Induced and Synchronized Spawning of Captive Broodstock

Using Ovaplant and Ovaprim

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Abstract

In a large population of salmon, maturation occurs at different rates and fish spawn at different times. In some cases, spawning dates within a group of fish may be spread over months. In these fish, it would be a distinct advantage to synchronize and compress the spawning season. In other circumstances, it is desirable to have fish mature ahead of cohorts. This advanced maturation provides greater flexibility in the hatchery and seawater entry dates. As well, early gametes permits the out-crossing of different strains. With regard to alternate species, several obstacles to research and development could be overcome if fish spawned in a predictable fashion. Under ESC (Health Canada) approval we have developed a method to advance and synchronize maturation in captive broodstock. In controlled studies and trials conducted in B.C., New Brunswick and Chile, coho, chinook and Atlantic salmon, trout and sablefish were induced to mature using peptide implants. In treated coho salmon (*Oncorhynchus kisutch*), spawning dates were significantly ($P<0.05$) advanced, the spawning season shortened, milt quality was increased and fry were quicker to first feeding. In several trials, this method has proven both effective and safe (for humans and fish). The implications of this technology for producers are profound in the development of a broodstock management programme.

Introduction

In the practice of inducing maturation of captive fishes, there are several different methods such as: injection of gonadotropin hormones (GTH) or pituitary extracts containing GTH[1], human chorionic GTH and gonadotropin-releasing hormones (GnRH). All of these methods either supply GTH, GTH-like peptides or elicit the liberation of native GTHs from the pituitary which then induce maturation[2]. Recently, hypothalamic hormones such as native GnRH or their analogues have gained favour among commercial fish producers. GnRH peptides are smaller, easier to prepare and are more effective at inducing maturation than other peptides. Because they are naturally occurring hormones that use the fishes own internal machinery, they are more reliable and are more potent without harmful side effects to the fish. As well, analogues of both the mammalian and salmon GnRH (sGnRHa) forms are more potent and degrade slower than natural hormones[3]. Methods for administration of GnRH peptides to fish include topical absorption[4], injection of soluble GnRH in vehicle, intubation[5] or in a sustained release preparation[6]. Of these methods, three are most commonly used: saline solutions of GnRH[7], injection of the commercial preparations that contain a salmon GnRH analogue such as Ovaprim[8] or sustained released pellets[2] (Ovaplant; Syndel Laboratories, Vancouver, B.C.). Pure synthetic GnRH analogues have been on the commercial market for over a decade, but require field preparation and refrigeration to prevent degradation. Preparations such as Ovaprim or Ovaplant are preferred because of handling and storage considerations without loss of efficacy.

In a study to demonstrate the efficacy of induced maturation using liquid injectable sGnRHa or implants that contain sGnRHa, coho salmon were either injected with Ovaprim, implanted with Ovaplant, or administered both treatments. To determine species differences, rainbow trout (*O. mykiss*) were treated with Ovaplant to induce spawning. The objective of this study was to determine whether treatment to induce spawning causes an increase in prespawn mortality, advances spawning date, compresses the spawning season and has any deleterious effect on the progeny. The study was conducted under controlled conditions and in a commercial production setting.

Methods and Materials

Fish

One month prior to normal spawning, 250 maturing male and female coho salmon (*O. kitsuch*) were transferred from seawater netcages to freshwater raceways at the Chiloe Aquaculture

Research Center, University of Chile, Castro, Chile. Four earthen raceways (4 X 2 X 25m) were divided in half by a screen placed mid way along the length to which 25 males and 25 females were randomly selected and placed in each section of the divided raceways. Fish were acclimated to the holding condition for two weeks at water temperatures of 8^{o}C and at flow rates sufficient to provide saturated levels of dissolved oxygen. After two weeks, groups fish were assigned treatment in the following manner: groups closest to the inlet were designated controls all other groups were randomly assigned treatments. Control fish were so designated to prevent possible effects of pheromones released from treated fish upstream. Group designations and treatments appear in Table 1. Average weight of coho was 3.5kg.
Trout (*O. mykiss*) were transferred from seawater netcages approximately 6 weeks prior to the normal spawning date. Fish were randomly placed in partitioned raceways to form two groups containing 32 fish each. Average weight of trout was 5kg.

Table 1: Group and treatment designations for fish.

Group	Treatment	Number of Males	Number of Females
Coho			
1	Ovaplant & Ovaprim	25	25
2	Ovaplant	25	25
3	Ovaprim	25	25
4	Placebo Implant	25	25
5	No Treatment	25	25
Trout			
6	Ovaprim & Ovaplant	13	19
7	No Treatment	15	17

Procedure for Ovaplant Experiment

As detailed in Table 1, coho and trout were implanted with Ovaplant implants (Groups 1, 2, and 6) or placebo implants (Group 4) containing no peptide. Ovaplant implants each contained 150m g of sGnRHa (sGnRH-D-Arg^{6}-Pro^{9}-Net) in inert, biodegradable vehicle. Final doses for fish averaged 43m g/kg for coho and 30m g/kg for trout. The pellet has a life expectancy of 21 days making the daily average dose of sGnRH analogue 2m g/kg/day for coho and 1.4m g/kg/day for trout.

Fish were crowded in one area of the raceway and removed by dip net to an anesthetic bath containing freshwater, salt and 500 ppm benzocaine. Anesthetized fish were transfer to a scale and weighed. After weighing, fish were implanted, the affected area swabbed with a topical disinfectant and the fish replaced in the raceway to recover.

Checking the fish

All fish were checked for maturation twice a week after implantation. Maturation was considered achieved when fish expressed gametes upon receiving gentle pressure to the abdomen. Once spawning was initiated, fish were checked for ripeness daily.

Ovaprim injection

When the first coho expressed gametes, all fish in Groups 1 and 3, were anesthetized, weighed and received 0.5ml/kg of Ovaprim delivered interperitonealy using a 22G needle connected to a 3 ml syringe. Trout in Group 6 were treated similarly. After treatment, fish were returned to the raceway and checked for maturity every day thereafter.

Incubation Protocol

Paired mating is the use of milt from one male to fertilize the eggs of one female, which permits a more accurate evaluation of gamete viability. Egg incubation at the Chiloe Aquaculture Research Center was carried out using routine procedure. Fertilized eggs from paired matings were incubated separately o the hatching stage at which time they were pooled with cohorts from the same experimental group until the experiment terminated.

Males

A positive response to treatment was the release of milt after palpation of the fishes abdomen. After each fish expressed milt, the adipose fin was clipped in order to determine the number of newly expressing males for each sampling period. Activation of milt was with the addition of Billard's solution. Samples were repeated in triplicate. Parameters measured appear in Table 3.

Females

Spawning in females was defined as the free flowing of eggs from the genital papillae with slight pressure to the abdomen of the fish. Fish that released eggs were dispatched with a blow to the head, weighed, exsanguinated and dried with towels. Eggs were stripped from the carcass and enumerated volumetrically. Numerical tracking of individual egg batches was by Group number and spawning order. Parameters measured appear in Table 3.

Table 3: Parameters measured in spawning fish. Units of measurement for milt are: Motility in seconds, number in cells per ml and volume in ml. Units of measurement for eggs are volume in liters, size in mm, survival and hatch in percent and time to first feeding in days post treatment.

Parameter	**Measurement**
Males	
Motility	Time to cessation of movement
Cell Count	Neubauer Bright line chamber; cells/ml
Volume	Volumetric measure
Females	
Volume of eggs	Volumetric measure
Egg size	Measure of subsample

Fertilization rate	Cell division 24h post-fertilization
Survival to eyed, hatch and first feeding	Survival of total eggs received per female
Hatching	Time from treatment to hatch
First feeding	Time from treatment and weight

Statistical analysis

Statistical analysis conducted on the data were one-way analysis of variance (ANOVA) using Tukeys and Kruskal-Wallis methods for parametric data and ranked Dunn's method for non-parametric data. Significance accepted at the P<0.05 level unless otherwise noted.

Results

Prespawn mortality

After 48 hours post-treatment, there were no mortalities in any group. There were no differences in overall mortality between treatments.

Time to spawning

Coho

Treatment with either Ovaplant, Ovaprim or in combination significantly (P<0.05) advanced spawning date. Further, the spawning season was significantly compressed using experimental treatments (Table 4). The remaining two fish in the group spawned on the following days to extend the spawning season in this group to four days. In Group 2 (Ovaplant), all fish spawned within 6 days of the star of spawning on 12 May. Group 3 (Ovaprim) completed in six days after beginning spawning two days post treatment. Placebo control fish began to spawn 8 days after the treated groups began to spawn and continued spawning for 14 more days. Untreated fish began to spawn 6 days after other fish were treated and continued to spawn for 16 more days. There was no difference in spawning time for control groups.

Trout

Treatment with Ovaplant and Ovaprim significantly advanced spawning date in trout. Treated trout began to spawn two weeks post treatment and the spawning season lasted 17 days with 89% of fish spawning in 10 days. Control fish began to spawn as the last treated fish was spawned, 31 days post treatment. The spawning season for control trout spanned 25 days. The time to 50% spawning for both populations is separated by one month (Table 4).

Table 4: Comparison of spawning rate in coho and trout. Treatment for coho salmon was 2 May for coho and 30 April for trout. Shown are figures for days post treatment to the initial spawn in the Group in days, the number of days when 50% of the Group had spawned and the days to completion of spawning within the Group. Duration of the spawning season in days for each group is also shown.

Group	Initial Spawning	50% Spawning	End of Spawning	Duration
Coho				

1	10	10	14	4
2	10	12	16	6
3	11	11	17	6
4	17	25	31	14
5	16	24	32	16
Trout				
6	14	15	31	17
7	31	47	56	24

Characteristics of milt

Coho males in Group 1 and 3 had lower volumes of milt that control fish, but had higher sperm counts (Table 5). In trout, treated males gave a higher ($P<0.053$) milt volume than control fish. There were no other differences seen in milt.

Table 5: Parameters of milt characteristics for coho and trout. Volume of milt in ml is for single attempts at time of spawning. Sperm density or count is expressed as millions of cells per ml. Motility of sperm is expressed in seconds duration of activity after adding Billards solution to a small sample of milt. Data expressed as mean value and standard error of the mean

Group	Volume	Count	Motility
Coho			
1	34.8±3.4	45.4±4.6	1929.8±23.4
2	44.4±3.8	41.8±6.1	1742.7±36.4
3	41.1±2.3	49.1±3.3	1905.0±28
4	47.2±3.4	43.2±4.1	1778.5±178.5
5	49.8±3	50.4±0.5	1834.0±172.3
Trout			
6	37.9±10.5	46.25±3.1	15107±181.9
7	27.9±13.2	48.7±1.4	1824.2±106.8

Eggs

There were no differences between treatment for the following parameters: volume of eggs, size of eggs, survival to eyed stage, survival to hatch and survival to first feeding. Experimental groups were significantly faster to first feeding than control groups. When considered in relation of time from spawning to first feeding, treatment further increased this effect. The duration of first feeding to fish also differed with respect to treatment (Table 6)

Table 6: Days to first feeding and duration of first feeding for coho salmon fry from the start of spawning for the group and from treatment.

Group	From start of spawning	From treatment	Duration
1	65	75	5
2	66	73	8
3	67	83	10
4	71	96	19
5	72	106	23

Discussion

Fish treated with either Ovaplant and or Ovaprim spawned in advance of control fish. Eggs and larvae from treated groups were no different in survival and growth than control stocks. This demonstrates that there is no latent effect of treatment on gamete viability. As such, this represent a significant advantages to freshwater culture operations. The reduced residency of spawning fish in freshwater decreases the potential of disease transmission to the present and next generations by removing a pool of potential pathogens and decreases the need for therapeutic use. Advanced spawning also permits groundwater hatcheries to take advantage of warmer water temperatures which, in turn, promotes better growth and feed conversion in offspring.

Treatment of fish also compressed the spawning season. That is, the duration of the spawning season from first to last spawner in a group was less than control groups. In the case of trout, spawning was complete by the time the control fish had begun to spawn. This is also mirrored in the case of coho where the spawning season for groups averaged five days compared to 15 days for control fish. Compressed spawning season represents considerable economic advantage from a labour perspective as it decreases the amount of time crews must attend adult fish.

Batch spawning of fish also permits batch ponding and first feeding of fry as exemplified by the current study. This can permit uniform growth of fish within a population by decreasing the duration of the first feeding phase. Uniformity of fish is desired because husbandry practices such as grading and mixing several size feeds are reduced if fish are of uniform size. It is commonly held that smolts of a uniform size also perform better in seawater than smolts of varied sizes.

There are several implications of this study that induced maturation and coordinated spawning is a valuable tool for fish culturists. Firstly, this study indicates a greater efficiency of hatchery operations with regard to operational and capital cost savings. These savings are realized both immediately with adults and downstream with juvenile production. Secondly, there are implications for fish health as the freshwater residency period of adult fish is reduced, and hence the use of therapeutants and horizontal transmission of disease is reduced. Thirdly, the genetic potential of broodstock can be maximized. By manipulating spawning date, a greater number of viable gametes can be introduced into the breeding programme. As well, these techniques permit the out crossing of strains and the maximization of single paired matings. In sum, advanced maturation helps maximize options in broodstock management.

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