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

## Introduction

The June sucker (*Chasmistes liorus*) is an endangered species of fish endemic to Utah Lake, Utah. A recovery program has been implemented with a goal of propagating captive brood stock for stocking 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 females ovulated however injected males expressed increased amounts of milt. Prior to the spawning season the water temperature was increased and the diet was changed (Mellenthin, 2002).

The purpose of this study was to evaluate the use of an additional hormone, Ovaprim, and water temperatures to induce ovulation with more consistent results (Rottmann et al., 1991A). Ovaprim is an analogue of salmon gonadotropin releasing hormone (sGnRHa) with a dopamine blocker (Syndel, 2003B).

## Methods

Females 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). Attempts to sample eggs with a catheter in 2002 for less subjective selection of ripe females were not successful (Mellenthin, 2002). These characteristics were relative to the other fish in the individual holding tank. Fish were sorted for selection June through September 2003 some tanks were sorted several times. All females were given intraperitoneal injections of Ovaprim and HCG (Rottmann et al., 1991E). Five different dosages were used, 0.5 ml's Ovaprim/kg fish body weight (BW), 1.0 ml's Ovaprim/kg BW, 0.5 ml's Ovaprim+1000 international units (IU's) HCG /kg BW, 1.0 ml's Ovaprim+1000 IU's HCG /kg BW, and 1000 IU's HCG/ kg BW (Rottmann et al., 1991E; Syndel, 2003A). The 1000 IU's HCG/ kg BW was only used on re-injections of fish, which partially ovulated. 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 selected from 3 temperature regimes, 56 degrees Fahrenheit (F) since February 2003, 56 degrees F for 1 week prior to first sorting for injections, and 64 degrees F water. 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).

Males were selected and injected during the months June and July according to three protocols. Prior to and during the spawning season all of the

sorted and injected males were held on 65 degree F water. Males in all protocols were monitored for an increased in the amount of milt extruded for up to 7 days. Protocol 1: Males were selected if a small amount of milt was extruded at sorting. All 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). Injections were either intraperitoneal or intramuscular (Rottmann et al., 1991E). Protocol 2: Males were selected if a small amount of milt was extruded at sorting or if a high amount of tubercles were found on the anal fin, relative to the other fish in the tank without giving milt. All injections were one time only and intraperitoneal at dosages of 750 IU's HCG/kg BW or 1.0 mls Ovaprim/kg BW (JSRIP, 2003; Syndel, 2003C). Protocol 3: Males were selected if a high number of tubercles were found on the anal fin, relative to the other fish in the tank without giving milt. All injections were 1 time only and either intraperitoneal or intramuscular, with dosages of 750 IU's HCG/kg BW or 1.0 mls Ovaprim/kg BW.

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

> The male and female are anesthetized in water with a 1% salt with MS222, 4-5ml/gallon of water. 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 50ml 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 female is 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 bag (Rottmann et al., 1991F). Eggs are rinsed thoroughly and then water is added and water hardening occurs for and additional 45 minutes in tempered water (Piper et al., 1982).

**Results**

A total of 30 females were injected with complete ovulation occurring in six fish and partial ovulation occurring in three fish (Table 1). The four fish with complete ovulation and the three fish with partial ovulation had been on coldwater since February. Complete ovulation occurred in 2 fish held in warm water. Partial and complete ovulation occurred at three of the dosage levels (Table 2). At 0.5 mls Ovaprim/kg BW two fish experienced partial ovulation. Complete ovulation occurred in one fish at the 1.0 mls Ovaprim/kg BW, one fish at the 0.5 mls Ovaprim+1000 IU's HCG/kg BW, and two fish at the 1.0 mls Ovaprim+1000 IU's HCG/kg BW. One out of three fish re-injected experience

partial ovulation again. 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. Percent occurrence of partial and complete ovulation in injected fish by temperature regime.

| | | Partial Ovulation | | Complete Ovulation | |
|---|---|---|---|---|---|
| Temperature | # Injected | # | % | # | % |
| 56° F 4 months | 10 | 3 | 30.00% | 4 | 40.00% |
| 56° F 1 week | 3 | 0 | 0.00% | 0 | 0.00% |
| 64° F | 17 | 0 | 0.00% | 2 | 11.76% |
| Total | 30 | 3 | 10.00% | 6 | 20.00% |

Table 2. Percent occurrence of partial and complete ovulation in injected fish by dosage level.

| | | Partial Ovulation | | Complete Ovulation | |
|---|---|---|---|---|---|
| Dosage | # Injected | # | % | # | % |
| 0.5 mls/kg | 4 | 2 | 50.00% | 0 | 0.00% |
| 1.0 mls/kg | 4 | 0 | 0.00% | 1 | 25.00% |
| 0.5 mls+1000 IU's/kg | 3 | 0 | 0.00% | 1 | 33.33% |
| 1.0 mls+1000 IU's/kg | 19 | 1 | 5.26% | 4 | 21.05% |

A total of fifteen males were injected in all three protocols. Protocol 1 injected six males, three were intraperitoneal and three were intramuscular. Three fish had increased milt levels on day 2 and only received two injections, two were intraperitoneal injections and one was intramuscular. On day 3 the remaining three fish had an increase in the amount of milt stripped. Protocol 2 used four fish for injections, two extruded a small amount of milt and two had a high number of tubercles on the anal fin at sorting. Injected one fish of each selection description with 1.0 mls Ovaprim/kg BW and the opposite selections with 750 IU's HCG/kg BW. An increased amount of milt was stripped on the fourth and fifth day after injections. Protocol 3 used five fish for injections, all five fish were selected by a high number of tubercles were found on the anal fin. Three fish were injected with 1.0 mls Ovaprim/kg BW and two with 750 IU's HCG/kg BW, alternating intramuscular and intraperitoneal sites for each type of hormone. None of the fish injected with Ovaprim gave milt, but both HCG injected fish released milt. One fish gave milt the first day after injections and the other fish gave a limited amount on the second day and an increased amount on the third day. Males injected intramuscularly with Ovaprim developed blackened areas at injection sites.

Three crosses were made from the injected males and females. Cross 1 used 1.0 mls Ovaprim+1000 IU's 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 0.5 mls Ovaprim+1000 IU's 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 1.0 mls 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 of crosses 1-3 versus the sum percent survival to swim up of three lots of eggs received from wild stock in 2003.

| Cross Number | Number of Eggs | Number of fish on Feed (swim up) | Percent survival to swim up |
|---|---|---|---|
| Cross 1 | 6875 | 3855 | 56.07% |
| Cross 2 | 7497 | 2219 | 29.60% |
| Cross 3 | 8162 | 5281 | 64.70% |
| Sum of 3 lots received | 87766 | 28594 | 32.58% |

## Conclusions

## Literature Cited


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