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

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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 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 1 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 fifteen 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. An independent sample T-test was used to analyze the average percent survival to swim up between hatchery stock and wild stock crosses. 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 1 fish from the 1994 year class. Partial ovulation occurred in one fish from 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.

	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 $_y$

* Fish at 56° F were used as replicates in Experiment 1.

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). There was not a significant difference in the average percent survival to swim up between hatchery and wild crosses.

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

Cross Number	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 at is the only dosage 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 weight 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 not detrimental to the progeny when the average percent survival to swim up compared to groups received from wild stock of June sucker.

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