UTAH DIVISION OF WILDLIFE RESOURCES
FISHERIES EXPERIMENT STATION
1465 WEST 200 NORTH
LOGAN, UT 84321

THE ICHTHYOGRAM

DECEMBER 2003

The Ichthyogram

Midway State Fish Hatchery Receives Clean Bill of Health…

Fish health biologists at the Fisheries Experiment Station are pleased to announce that fish health approval has been granted to the previously quarantined Midway State Fish Hatchery. The Midway facility was the first state hatchery in Utah to be contaminated with *Myxobolus cerebralis* (Mc), the parasite that causes whirling disease (see *Ichthyogram* volume 12, issue 1-2). The hatchery was taken out of production in the spring of 2000.

Since the hatchery closure, the Division of Wildlife Resources has taken considerable effort to clean up the hatchery. First, the facility was depopulated, and all of the outside raceways were decommissioned. Furthermore, since the springs that supply the hatchery were found to be contaminated, new production wells were drilled to serve as a clean source of water. Sentinel fish have been maintained on each of the successful wells, and polymerase chain reaction (PCR) techniques were employed to test for the presence of the parasite. In order to prevent infection from the nearby positive waters, all fish have been, and will be cultured in enclosed raceways. Water from the new wells is cooler and softer than the previous springs, and this will give culturists more options in the future fish production.

A representative sample of rainbow trout that had been maintained on the site for over six months were tested in March of this year for the presence of any of prohibitive pathogens; including Mc, as well as *Renibacterium salmoninarum*, and the viruses that cause Infectious Hematopoietic Necrosis or Viral Erythocytic Necrosis. No evidence of any of these agents was found. Again, in late July 2003, 120 more rainbow trout from two production wells tested negative for the pathogens. Once it was determined that the second inspection revealed no prohibitive pathogens, the state hatchery was granted fish health approval.

Now that the quarantine of the Midway Hatchery has been lifted, the Division is continuing its effort to get the hatchery back into production. An interim facility has been built, and hatchery superintendent Chuck Bobo and Tom St. John are currently assembling indoor raceways to rear a limited number of trout. These disease-free fish should be available for stocking into waters throughout the regions next fall. Long-term plans include a multi-million dollar culture facility comparable to the Kamas State Fish Hatchery. The new hatchery will be supplied by protected wells, and disease monitoring will continue. Researchers at FES are also working on technologies to filter water from the contaminated spring, in order to reclaim this valuable source of water as well. Progress on this research and the construction of the new hatchery buildings will be discussed in future *Ichthyogram* articles.

Patrick Goddard

Anna Miller and Patrick Goddard look over new raceways being assembled by Midway superintendent Chuck Bobo

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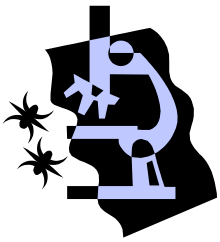
New Detection Methods for Whirling Disease in Fish



Current detection methods for diagnosis of whirling disease in fish rely on traditional histology and enzymatic digest or polymerase chain reaction (PCR). Enzymatic digest is very time and labor intensive and becomes tedious when large lots of fish are tested. Traditional histology can take weeks before results are available; in addition a trained pathologist is required to evaluate the samples. PCR is very fast and sensitive, but it cannot be performed in most laboratories. PCR samples must be treated with care to prevent cross contamination and false positive or false negative results.

Researchers at Utah State University, using Federal Aid funds provided by the Utah Division of Wildlife Resources, have been testing monoclonal antibodies against *Myxobolus cerebralis* for use in diagnostic assays for detection of whirling disease in fish. Dr. Nabil Youssef's lab has developed more than 20 antibodies with activity against *M. cerebralis*, the causative agent of whirling disease. Antibodies were developed in mice using *M. cerebralis* myxospores or triactinomyxons.

Carey Wicks, a graduate student in the Biology department, has conducted tests on the monoclonal antibodies to develop an assay system for rapid detection of infection in fish tissue sections. Preliminary tests using myxospore suspension or sections of fixed myxospores showed that twelve antibodies could detect myxospores in an indirect immunofluorescent assay (IFA) where antibody reaction was measured through a

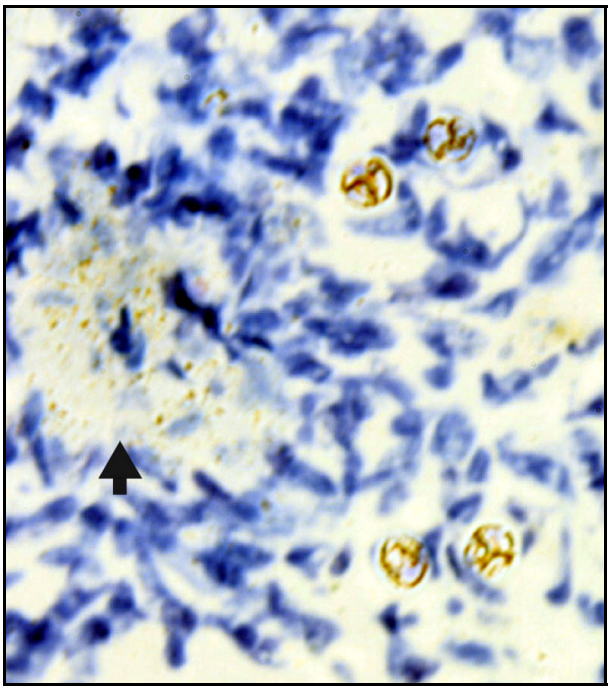


Figure 1. MC7-15 binding to mature spores and trophozoites (arrow), reaction with antibody causes brown color, in frozen tissue section treated with immunohistochemistry procedure and counterstained with Mayer's hematoxylin.

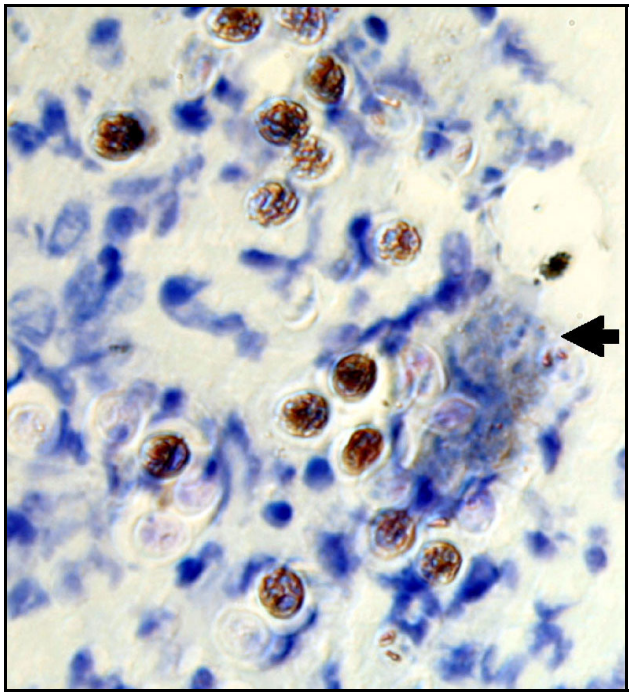


Figure 2. MC4-4.2 binding to both mature spores and trophozoites (arrow), in frozen tissue section treated with immunohistochemistry procedure and counterstained with Mayer's hematoxylin

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fluorescent probe. These antibodies were characterized by isotype, binding site on myxospores using IFA and electron microscopy, whether the antigen is carbohydrate or protein, and specificity against *M. cerebralis* by testing antibodies against neurotropic myxospores from rainbow trout.

Monoclonal antibodies with positive results from the characterization study were tested on frozen sections from rainbow trout to determine the optimum dilution and assay conditions. Antibodies were further tested on sections containing both mature spores and trophozoites. Antibodies were evaluated for utility on frozen sections of infected rainbow trout head using both IFA and immunohistochemistry, where antibody binding was measured by production of a colored product on sections. This procedure can produce slides for evaluation in less than a day. Slides can be examined on a light microscope or a microscope with fluorescence attachment depending on the detection system used. Several antibodies reacted with trophozoites and/or mature spores in these tests. Future research will include more specificity testing against other myxosporea and tests using cocktails of antibodies to determine if a combination of antibodies can detect lower spore counts or detect infection before the formation of trophozoites.

Valerie Hubbard, a research technician at Utah State University, has been conducting tests using monoclonal antibodies in an ELISA for detection of whirling disease in fish homogenate. In this system, fish heads are homogenized, centrifuged, and boiled before they are applied to a 96-well ELISA plate. A combination of antibodies is added to detect myxospores. Antibody reaction is measured by the production of a colored substance in the wells. Color is measured with an ELISA plate reader and absorbance values for each well are taken. The absorbance values show the amount of reaction in the well, more reaction yields a darker color and higher absorbance value. A typical data set is presented on the following table:

TABLE OF ABSORBANCE VALUES

Tissue	Test 1	Test 2
Not Infected (Control)	0.253	0.194
Infected	1.252	1.226

This table lists two separate tests (test 1 and test 2). The tissue tested was infected trout tissue from a laboratory infection or uninfected trout tissue. The values under column 1 demonstrate that the reaction in the infected tissue wells was almost 5 times greater than the background noise from the uninfected tissue. The pattern is similar for the test in set 2. The results have been very positive in determining whether a fish is infected or not. Future research will involve optimization to determine the lowest number of spores detectable with this assay.

Carey Wicks, Utah State University

Does Sperm Motility Affect Spawning Success of Colorado River Cutthroat Trout in Dougherty Basin Lake?

In Dougherty Basin Lake, the viability of the milt was of interest to see if poor motility rates might be affecting the spawning success of Colorado River cutthroat trout. During the first egg take, a microscope was carried into the lake (about 1 km trek), but the flashlight did not produce enough light to make it work and no viability assay was conducted. For the second take, a sperm extender solution was used to maintain the sperm until it could be transported back to a recreational vehicle trailer where there was power. Motility was examined by mixing the sperm and extender solution (7 uL) with a salt diluent solution (7 uL) on a pre-focused microscope slide. The percentage of sperm that were motile was low for each of the 5 lots: 0 to 25% for Lot 1, 5% or less for Lot 2, <5 to 50% for Lot 3, 5 to 99% for Lot 4, and <5% for Lot 5. The duration of motility (defined as forward motion) ranged from 0 to 44 sec.

Since it was unclear if the low viability of the sperm was related to the extender solution and the delay in testing, a follow up test was conducted at Mantua State Fish Hatchery using cutthroat trout males. Three 3-year-old males that had not been spawned in 2003 (males #1 to 3)and two moribund 5-year-old males that were being culled were used for the test. Milt was expressed after the vent area had been dried. Most samples were clean with the exception of the first, which contained blood. As soon as samples were collected, a subsample was taken and diluted with Hank's buffered saline solution (HBSS) on a 1:2 milt:extender basis. All samples were kept on ice until analysis was complete. All five unextended samples were assayed for motility and duration within 30 min of collection (initial assay). Samples were subsequently transported back to the FES where samples were assayed in both their extended and unextended form about 1 h after the samples were collected. Results are presented in Table 1.

After transport, all five samples either had the same (1 fish) or lower percentages of motile sperm, whether extended or not. The duration of motility was lower after transport for 4 of the 5 samples in the extender solution. The unextended samples were variable, with 3 showing a decrease and the two samples from the 5-year-olds showing an increase after transport. Within the post-transport samples, males 1 and 3 had better quality in the extended form compared to unextended. However, male 2 had a dramatically higher percent motility when left unextended compared to extended. Overall, the use of extender did not dramatically improve the viability of sperm in the short term.

Table 1. The percentage of motile sperm and the duration of motility for Bear Lake cutthroat trout males from Mantua Hatchery on 19 June 2003 before and after about 1 h of transport in extender solution or without.

	Pre-transport		Post-transport			
			Extended		Unextended	
Male	Motile (%)	Duration (sec)	Motile (%)	Duration (sec)	Motile (%)	Duration (sec)
1	95	52	50	47	25	29
2	75	56	10	37	90	36
3	90	57	75	54	25	31
4	75	30	75	32	60	36
5	30	46	10	29	10	77

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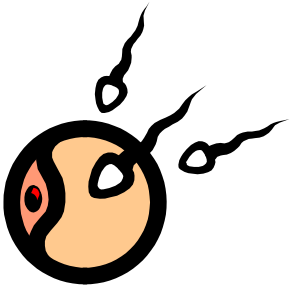

For the third egg take, sperm viability was assayed at the lake using a microscope that was lit with a 12 V spotlight hooked up to a car battery. The light was shone on the microscope slide obliquely from above, permitting observation at 400x. Sperm (0.7 uL) was put on the slide and another drop or two of the salt diluent solution was located next to it. The cover slip was used to quickly mix the diluent and sperm and then cover the sample. The percentage of motile sperm was estimated visually and the duration of motility was noted with a timer. In this case motility was defined as any movement of the sperm. Sperm was taken from four individual males since the milt was not pooled for fertilization of the eggs. The drop of sperm for testing was taken from either the fin of the fish, the side of the pan, or on the metal screen that kept feces out, and transferred to a microcentrifuge tube. Air temperature was 8 to 9 C, so no chilling was needed. The tube was held by the rim to prevent heating of the sample by body heat. The percentage of motile sperm for the four males was 15, 90, 40, and 100%; the duration of motility was 4:32, 2:29, 1:27, and 4:27 min. The results indicated that there was a wide range of sperm motility among individual males, underscoring the need to use multiple males in the spawning process. There was sufficient motility to indicate that problems related to sperm are not a major concern for the egg take at Dougherty Basin.

Eric Wagner and Ronney Arndt

Other News . . .

Vaccine Project Begins at State Hatcheries

Bacterial coldwater disease (BCWD) is an annual problem at Utah hatcheries. The disease, caused by *Flavobacterium psychrophilum*, presents in two main forms: 1) rainbow trout fry syndrome (RTFS), and 2) peduncle disease. In an effort to reduce the prevalence of RTFS (i.e. reduce host susceptibility) in our triploid Sand Creek strain of rainbow trout (RTSC) we have contracted with Novatis Aquahealth, LTD to produce a whole-cell formalin-killed autogenous bacterin from a strain of *F. psychrophilum* found at a Utah hatchery and stored at the Fisheries Experiment Station. Three state hatcheries were selected using historical occurrence of disease, water temperature and culture of RTSC. Fifty-day-old rainbow trout from the vaccinate groups will be immersed in a 1/10 solution of bacterin for one minute and a booster applied 14 days later. To simulate normal production, the control groups will not be sham vaccinated, but will otherwise be treated the same as vaccinates. The longevity of protection for an immersion bacterin is fairly short lived (≤ 3 months). If RTFS occurs by the end of three months, the field trial will be evaluated by comparing the mortality and disease occurrence between hatcheries. If no epizootic occurs, 60 fish will be lethally sampled per treatment group and prevalence evaluated by inoculating kidney or brain tissue onto selective agar, and the production lot histories for each treatment will also be compared. Remember that disease management is accomplished through controlling the above three factors related to disease occurrence. By reducing host susceptibility by vaccination we hope to reduce or eliminate RTFS epizootics. *David Thompson*

Fish Culture Class Planned for March 22 - April 2

Plans are underway to hold an intensive two-week fish culture class for new UDWR hatchery technicians, beginning March 2004 in Logan, Utah. The class is held periodically to provide newer employees with a basic education of various fish culture techniques, along with water quality and fish health concepts. In addition, this new class will include information on use of GPS, new hatchery technology such as low head oxygenation, genetics, and computer technology.

A number of instructors will participate in teaching the course, including Tim Miles, Doug Routledge, Patrick Goddard, Anna Miller, David Thompson, Quentin Bradwisch, Ronney Arndt, Joe Valentine, Gordon Nelson, Eric Wagner and Chris Wilson.

Although the course if designed primarily for UDWR fish culture workers, consideration is being given to extending an invitation to a limited number of University, federal or tribal members as well.

Evaluation of Induced Spawning Techniques and Requirements in Captive June sucker (*Chasmistes liorus*)

Introduction

The June sucker (*Chasmistes liorus*) is an endangered fish species endemic to Utah Lake, Utah. A recovery program has been implemented with a goal of propagating captive brood stock for subsequent stocking of progeny into Utah Lake. Spawning of captive brood stock has been induced at the Fisheries Experiment Station (FES), in Logan, Utah, but with limited success for female June sucker.

In previous spawning attempts ovulation rarely occurred without the use of human chorionic gonadotropin (HCG) (Mellenthin, 2002). In 2002 HCG dosage levels were evaluated; none of the injected females ovulated, however injected males expressed increased amounts of milt. Possible causes of no successful spawning in 2002, were an increase the water temperature and a change in diet prior to the spawning season (Mellenthin, 2002).

The purpose of the first part of this study was to evaluate the use of additional hormone types, dosages, and holding temperatures to induce ovulation with more consistent results for female June sucker (Rottmann et al., 1991a). The purpose of the second part of this study was to evaluate selection characteristics, injection locations, dosages and hormone types to induce higher levels of spermiation in males. Ovaprim is an analogue of salmon gonadotropin releasing hormone (sGnRHa) with a dopamine blocker (Syndel, 2003b).

Methods

Females of 1989 through 1994 year classes were selected for injections based on external characteristics: swollen vent and a soft rounded abdomen. Although these methods are subjective, more accurate methods have yet to be established in June suckers (Rottmann et al., 1991c). These selection characteristics were relative to the other fish in the individual holding tank. Attempts to sample eggs with a catheter in 2002 for less subjective selection of ripe females were not successful (Mellenthin, 2002). Fish were sorted for selection by characteristics June through September 2003; some tanks were sorted several times. All fish were anesthetized with MS-222 in a 1% salt bath for sorting, injections and monitoring (Piper et al., 1982; Rottmann et al., 1991b). Two experiments were conducted, one compared the effects of different hormones and dosages on ovulation and the second compared different temperatures. All females were given intraperitoneal (IP) injections of Ovaprim and/or HCG (Rottmann et al., 1991e). Experiment 1 used four different dosages: three fish at 0.5 ml Ovaprim/kg fish body weight (BW), two fish at 1.0 ml Ovaprim/kg BW, two fish at 0.5 ml Ovaprim+1000 international units (IU) HCG /kg BW, and three fish at 1.0 ml Ovaprim+1000 IU HCG /kg BW (Rottmann et al., 1991e; Syndel, 2003a). Three fish partially ovulated and were re-injected, after complete ovulation did not occur, with 1000 IU HCG/ kg BW in an attempt to produce FES progeny lots. Injected fish were monitored for ovulation once a day for up to ten days (Syndel, 2003c). Re-injected fish were monitored additional days. The fish were held on 56° F water for four months prior to injections. Experiment 2 used fish from three temperature regimes: 1) 56° F since February 2003 (2 fish), 2) 56° F for 1 week prior to first sorting for injections from 64° F (3 fish), and 3) 64° F water for over a year (17 fish). All fish were injected with 1.0 ml Ovaprim+1000 IU HCG /kg BW. Two fish from Experiment 1 injected with 1.0 ml Ovaprim+1000 IU HCG /kg BW were included as replicates in this experiment.

Males of 1989 through 1995 year classes were selected and injected during the months of June and July according to three protocols. Prior to and during the spawning season all of the sorted and

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injected males were held on 64° F water. Males in all protocols were monitored for an increased amount of milt extruded for up to 7 days. A total of fifteen males were injected in all three protocols. Protocol 1: Males were selected if a small amount of milt was extruded at sorting. Six males were injected at 500 IU HCG/kg BW up to three times, but only once per day (if an increased amount of milt was extruded at second day of monitoring the third injection was cancelled). Three males received IP injections and three received intramuscular (IM) injections (Rottmann et al., 1991e). This is the same protocol used in previous years except for the comparison of the performance of IM injections (Mellenthin, 2002). Protocol 2: four fish were selected for injections, two of which extruded a small amount of milt and two had a high number of tubercles on the anal fin and did not give milt at sorting. One fish of each selection characteristic was injected with 1.0 ml Ovaprim/kg BW and 750 IU HCG/kg BW (JSRIP, 2003; Syndel, 2003c). All injections were one time only and IP. Protocol 3: five fish were selected for injections, all with a high number of tubercles on the anal fin relative to the other fish in the tank without giving milt at sorting. Three fish were injected with 1.0 ml Ovaprim/kg BW and two with 750 IU HCG/kg BW, alternating IP and IM sites for each type of hormone. All injections were one time only.

When appropriate crosses of ripe females and males were available, eggs were fertilized according to the following spawning procedure:

The male and female are anesthetized with MS222 in a 1% salt bath. The vent area on both fish is patted dry prior to stripping (Piper et al., 1982; Rottmann et al., 1991c). The milt is stripped into a 50 ml centrifuge tube containing a small amount of tempered Hank's Balanced Salt Solution (HBSS); if necessary, additional HBSS is added for an equal volume of HBSS and milt. The females' eggs are stripped into a Ziploc bag (quart or gallon) containing tempered HBSS, enough to cover the expected amount of eggs. The HBSS is then drained and the milt is added along with water for activation and mixed by gently rocking the bag for 3 minutes. The water is poured off and the fertilized eggs are rinsed several times (24 Roads Hatchery staff, personal communication). A tempered bentonite solution (50 grams/liter water) is added to the eggs for 15 minutes while occasionally gently rocking the bag (Rottmann et al., 1991f). Eggs are rinsed thoroughly, then water is added and water hardening occurs for an additional 45 minutes in tempered water (Piper et al., 1982).

The data was analyzed using SPSS. Chi squared tests using maximum likelihood ratios were used to analyze paired treatments in Experiment 2. The level of significance 0.05 was used for all tests. Experiment 1 was not statistically analyzed due to the lack of replication in the treatments, and due to the alteration of the treatments with the re-injections.

Results

A total of 30 females were injected with complete ovulation occurring in six fish and partial ovulation occurring in three other fish. In Experiment 1, ten fish total were injected; four fish completely ovulated and three fish partially ovulated. At 0.5 ml Ovaprim/kg BW, two fish experienced partial ovulation. Complete ovulation occurred in one fish at the 1.0 ml Ovaprim/kg BW, one fish at the 0.5 ml Ovaprim+1000 IU HCG/kg BW, and two fish at the 1.0 ml Ovaprim+1000 IU HCG/kg BW (Table 1). Partial ovulation occurred in one fish injected at 1.0 ml Ovaprim+1000 IU HCG/kg BW. One of three fish re-injected experienced partial ovulation again; the fish was initially injected with 0.5 ml Ovaprim/kg BW. In Experiment 2, twenty-two injected fish were used. Complete ovulation occurred in both fish at 56° F and in two fish at 64° F. Ovulation did not occur in any fish on the 64 F - 56° F regime. There was a significant difference in the number of fish that completely ovulated in 56° F regime than the other two temperatures. There was not a significant difference between the 64 F - 56° F and the 64° F regimes (Table 2). Complete ovulation occurred in 5 fish from the 1991 year class and

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1 fish from the 1994 year class. Partial ovulation occurred in one fish from each of the 1989, 1992, and 1994 year classes. The vents on the females held at 56° F for four months appeared to be swollen and distended to a greater extent than the females on the other temperature regimes.

Table 1. Experiment 1, percent occurrence of partial and complete ovulation of female June sucker held at 56° F by dosage type and level.

	No Ovulation		Partial Ovulation		Complete Ovulation	
Dosage	#	%	#	%	#	%
0.5 ml Ovaprim	1	33.33	2	66.67	0	0.00
1.0 ml Ovaprim	1	50.00	0	0.00	1	50.00
0.5 ml Ovaprim + 1000 IU HCG	1	50.00	0	0.00	1	50.00
1.0 ml Ovaprim + 1000 IU HCG	0	0.00	1	33.33	2	66.67

Table 2. Experiment 2, percent occurrence of ovulation with the 1.0 ml Ovaprim + 1000 IU HCG dosage level at three temperature regimes. Matching subscripts among treatment percentages depict no significant difference between treatments. * Fish at 56° F were used as replicates in Experiment 1.

	No Ovulation		Complete Ovulation	
Temperature Regime	#	%	#	%
56° F*	0	0.00	2	100.00 $_z$
64° F - 56° F	3	100.00	0	0.00 $_y$
64° F	15	88.24	2	11.76 $_y$

With the male injections twelve of the fifteen males increased the amount of milt expelled. In Protocol 1 three fish had increased milt levels on day 2 and only received two injections, two were IP injections and one was IM. On day 3 the remaining three fish had an increase in the amount of milt stripped. In Protocol 2 an increased amount of milt was stripped on the fourth and fifth day after injections in all four fish. In Protocol 3 none of the fish injected with Ovaprim gave milt, but both HCG injected fish released milt. One fish expressed milt the first day after injections and the other fish expressed a limited amount on the second day and an increased amount on the third day. All year classes injected had at least one fish express milt. Males injected intramuscularly with Ovaprim developed blackened areas at injection sites.

Three crosses were made from the injected males and females. Cross 1 used a 1.0 ml Ovaprim+1000 IU HCG/kg BW injected female with a male injected two times at 500 IU HCG/kg BW, producing 6875 eggs with a 56.07% survival to swim up. Cross 2 used a 0.5 ml Ovaprim+1000 IU HCG/kg BW injected female with a male injected two times at 500 IU HCG/kg BW, producing 7497 eggs with a 29.60% survival to swim up. Cross 3 used a 1.0 ml Ovaprim/kg BW injected female with a male injected three times at 500 IU HCG/kg BW, producing 8162 eggs with a 64.70% survival to swim up (Table 3).

Table 3. Comparison of percent survival to swim up among three hatchery crosses and three crosses received from wild stock in 2003.

Cross mumber	Number of eggs	Number of fish on feed (swim up)	Percent survival to swim up
Hatchery Cross 1	6875	3855	56.07
Hatchery Cross 2	7497	2219	29.60
Hatchery Cross 3	8162	5281	64.70
Wild Cross 1	23750	9371	39.46
Wild Cross 2	32487	17391	53.53
Wild Cross 3	31529	1832	5.81

*Two lots received from wild stock were not included in this comparison since they were discarded prior to projected hatch date due to poor egg quality and condition.

Conclusions

The results from the second experiment on female June sucker show that there is a significant difference in the effect in holding fish in 56° F water for an extended period of time compared to a short exposure to this temperature or 64° F water on complete ovulation. The various dosage levels all resulted in partial or complete ovulation. Seventy percent of the fish in 56° F water had a degree of ovulation occur indicating that temperature effects might be more important than dosage level, though 1.0 ml Ovaprim + 1000 IU HCG/kg BW is the only dosage at which every female ovulated. The success with 1.0 ml Ovaprim + 1000 IU HCG/kg BW at 56° F, and that prior to 2002, spawning was induced with HCG in 60° F water; indicates temperature effects should be investigated with 1.0 ml Ovaprim +1000 IU HCG/kg BW before hormone types and dosage levels are evaluated further. The results showed that both IP and IM injections are both effective locations for producing milt with 500 and 750 IU HCG. The selection characteristic using tubercles is effective for producing milt with 750 IU of HCG. The use of Ovaprim showed promise with IP injection of males, which expressed milt at sorting, but milt production was poor from males injected intramuscularly. Fish with tubercles only were not as effective for producing milt with the Ovaprim injections. The success with HCG in this study and in previous years indicates that future injections should use HCG and not Ovaprim. The different methods with HCG should be evaluated further by comparing volume or condition of the milt in relation to fish length and or weight. The use of hormones and the procedure for spawning captive June sucker is effective when comparing the percent hatch to eggs received from wild stock of June sucker. *Eriek Hansen*

Literature Cited

24 Road Hatchery Staff. 2003. Personal Communication. U.S. Fish and Wildlife Service, Grand Junction, Colorado. June Sucker Recovery Implementation Program (JSRIP). February 25, 2003. Native aquatic species culture workshop. Salt Lake City, Utah.

Mellenthin, R. 2002. 2002 HCG trials. Utah Division of Wildlife Resources: Fisheries Experiment Station. Logan, Utah.

Piper, R.G., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G., Leonard, J.R. 1982. Fish Hatchery Management. United States Department of the Interior, Fish and Wildlife Service. Washington, D.C.

Rottmann, R.W., Shireman, J.V., Chapman, F.A. November 1991a. Introduction to hormone-induced spawning of fish. Pub. No. 421. Southern Regional Aquaculture Center.

Rottmann, R.W., Shireman, J.V., Chapman, F.A. November 1991b. Capturing, handling, transporting, injecting and holding brood fish for induced spawning. Pub. No. 422. Southern Regional Aquaculture Center.

Rottmann, R.W., Shireman, J.V., Chapman, F.A. November 1991c. Determining sexual maturity of broodstock for induced spawning of fish. Pub. No. 423. Southern Regional Aquaculture Center.

Rottmann, R.W., Shireman, J.V., Chapman, F.A. November 1991d. Hormonal control of reproduction in fish for induced spawning. Pub. No. 424. Southern Regional Aquaculture Center.

Rottmann, R.W., Shireman, J.V., Chapman, F.A. November 1991e. Hormone preparation, dosage calculation, and injection techniques for induced spawning of fish. Pub. No. 425. Southern Regional Aquaculture Center.

Rottmann, R.W., Shireman, J.V., Chapman, F.A. November 1991f. Techniques for taking and fertilizing the spawn of fish. Pub. No. 426. Southern Regional Aquaculture Center.

Syndel International Inc. 2003a. Ovaprim info sheet. http://www.syndel.com/ spawning/ovaprim_information_sheet.html.

Syndel International Inc. 2003b. Using Ovaprim to induce spawning in cultured fish. http://www.syndel.com/spawning/using_ovaprim.html.

Syndel International Inc. 2003c. Induced spawning of cultured fish using Ovaprim. http://www.syndel.com/spawning/ovaprim_product_ information.html.

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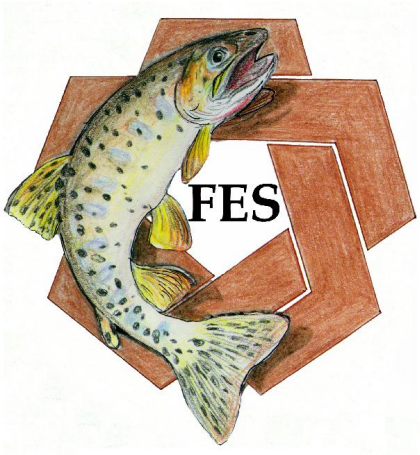

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