and 7.22 m^3, respectively, to achieve new rearing density indices of 0.16 and 0.19 to 0.22, respectively. Water flow was increased to 170 L/min on day 36 (Piper Flow Index = 0.64) and day 74 (Flow Index = 0.95 to 1.11).

On day 74, examination of moribund fish in the raceways indicated that fish were fighting an infection with *Columnaris*, a bacterial disease. The fish were taken off feed and all raceways were treated with 50 ppm of hydrogen peroxide for 60 min. During the following two days, the dose was increased to 75 and 100 ppm for the same duration. On day 85, continuing mortality and morbidity in two raceways (one raceway from each treatment) prompted another treatment, followed by treatment of all raceways for the following three days. The fish continued to have problems after the treatment in both experimental groups.

Near the close of the study, we sent samples of fish oil to an independent laboratory for analysis of rancidity (Eurofins, Des Moines, IA). Both the peroxide test and free fatty acid test were conducted (AOAC 1990). Also, proximate analysis of both experimental diets was conducted by a commercial lab using standard methods.

The study was concluded after 105 days of feeding. At this time, Health Condition Profiles (HCP; Goede and Barton 1990; Goede 1991) were conducted on 10 fish from each raceway (30 per treatment). In addition, ten fish from each of two raceways containing non-study fish from the same cohort were also dissected according to the HCP methodology so that non-statistical comparisons to the treatment and control raceways could be made.

Water quality was measured at the end of the study using standard methods (APHA et al. 1989). Variables measured included total alkalinity, total hardness, pH (tail end of raceway), total gas saturation (saturometer), temperature, ammonia, and dissolved oxygen. Dissolved oxygen was also monitored biweekly during the study. Raceways were given supplemental oxygen during the experiment via low-head oxygen injection devices (Watten and Boyd 1989).

Average weights were determined by monthly sample counts of fish in each raceway. The mean of these three grab samples were used to project feed needs for the following month. Fish for this sample were crowded to the head end of the raceway with a screen, netted, and weighed in water. Specific growth rates were calculated using the formula: $SGR = (\log_e(W_f) - \log_e(W_i))/d \times 100$, where W_f = final weight and W_i is initial weight.

Statistics—For analysis of final weight, feed conversion, specific growth rate, and mortality rates, *t*-tests were used. Mortality percentages were arc-sine transformed prior to analysis. Levene's test was used to test the homogeneity of variance assumption. Health Condition Profile data were analyzed using SigmaStat 3.0.1. Non-categorical data were analyzed for significance by a two-tailed *t*-test, and when equal variance and normality tests failed, data were analyzed by the Mann-Whitney Rank Sum test. Non-parametric ordinal data were analyzed by the Wilcoxon Signed Rank test, and categorical data were tabulated and analyzed by the Fisher Exact test.

Results and Discussion

The final mean weight, feed conversion, specific growth rate, and mortality rates did not significantly differ between feed treatments (Table 1). Despite, disease problems and several days off feed, specific growth rates were higher in this study than in previous studies conducted here using Fish Lake-DeSmet strain rainbow trout (1.58 to 1.77; Arndt et

al. 1998) or Sand Creek strain (1.65 to 2.10; Arndt et al. 2001). This was likely due to the higher protein levels in the steelhead diet (>45%). The final weights for the *Ration Plus* fish were 14.0 g/fish, for the controls was 14.3 g/fish, and for the non-study controls was 13.7 g/fish. Mesenteric fat scores were 3.5 for the *Ration Plus* fish and 3.6 for the controls. Mesenteric fats are ranked according to the following scale: 0 = no visceral fats, 1 = approximately 25% of pyloric caeca covered with fat, 2 = approximately 50% covered, 3 = more than 50% covered, 4 = caeca completely covered. Feed conversion ratios were similar to Arndt et al. (2001), but feed was converted more efficiently in this study than in the study by Arndt et al. (1998; 1.23 to 1.34).

Table 1. Comparison of hatchery performance variables between fish fed a diet supplemented with the feed additive *Ration Plus* and a control diet (Silvercup steelhead).

Parameter	RationPlus	Control	Non-Study control
Length (mm)	110.5*	102.2	102.8
Weight (g/fish)	14.4	12.4	10.9
Ktl	1.019	1.002	0.958
Hematocrit (%)	35.8*	33.6	36.5
Leucocrit (%)	0.93	0.89	1.30
Plasma protein	4.04	3.72	3.83
Eyes	100% N	100% N	95% N, 5% B1
Gills	100% N	93% N, 7% F	95% N, 5% F
Pseudobranch	100% N	100% N	100% N
Thymus	0.87	1.03	0.85
Fat	3.53	3.60	3.40
Spleen	97% R, 3% G	93% R, 7% G	95% R, 5% G
Hind Gut	100% 0	100% 0	100% 0
Kidney	97% N, 3% S	100% N	100% N
Liver	17% A, 60% B, 23% C	7% A, 77% B, 17%C	10% A, 60% B, 30% C
Bile	2.27	2.36	2.30
Fins	0.33	0.27	0.20
Opercle	100% 0	93% 0, 7% 1	100% 0

Mortality rates were higher in this study than in previous experiments (typically < 2%), largely due to the *Columnaris* outbreak. It was opportune that the disease struck when it did, giving us an opportunity to evaluate the potential benefits of the feed additive for improving immunity. Unfortunately, the additive did not significantly reduce mortality.

Despite the fact that most raceways had experienced epizootic-related mortalities, the HCP-related data indicated test fish were generally healthy with few significant differences found. Looking at the blood-related parameters, the percent hematocrit was significantly higher ($P = 0.026$) for the treatment fish (36%) than for control fish (34%). The average hematocrit reading from the twenty non-study fish was 37%. Even with a significant difference, all three hematocrit values fall within the 24-43% range of normality discussed by Wedemeyer et al. (1990) for juvenile salmonids. For our study the averages of 3.9 g/dL for plasma protein, and 0.9% for leucocrit also fall within the range of normality (Wedemeyer et al.1990). One fish among the treatment fish exhibited a swollen kidney, although fish from all raceways exhibited kidneys that were slightly swollen beyond what might be normally expected. Whether or not this swelling could be attributed to the mentioned disease outbreak cannot be ascertained. There was also no relationship between the presence of swollen kidneys and dietary treatments.

Table 2. Proximate analysis of two fish feeds, Silvercup Steelhead (control diet) and Silvercup Steelhead top-dressed with the feed additive RationPlus. Both were top-dressed with fish oil

	RationPlus diet	Control diet
Water (% ± SE)	7.4 ± 0.02	8.6 ± 0.02
Ash (% ± SE)	7.7 ± 0.01	7.6 ± 0.02
Protein (% ± SE)	60.9 ± 0.79	55.0 ± 0.70
Lipid (% ± SE)	23.2 ± 0.06	22.3 ± 0.12

Because the *Ration Plus* was added to the diets by top dressing with fish oil, there was interest in the possibility of the dietary lipid levels affecting growth in some way. All fish were fed extruded floating feeds formulated for steelhead (Silver Cup). The steelhead ration contains 45% protein and 16% lipid compared to 40% protein and 10% of the normal trout grower. Proximate analysis of the feeds indicated that the *Ration Plus* diet had higher levels of protein (60.9%) than controls (55.0%) and slightly higher levels of lipid (23.2 vs. 22.3; Table 2). The additional protein and lipid did not improve the growth of fish fed the additive diet.

Interestingly, all three groups of fish exhibited a higher incidence of fatty livers than one would normally expect (Table 3). From previous research at the FES, a sample size of 20-30 fish might contain 0-6% with fatty livers. For this test, 23% of the Ration Plus fish exhibited fatty livers, the controls had 17%, and the non-study controls had 30%.

When the fish began experiencing elevated mortalities, one initial theory of the cause was that the oil we were using for

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Comparing Dosage Levels Of Ovaprim, HCG or a Combination Of the Two to Induce Spawning of Mature June Sucker Females *(Chasmistes liorus)*

Introduction

The June sucker (*Chasmistes liorus*) is an endangered fish endemic to Utah Lake, Utah. To aid in recovery efforts, a warm water sport fish and native aquatic species hatchery is scheduled to be built. Broodstock will be held at the new facility and they will be used to produce eggs to be hatched for future production/research fish to be stocked into Utah Lake, Utah.

Some species of fish will not produce mature gametes when held in captivity (Mittelmark and Kapuscinski 1993). The required environmental conditions such as temperature, substrate, flow rates, diet and light may not be present. Stress may be the result and this could prevent the necessary responses to complete the maturation of gametes. From past experiences at the Fisheries Experiment Station (FES), very few captive brood stock females have ovulated without the use of Human Chorionic Gonadotropin (HCG). Hormone studies have also been done annually at FES from 2002 – 2005. During this period we have found that the June sucker females need to be moved from the 65^{o}F water they are being held in and placed in colder 56F water at least 5-6 months prior to spawning in June.

The purpose of this study was to compare dosage levels of Ovaprim, a Gonadotropin Releasing Hormone (GnRHa) with a dopamine blocker, HCG (Human Chorionic Gonadotropin) or a combination of the two to induce spawning of mature June sucker females held in captivity.

Methods:

The June sucker brood stock used in this study, are from crosses made on the Provo River, Utah from 1989-1995. From July – November 2004, the brood stock were in 65F water. In December 2004, they were switched to 56^{o}F water to aid in the egg maturation process.

On June 1, 2005 we began our hormone study on the June sucker. We used 6 treatments. The fish were checked for 9 consecutive days to see if the eggs were ripe or not. We injected 19 fish in most treatments. For treatment 3 we injected 18 fish, but one of them ended up being a male so only 17 were used in the study.

Treatments were evaluated on the following basis:

Treatment 1	One injection of Ovaprim @ 1 ml/kg of body weight and HCG @ 1000 IU/kg of body weight
Treatment 2	One injection of Ovaprim @ 1 ml/kg of body weight and then injections of HCG @ 400, 750 and 1000 IU/kg of body weight respectively
Treatment 3	HCG @ 1000 IU/kg of body weight for 3 consecutive days
Treatment 4	HCG @ 400, 750 and 1000 IU/kg of body weight respectively.
Treatment 5	Controls = one injection of saline @ 1 ml/kg of body weight
Treatment 6	One injection of Ovaprim @ 1 ml/kg of body weight

All fish were anesthetized with MS-222 in a 1% salt bath for sorting, injections and spawning. (Piper et al., 1982; Rottmann et al., 1991a). The oxygen was kept above 8 mg/L and the water was exchanged if the water temperature increased by 4^{o} C. All females were given intraperitoneal (IP) injections of Ovaprim and/or HCG (Rottmann et al., 1991b).

During our evaluation, if we stripped the female of her eggs we said that we spawned her. If she only gave a few eggs, but was not ripe enough, we said this was partial ovulation. If she gave no eggs at all,

this was called no ovulation.

Table 1. The effect of various hormone injection protocols on ovulation success of June suckers. Matching subscripts among treatments depict no significant difference between treatments.

Treatment	Spawned		Partial Ovulation		No Ovulation		Spawned + Partial Ovulation	
	Number	%	Number	%	#	%	#	%
Ovaprim + HCG @ 1000 IU/kg	9 a	47.4	3 ab	15.8	7 ab	36.8	12 ab	63.2
Ovaprim HCG @ 400,750,1000 IU/kg	7 a	36.8	7 a	36.8	5 ab	26.3	14 a	73.6
HCG @ 1000 IU/kg	8 a	47.1	2 ab	11.8	7 ab	41.1	10 ab	58.9
HCG @ 400,750,1000 IU/kg	7 a	36.8	9 a	47.4	3 a	15.8	16 a	84.2
Saline Controls	0 b	0	1 b	5.3	18 c	94.7	1 c	5.3
Ovaprim @ 1 ml/kg	8 a	42.1	1 b	5.3	10 b	52.6	9 b	47.4
Totals	**39**	**34.8**	**23**	**20.5**	**50**	**44.6**	**62**	**55.4**

Notes: Some of the females that partially ovulated gave a few eggs several days in a row. Some of them actually gave a small stream of eggs, so we figured she was ripe. We would keep her in the anesthetic to get ready for spawning her and when we tried to strip her eggs into the Ziploc bag, no eggs would come out. It seemed like the stress of checking her prevented her from releasing her eggs.

Table 2. Percent mortality for June suckers injected with ovulation inducing hormones

Treatment Type	Number in Treatment	Number that Died	Percentage
Ovaprim + HCG @ 1000 IU/kg	19	3 ab	15.8
Ovaprim + HCG @ 400, 750, 1000 IU/kg	19	2 ab	10.5
HCG @ 1000 IU/kg	17	0 a	0.0
HCG @ 400, 750, 1000 IU/kg	19	0 a	0.0
Saline Controls	19	0 a	0.0
Ovaprim @ 1 ml/kg	19	5 b	26.3

Conclusions

Except for the control group, the study indicates that there is not a significant difference in treatment types for the number of females that ovulated. The study did show that there are significant differences in those females that partially ovulated, did not ovulate, or spawned or partially ovulated. For those females that partially ovulated, the injections of Ovaprim + HCG @ 400, 750, and 1000 IU/kg; and HCG @ 400, 750 and 1000 IU/kg, showed that these two treatments were significantly better than the other treatments. For those females that did not ovulate, the saline treatment was significantly worse than all other treatments and the HCG @ 400, 750 and 1000 IU/kg was significantly better than all other treatments. For those females that spawned or partially ovulated, the injections of Ovaprim + HCG @ 400, 750 and 1000 IU/kg; and HCG @ 400, 750 and 1000 IU/kg were significantly better than the other treatments, with the control group being significantly worse than all other treatment types.

More than likely, injecting the females with HCG @ 400, 750 and 1000 IU/kg of body weight, will be our standard protocol. The females injected using this protocol, had significantly fewer females that did not ovulate and this treatment had the higher percentage of females that spawned or partially ovulated and no mortalities occurred.

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topdressing the feed was rancid. There has been a link between oxidized dietary lipids and the development of cold-water disease, or that fish fed such diets develop a similar "syndrome" as cold-water disease (Daskalov et al. 2000). The peroxide test for rancidity gave a peroxide value of 12 meq/kg. To put this value in perspective, other studies have shown oxidized peroxide values of 44-90 meq/kg compared with control values of unoxidized oil of 10-24 meq/kg (Forster et al. 1988, Daskalov et al. 2000). The data suggested that the oil we used was of good quality and should not have contributed to the epizootic experienced.

Table 3. Comparison of Health and Condition Profile parameter means between rainbow trout fed a diet with the *Ration Plus* additive or without (controls). Means that are significantly different are indicated with an asterisk.

Diet	Final weight (g ± SD)	Specific growth rate (%/d ± SD)	Feed conversion ratio ± SD	Cumulative mortality rate (% ± SD)
Ration Plus	14.0 ± 0.74	2.77 ± 0.05	1.09 ± 0.100	9.8 ± 5.44
Steelhead	14.3 ± 0.88	2.79 ± 0.06	1.04 ± 0.044	7.8 ± 2.21

Water quality measurements indicated that these should not have contributed to mortality. Dissolved oxygen was relatively high throughout the study in all raceways, ranging from 7.9 to 12.0 mg/L among all the measurements made. Among all six raceways, total alkalinity was 188 to 205 mg/L, total hardness was 222 to 239 mg/L, pH was 7.1 to 7.2, total gas saturation was 106.3 to 106.9%, un-ionized ammonia was less than 0.0037 mg/L, and carbon dioxide at the tail end of the raceway was 30.8 to 37.3 mg/L.

In summary, the results indicated little benefit to the use of the feed additive. It is possible different results may have been obtained with trout chow, which has less protein. The additional protein added by *Ration Plus* might then have been enough to generate significantly higher growth in that treatment. The additive's inability to aid in the defense against the bacterial infection indicated that any potential improvements in immune function were insufficient to significantly reduce mortality.

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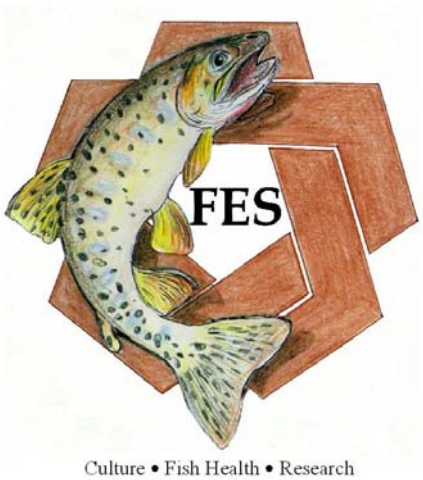

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It is not clear why mortalities only occurred in those females that were in treatments injected with ovaprim. The ovaprim is fairly viscous, so we needed to use a larger diameter needle to allow us to inject the hormone more freely into the female. The HCG was injected using a 25G 5/8" needle compared to the 21G 1.5" needle used for the Ovaprim. It could be that the 21G 1.5" needle was being inserted too far into the body cavity, damaging internal organs.

Doug Routledge

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