Temperature Requirements of Captive June sucker (*Chasmistes liorus*) in Combination with Hormones to Stimulate Spawning.

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Introduction

This study is a continuation of research evaluating the requirements for inducing spawning in captive June sucker (*Chasmistes liorus*), an endangered fish species endemic to Utah Lake, Utah. Spawning research is being conducted to meet a recovery goal of propagating captive broodstock for subsequent stocking of progeny into Utah Lake.

Hormones have been used in the past to compensate for missing environmental stimuli cueing egg development and ovulation (Rottmann et al., 1991d). The use of hormones alone has not been completely successful; a previous study conducted by Hansen (2003) indicated that there is a significant higher ovulation frequency for females cultured on water colder than the current temperature of 65° Fahrenheit (F). The goal of this study was to determine the length of time needed in coldwater to facilitate the induction of ovulation in conjunction with hormones. Two hormones were utilized, Ovaprim, an analogue of salmon gonadotropin releasing hormone (sGnRHa) with a dopamine blocker manufactured by Syndel International Inc. (Syndel, 2003b, c), and Chorulon®, a human chorionic gonadotropin (HCG) manufactured by Intervet Inc. (Intervet, 2004).

Methods

Females were selected and injected in three rounds. Females in Round 1 were selected from ten lots (sibling groups) in the 1989 through 1995 year classes, for ten treatments of temperature regimes (Table 1.). Females in treatments 1-3 were fish that did not ovulate in previous studies, and were still being held in 56 F water. The previous studies used external characteristics: vent and anal fin size to determine gender and a swollen vent with a soft rounded abdomen to select females with a greater spawning potential. Treatments 4-10 were selected by lot to obtain equal ratios in treatments and by external characteristics to determine gender, but not spawning potential.

Table 1. Temperature regimes for culturing female June sucker by treatment.

Treatment	Name	# Fish	Temperature Regime
1	16-months	2	56° F 16 months prior to injections
2	12-months	4	56°F 12 months prior to injections
3	8.5-months	8	56°F 8.5 months prior to injections
4	6-months	10	56°F 6 months prior to injections
5	5-months	9	56°F 5 months prior to injections
6	4-months	10	56°F 4 months prior to injections
7	6-months/ 1-week	5	56°F 6 months followed by 62° F 1 week prior to injections
8	5-months/ 1-week	5	56°F 5 months followed by 62° F 1 week prior to injections
9	4-months/ 1-week	4	56°F 4 months followed by 62° F 1 week prior to injections
10	Warm-water	17	65°F > 1 year prior to injections

Females were injected during the second week of June, the time period when fish in Utah Lake have been observed spawning (Shirley, 1983; Sigler and Sigler, 1996). All fish were anesthetized using MS-222 in a 1.0 % salt bath during handling (Piper et al., 1982; Rottmann et al, 1991b,c). Injections were intraperitoneal (IP) and each hormone was administered separately but during the same handling sequence (Rottmann et al., 1991e). The Ovaprim dosage was one-milliliter (ml) hormone per kilogram (kg) of fish body weight (BW), (Syndel, 2003a). The Chorulon® dosage was 1000 international units (IU) hormone per kg BW. Females were checked daily for up to ten days post injection for ovulation. Females that partially ovulated were monitored with the remaining fish. Injections for Round 2 occurred during the fourth week of June, and consisted of females that did not ovulate in Round 1, but exhibited the external characteristics: a swollen vent with a soft rounded abdomen; hence a greater spawning potential. These fish were injected again with 1000 IU Chorulon® per kg fish BW. Females were checked daily for up to ten days including the day of injection for ovulation. Injections for Round 3 occurred during the final week of June, and consisted of females from round two that had partially ovulated in either of the two previous rounds. Fish in Round 3 were injected again with 1000 IU Chorulon® per kg fish BW on day six and seven of Round 2 and monitored until the ten days of Round 2 had expired.

Males were selected from the same ten lots as the females, if a small amount of milt was expressed when massaged. Injections were IP and 500 IU Chorulon® per kg fish BW. Two consecutive injections were given twenty-four hours apart, and if the amount of milt did not increase by the second injection a third injection was administered after twenty-four additional hours (Hansen, 2003). Males were divided into two groups to correspond with females in the follow up rounds. Group 1 consisted of 19 fish that were injected during the second week of June, and group 2 consisted of 18 fish injected during the fourth week of June.

Crosses were made when eggs from females and milt from males from the appropriate lots were available. Eggs were fertilized according to the following procedure:

The male and female are anesthetized with MS222 in a 1% salt bath (Piper et al., 1982; Rottmann et al, 1991b,c). 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 Cellgro® Hank's Balanced Salt Solution 1X without calcium and magnesium (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 a tempered 0.75% rock salt diluent for activation described by Arndt (2002); the eggs are 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., 1991a,f). Eggs are rinsed thoroughly, then water is added and water hardening occurs for an additional 45 minutes in tempered water (Piper et al., 1982).

A swollen protruding vent along with a soft rounded abdomen has been used to select fish for spawning potential, although these methods can be very subjective (Rottmann et al., 1991c). To determine if vent characteristics are related to spawning potential in captive female June sucker measurements of the vent length and width were taken. Measurements of the vent were taken prior to injections, during the same handling time injections occurred. Vent measurements were taken post injection when a female readily expelled eggs; measurements were prior to stripping the female of eggs. Post injection measurements were not taken on females that did not expel eggs.

The data was analyzed using SPSS® (SPSS®, 1993). Chi-square tests using maximum likelihood ratios were used in comparing the presence/absence of ovulation and the level of ovulation (no, partial and complete). Variables with a significant difference were subsequently analyzed in paired treatments (partial tables) with chi-square maximum-likelihood ratio statistics. An independent sample T-test was used to compare the pre-injection vent measurements between fish that ovulated and did not ovulate. A T-test for paired (dependent) samples was used to compare vent measurements of females that ovulated pre and post injection. The level of significance 0.05 was used for all tests.

Results

A total of seventy-four females were injected and monitored, fourteen fish partially ovulated and nineteen fish completely ovulated for a total of forty-five percent ovulation. Fifty-seven fish were on temperature regimes involving cold-water in which thirteen fish partially ovulated and nineteen fish completely ovulated for a total of fifty-six percent ovulation (Table 2). The daily monitoring in Round 1 of females post injection showed that ovulation did not occur until day three. Complete ovulation ended by day seven, and did not occur in fish held in warm-water, though partial ovulation continued through the last day of monitoring for fish in cold-water (Figure 1). In Round 2 cold-water fish ovulated beginning on day 2 and continued through day 6. Partial ovulation occurred on days 3 and 6 in the cold to warm-water treatments, and no ovulation occurred in the fish held in warm water. Ovulation occurred on all four days of Round 3, but only one fish completely ovulated.

Table 2. Summary of fish numbers in ovulation levels by treatment.

Treatment	No Ovulation	Partial Ovulation	Complete Ovulation	Total
16-months	0	1	1	2
12-months	2	1	1	4
8.5-months	2	3	3	8
6-months	2	2	6	10
5-months	5	1	3	9
4-months	8	2	0	10
6-months/1-week	2	1	2	5
5-months/1-week	1	1	3	5
4-months/1-week	3	1	0	4
Warm-water	16	1	0	17
Total	41	14	19	74
Percentages	55%	19%	26%	100%

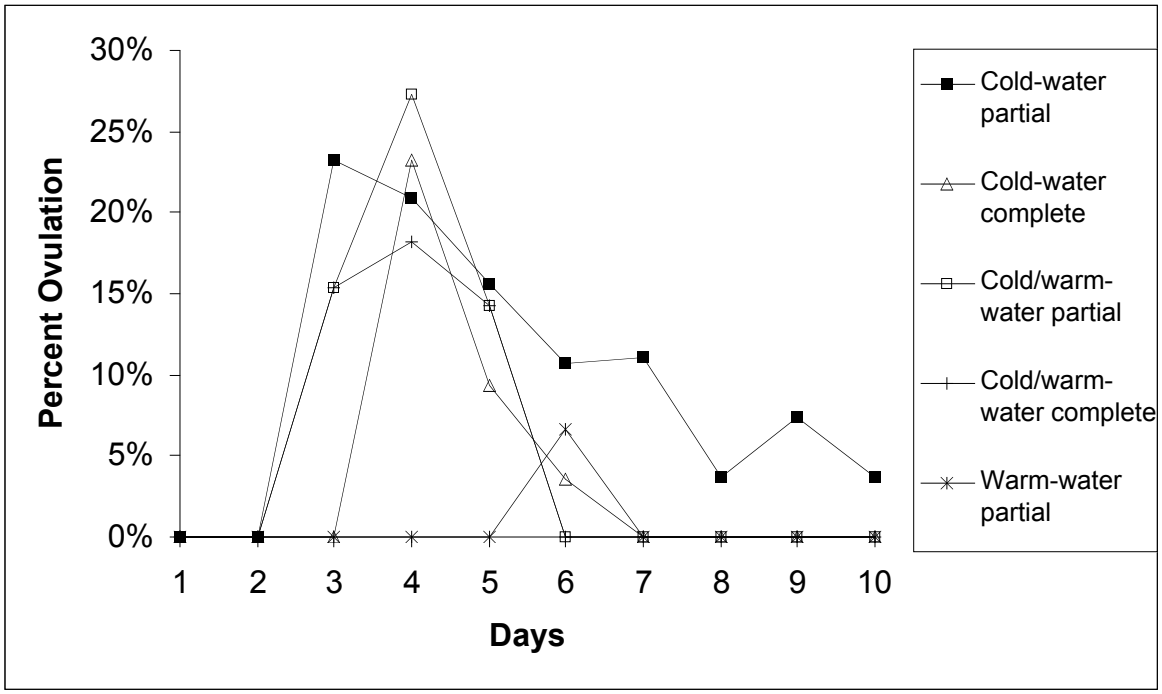

Figure 1. Daily percentage of females that partially and completely ovulated during Round 1 monitoring period following hormone injections.

A significant difference for ovulation levels between treatments was only found in Round 1 where 47.3% of females either partially or completely ovulated, whereas in Round 2, 12.5% ovulated and Round 3, 20.0% ovulated. In Round 1 the percentage of ovulation ranged from 100.0% in the 16-months treatment to 5.9% in the warm-water treatment with a significantly higher occurrence from all of the treatments except the 4-months/1-week treatment than the warm-water treatment. Significant differences were also found between treatments comparing paired ovulation levels. Comparing partial

versus no ovulation the percent partial ovulation ranged from 100.0% in the 16-months treatment to 5.9% in the warm-water treatment. Treatments 16-months, 8.5-months and 6-months had a significantly higher occurrence of partial ovulation than the warm-water treatment. Comparing complete versus no ovulation the percent complete ovulation ranged from 100.0% in the 16-months treatment to 0.0% in the 4-months, 4-months/1-week and warm-water treatments. Complete ovulation had a significantly higher occurrence in the 16-months, 8.5-months, 6-months and 5-months/1-week treatments than in the 4-months, 4-months/1-week and warm-water treatments. Comparing complete versus partial ovulation the percent complete ovulation ranged from 75.0% in the 6-months, 5-months and 5-months/1-week treatments to 0.0% in the 4-months, 4-months/1-week and warm-water treatments. The percent complete ovulation was significantly higher in the 6-months treatment than in the 4-months treatment (Table 3).

Table 3. Comparison of grouped ovulation levels by treatment. Matching subscripts within columns depict no significant difference.

Treatment	Ovulation (partial/ complete) vs. no ovulation	Complete vs. partial vs. no ovulation	Partial vs. no ovulation	Complete vs. no ovulation	Complete vs. partial ovulation
16-months	100.0%$_{z}$	50.0%$_{zy}$	100.0%$_{z}$	100.0%$_{z}$	50.0%$_{zy}$
12-months	50.0%$_{z}$	25.0%$_{zyxw}$	33.3%$_{zy}$	33.3%$_{zyx}$	50.0%$_{zy}$
8.5-months	75.0%$_{z}$	37.5%$_{zyx}$	60.0%$_{z}$	60.0%$_{z}$	50.0%$_{zy}$
6-months	80.0%$_{z}$	60.0%$_{z}$	50.0%$_{z}$	75.0%$_{z}$	75.0%$_{z}$
5-months	44.4%$_{z}$	33.3%$_{zyx}$	16.7%$_{zy}$	37.5%$_{zy}$	75.0%$_{zy}$
4-months	40.0%$_{z}$	0.0%$_{xw}$	20.0%$_{zy}$	0.0%$_{xw}$	0.0%$_{y}$
6-months/ 1-week	60.0%$_{z}$	40.0%$_{zyx}$	33.3%$_{zy}$	50.0%$_{zy}$	66.7%$_{zy}$
5-months/ 1-week	80.0%$_{z}$	60.0%$_{zy}$	50.0%$_{zy}$	75.0%$_{z}$	75.0%$_{zy}$
4-months/ 1-week	25.0%$_{zy}$	0.0%$_{yw}$	25.0%$_{zy}$	0.0%$_{yw}$	0.0%$_{zy}$
Warm-water	5.9%$_{y}$	0.0%$_{w}$	5.9%$_{y}$	0.0%$_{w}$	0.0%$_{zy}$

In Group 1 all nineteen males had an increase in the amount of milt expelled. Seven of the fish required and received a third injection. In Group 2 seventeen of eighteen males had an increase in the amount of milt expelled. Only one male required a third injection; milt was being expressed but not at the expected increased level. This male was not administered the third injection though, because a sufficient number of males were available. Even though milt amounts increased with injections milt was not always available for a specific cross; during handling looking for crosses males would often expel their milt prior to collection.

In the comparison of vent lengths and widths between fish that ovulated and fish that did not ovulated there was no significant difference for either variable. The vent measurements for fish that ovulated was a mean length of 15.2 mm, and a mean width of 9.1 mm, and for fish that did not ovulate was a mean length of 12.8 mm, and a mean

width of 7.9 mm (Table 4). There was a significant difference in both vent length and widths for pre versus post injections for fish that ovulated with a mean length difference of 2.9 mm (2.0 S.D.) and a mean width difference of 1.4 mm (1.2 S.D.).

Table 4. Summary of vent mean lengths and mean widths prior to hormone injections for ovulating and non-ovulating female June sucker.

Treatment	Ovulating fish	Non-ovulating fish
Vent Length (mm)	15.2	12.8
Standard Deviation	2.9	3.0
Vent Width (mm)	9.1	7.9
Standard Deviation	1.7	1.6

In total only eight crosses were made due to hatching/rearing space restraints from the fish injected for production. The numbers of eggs ranged from 5,150 in cross 6 to 16,660 in cross 5. Percent survival to first feeding ranged from 50% in cross 1 to 0% in crosses 2 and 5 (Table 5). Crosses 2 and 5 were discarded due to poor fertilization and low numbers of viable eggs likely due to the small amount of milt available. The milt used for fertilization of cross 6 was held overnight in HBSS. Only a small amount of milt was available for crosses 7 and 8.

Table 5. Summary of egg numbers and survival to initial feeding by cross.

Cross	1	2	3	4	5	6	7	8	Total
Number of Eggs	6750	12138	11067	13423	16660	5150	8330	11875	85393
Number on Feed	3344	0	4108	5623	0	968	520	505	15068
Percent Survival	50%	0%	37%	42%	0%	19%	6%	4%	18%

Conclusions

The results of the study confirm that exposure to colder water is necessary to promote egg development in order to stimulate ovulation with hormones. The data also shows that there was not a significant difference in the amount of ovulation for fish held in colder water five months and longer. Though fish in the 4-months treatment were not significantly different in the ovulation versus no ovulation comparison, complete ovulation did no occur in the treatment. Fish that have been on colder water for a sufficient time prior to being moved to warm-water did not result in a significant difference in the occurrence of ovulation. A period of six months in 56° F water is likely the preferred option; it allows sufficient stimuli for egg development and allows a warmer temperature the remainder of the year to improve overall fish condition. Nineteen percent of the fish only partially ovulated; the reason for this is unknown. A possible remedy is to use multiple doses or an initial dose and a resolving dose. Split doses have been beneficial in other species of fish. Using Ovaprim, some catfish require an initial dose with a resolving dose a couple of hours later, where as some salmonids require the initial and resolving doses a few days apart (Syndel, 2003a). It has been

recommended when using Chorulon®on walleye (*Stizostedion canadense*), to use one to three injections seventy-two hours apart (Intervet, 2004).

Vent measurements prior to injections are not an effective method to determine the spawning potential for June sucker. Post injection measurements were not taken on fish that did not ovulate for comparison to measurements of those that did ovulate; this might be useful information to obtain in future studies to determine spawning potential or candidates for re-injection. The procedure for increasing milt amounts meets current needs, though a method needs to be developed to reduce milt loss prior to harvest. Additional benefits could be gained through continued development of milt extenders. Several lots had poor survival and hatch rates due to low fertilization. In an attempt to increase fertilization, milt percent motility and duration should be observed to determine quality prior to use. This information along with the percent eye-up will help determine egg quality and the ability to address any inconsistencies.

Literature Cited

24 Roads Hatchery Staff. 2003. Personal Communication. U.S. Fish and Wildlife Service, Grand Junction, Colorado.

Arndt, R. 2002. June sucker milt research-spring 2002. Ichthyogram 13-2:5-9. Utah Division of Wildlife Resources: Fisheries Experiment Station, Logan, Utah.

Hansen, E. 2003. Evaluation of induced spawning techniques and requirements in captive June sucker Chasmistes liorus. Ichthyogram 14-4:6-10. Utah Division of Wildlife Resources: Fisheries Experiment Station, Logan, Utah.

Intervet USA. 2004. Chorulon® Label Sheet. http://www.intervetusa.com/default.asp?C=7&SC=11&Sec=TechInfo&Summary=1&RecId=42.

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.

Shirley, D.L. 1983. Spawning ecology and larval development of the June sucker. Transactions of the Bonneville Chapter of the American Fisheries Society: 18-36.

Sigler, W.F., Sigler, J.W. 1996. Fishes of Utah: a natural history. University of Utah Press, Salt Lake City, Utah.

SPSS®. 1993. SPSS® base system syntax reference guide, release 6.0. SPSS Inc., Chicago, Illinois, USA.

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.