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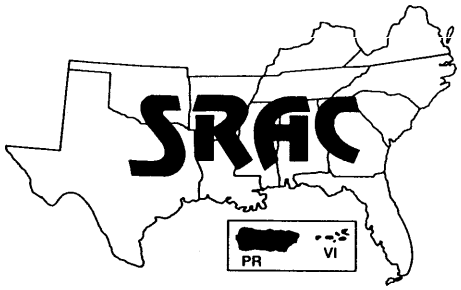

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Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish

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Induced spawning of fish often requires hormone injections. Preparations used for hormone-induced spawning must be mixed properly, and the correct amount must be used to be effective. This publication describes common techniques used to calculate the hormone concentration and dose, prepare hormone solutions, and inject the fish for induced spawning. To calculate the proper dosage, 1) the recommended dose, 2) approximate weight of the brood fish, and 3) desired volume of the injection must be determined. The quantity of hormone to be injected can then be calculated from the weight of each individual brood fish and appropriate injection schedule.

The hormones must be mixed and stored properly to prevent contamination and preserve potency. Always use sterile syringes, needles, vials, and utensils when mixing, injecting, or storing hormones. Nonsterile items should be boiled in water for at least 10 minutes. Water or saline solution (0.7 percent NaCl) should be boiled before mixing with the hormone for injections. If the hormone is to be stored after mixing, it is recommended that bacteriostatic water or bacteriostatic physiological saline, which contains antibacterial ingredients, be used. Using sterile procedures,

At this time the Food and Drug Administration has failed to clear any hormones for use as spawning aids in food fish. Check with your state aquaculture specialist for the latest information on legal implications.

hormones that have been mixed with bacteriostatic water or saline can be stored in a freezer for several years without loss of potency. However, bacterial contamination or improper storage can quickly destroy the potency of the hormone. All hormone preparations must be properly labeled to avoid confusion. The volume of hormone should be divided into small (l-2 cc) plastic vials before storing in the freezer so that only the required amount need be defrosted, saving the potency of the remaining vials.

■ Recommended dose

To determine the amount of hormone to be injected, first check the literature for the recommended dose and injection schedule for the fish species. Hormone doses are given in units of: 1) weight such as kilogram (kg), gram (g), and microgram (µg); 2) volume such as cubic centimeter (cc) or milliliter (ml) and microliter (µl); or 3) biological activity such as International Units (IU). Recommended hormone doses vary considerably for different species of fish and even from different hatcheries spawning the same species. Table 1 provides doses for various hormones used for induced spawning. These values represent a sample of a number of studies conducted by many workers. The actual dose used may be tailored for specific conditions encountered at your hatchery. It is usually better to slightly over-estimate the dose. This is especially true when using pituitary material because potency can vary. When first evaluating a particular dose and schedule, it is best to try it on a small number of fish to determine its effectiveness.

■ Weight of brood fish

When calculating the hormone dose, it is necessary to determine the weight of the fish. Individual fish can be weighed in a container of water using a hanging scale or platform scale. However, this method may be impractical for large species. It is preferable to estimate the weight of the fish rather than inflicting damage by weighing. A length-weight relationship for the species of fish can often be obtained from the literature or can be calculated from fish that have died, fish not used for spawning, or fish that have already spawned. The length of brood fish can be determined with the fish under water in the holding tank using a measuring stick or tape. The approximate weight is calculated, averaged with the other brood fish, and rounded up to the next figure.

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Table 1. Commonly used hormone concentrations in weight and International Units (Ill) per fish body weight, time interval in hours (h) between injections, and percentage of total dose in each injection.

Hormone	Total dose	Interval	Percentage
Pituitary	4-8 mg/kg	6-24 h	20%-80% 33%-67%
HCG	300-1,800 IU/kg	0 h 24 h	100% 33%-67%
HCG + Pituitary	430-2,300 IU/kg 5-9 mg/kg	6-24 h 6-24 h	10%-90% + 100%
HCG & Pituitary Mixture	60-1,000 IU/kg + 2.5-12 mg/kg	6-24 h	20%-80% 50%-50%
LHRHa	5-100 µg/kg	0 h 6-18 h (48 -72 h for trout)	100% 20%-80% 50% -50 %
LHRHa+	5-100 µg/kg	6-18 h	20%-80% 50%-50% +
Haloperidol	0.1-1 mg/kg		100%

■ Volume of injection

The volume of the injection is usually measured in cubic centimeters (cc) which are identical in volume to milliliters (ml). Injections should be small enough to avoid injuring the fish, yet large enough to be accurately measured. The total volume of injections for large species such as striped bass, sturgeon, paddlefish or grass carp should be no more than 1-2 cc. If the volume is greater than 1 cc, it should be split and injected in different locations. Small ornamental aquarium fish should be injected with no more than 0.1-0.2 cc.

Preparation of hormone solutions

To calculate the concentration of hormone to be mixed, the recommended dose (Table 1), multiplied by the approximate weight of individual brood fish, is divided by the desired volume of the injection.

$$\text{Hormone Concentration} = \frac{\text{Recommended Dose} \times \text{Fish Weight}}{\text{Desired Injection Volume}}$$

1) Pituitary extract

Fresh or dried pituitary material is usually mixed with sterile or boiled water or saline (0.7 percent NaCl) **approximately 1 hour before injec**tion. When mixing pituitary material that is to be stored in a freezer, bacteriostatic water or bacteriostatic physiological saline is recommended to minimize contamination.

A mortar and pestle or hand tissue grinder is used to pulverize the dried pituitary material. The liquid is added a little at a time, mixing thoroughly to produce a uniform suspension. The tissue residue should be allowed to settle to the bottom of the vials while on ice or in a refrigerator, because once in solution, the hormone has a relatively short shelf life at room temperatures. If the preparation is to be stored in a freezer, the tissue residue will settle to the bottom during freezing. Only the liquid above the tissue residue is injected. Dried pituitary extract is usually injected into the muscle at 4-8 milligrams/kilogram (mg/kg) of fish body weight.

For example, if the recommended dose of pituitary extract is 4 mg/kg, the fish weigh approximately 6 kg, and the desired volume of injection is 1 cc. Then the concentration of hormone is equal to 4 mg/kg, multiplied by 6 kg, divided by 1 cc. In this case, 24 mg/cc.

$$\text{Hormone Concentration} = \frac{4 \text{ mg/kg} \times 6 \text{ kg}}{1 \text{ cc}} = 24 \text{ mg/cc}$$

■ Mixing hormone to be injected immediately

If the reconstituted hormone is to be used immediately, the total quantity of hormone required for all brood fish to be injected is calculated and mixed together. The quantity of hormone is then dispensed by volume according to individual brood fish weight. The quantity of hormone to be weighed is determined by multiplying the recommended dose by the total weight of brood fish.

$$\text{Total Weight of Hormone} = \text{Recommended Dose} \times \text{Total Weight of Fish}$$

How many milligrams (mg) of hormone are needed to spawn ten fish with an average weight of 6 kg, if the recommended dose is 4 mg/kg? The total fish weight is 10 fish x 6 kg or 60 kg. Then the total weight of hormone is equal to 4 mg/kg, multiplied by 60 kg, or in this case, 240 mg.

$$\text{Total Weight of Hormone} = 4 \text{ mg/kg} \times 60 \text{ kg} = 240 \text{ mg}$$

It is advisable to add an additional 10 to 15 percent to the total for loss or wastage while mixing and filling syringes.

$$\text{Additional 10\%} = 240 \text{ mg} \times 0.1 = 24 \text{ mg}$$

$$\text{Total Weight of Hormone} = 240 \text{ mg} + 24 \text{ mg} = 264 \text{ mg}$$

What volume of liquid solvent should be mixed with the hormone to obtain the desired concentration? The volume of liquid solvent required is equal to the total weight of hormone (264 mg) divided by the desired hormone concentration (24 mg/cc) or in this case, 11 cc.

$$\text{Volume of Liquid} = \frac{\text{Total Weight of Hormone} = 264 \text{ mg}}{\text{Hormone Concentration} \quad 24 \text{ mg/cc}} = 11 \text{ cc}$$

■ Mixing hormone to be stored

If the reconstituted hormone is to be stored in a freezer, the weight of

hormone to be mixed with a vial of bacteriostatic saline or water is calculated by multiplying the hormone concentration calculated in the example above (24 mg/cc) by volume of the vial (30 cc); 720 mg of hormone are then weighed and mixed in a 30 cc vial.

Quantity of Hormone=
Hormone Concentration x Volume

Quantity of Hormone=
24 mg/cc x 30 cc = 720 mg

The quantity of hormone should be divided into small (l-2 cc) plastic vials before storing in the freezer so that only the required amount need be defrosted, saving the potency of the remaining vials. The hormone preparations should be properly labeled according to hormone type, concentration and date mixed to avoid confusion.

2) Human chorionic gonadotropin (HCG)

HCG is measured not by weight but biological activity called International Units (IU). It is usually available in sterile vials containing 5,000 or 10,000 IU. The unopened vial of hormone should be stored in a refrigerator at 35-45ºF (2-7°C). HCG is mixed with bacteriostatic water, usually supplied with the hormone. The hormone solution should either be used immediately or divided into small volumes and kept in a freezer, or potency maybe reduced. Intramuscular injections (in the muscle) of 300 to 1,800 IU of HCG per kg of fish body weight are usually recommended.

What volume of liquid solvent should be added to a vial of HCG? Again, the concentration of hormone to be mixed, must be calculated from the recommended dose (Table 1), the approximate weight of the individual brood fish, and the desired volume of the injection.

$$\text{Hormone Concentration} = \frac{\text{Recommended Dose} \times \text{Fish Weight}}{\text{Desired Injection Volume}}$$

For example, if you have a 10,000 IU vial of HCG, the recommended dose is 400 IU/kg, the fish weigh approximately 1 kg, and the desired volume of injection is 0.2 cc. Then the desired concentration of hormone is equal to 400 IU/kg, multiplied by 1 kg, divided by 0.2 cc, in this case, 2,000 IU/cc.

$$\text{Hormone Concentration} = \frac{400 \text{ IU/kg} / 1 \text{ kg} = 2{,}000 \text{ IU/cc}}{0.2 \text{ cc}}$$

What volume of water must be mixed with the 10,000 IU vial to get the desired concentration? The required volume of liquid is equal to the amount of hormone in the vial (10,000 IU), divided by the desired concentration (2,000 IU/CC). This equals 5 cc.

$$\text{Volume of Liquid} = \frac{\text{Amount of Hormone}}{\text{Hormone Concentration}} = \frac{10{,}000 \text{ IU}}{2{,}000 \text{ IU/cc}} = 5 \text{ cc}$$

3) Luteinizing hormone-releasing hormone analog (LHRHa)

One of the synthetic LHRH analogs that has been used successfully is Des-GLY^{10}[D-Ala^{6}]-LH-RH Ethylamide. LHRHa is available in a pre-weighed quantity in a sterile vial. The unopened vial of hormone should be stored in a freezer. The hormone should be mixed with bacteriostatic water and either used immediately or divided into small volumes and kept in a freezer. LHRHa is usually injected in the muscle at 5 to 10 micrograms/kilogram (µg/kg) of fish weight. However, doses as high as 100 µg/kg and as low as 1 µg/kg have been successful.

What volume of liquid solvent must be added to a vial containing LHRHa to obtain the desired concentration of the hormone? Again, the concentration of hormone to be mixed is calculated from the recommended dose (Table 1), the approximate weight of the individual brood fish, and the desired volume of the injection.

$$\text{Hormone Concentration} = \frac{\text{Recommended Dose} \times \text{Fish Weight}}{\text{Desired Injection Volume}}$$

For example, if you have a 1 mg (1,000 µg) vial of hormone, the recommended dosage is 10 µg/kg, the fish weigh approximately 50 g each, and the desired volume of injection is 0.1 cc. Then the desired concentration of hormone is equal to 10 µg/kg, multiplied by 50 g, which is converted to 0.05 kg, divided by 0.1 cc (5 µg/cc).

$$\text{Hormone Concentration} = \frac{10 \text{ µg/kg} \times 0.05 \text{ kg} = 5 \text{ µg/cc}}{0.1 \text{ cc}}$$

The required volume of liquid is equal to the weight of hormone in the vial, divided by the desired concentration. This equals 200 cc.

$$\text{Volume of Liquid} = \frac{\text{Weight Of Hormone} = 1{,}000 \text{ µg}}{\text{Hormone Concentration } 5 \text{ µg/cc}} = 200 \text{ cc}$$

Rather than mixing such a large volume of hormone, three dilutions are made. First draw 10 cc of bacteriostatic water in a syringe and then mix the liquid with the vial of hormone. Nine cc of this mixture are then placed in sterile l-cc vials and labeled (100 µg/cc).

The remaining 1 cc of hormone is mixed with 9 cc of bacteriostatic water. Nine cc of this mixture are then placed in sterile l-cc vials and labeled (10 µg/cc).

This time, the remaining 1 cc of hormone is mixed with 1 cc of bacteriostatic water to obtain the desired concentration of 5 µg/cc. This mixture is placed in sterile l-cc vials and labeled (5 µg/cc). This dilution is used to inject the fish. The remaining vials of 10 µg/cc and 100 µg/cc concentration are diluted as needed.

4) Haloperidol

Haloperidol {4-[4-(4-chlorophenyl)-4-hydroxy-piperidino] -4'-fluorobutyrophenone} powder should be mixed with bacteriostatic water and dissolved in solution by acidifying with lactic acid. Once in solution, haloperidol can be used immediately or divided into small volumes and kept in a freezer.

For example, if the recommended dose of haloperidol is 0.5 mg/kg, the fish weigh approximately 10 kg, and the desired volume of injection is 1 cc. Then the concentration

of hormone is equal to 1 mg/kg, multiplied by 5 kg, divided by 1 cc. In this case, 5 mg/cc.

$$\text{Hormone Concentration} = \frac{0.5 \text{ mg/kg} \times 10 \text{ kg}}{1 \text{ cc}} = 5 \text{ mg/cc}$$

The weight of hormone to be mixed with a vial of bacteriostatic water is calculated by multiplying the hormone concentration (5 mg/cc) by the volume of the vial (30 cc); 150 mg of haloperidol are then weighed and mixed in a 30 cc vial of bacteriostatic water.

$$\text{Quantity of Hormone} = \text{Hormone Concentration} \times \text{Volume}$$

$$\text{Quantity of Hormone} = 5 \text{ mg/cc} \times 30 \text{ cc} = 150 \text{ mg}$$

Lactic acid is gradually added to adjust the pH to 3.0 to 3.6. Use pH paper to determine the end point or add lactic acid, drop by drop, until all the haloperidol is in solution and the mixture is clear, not cloudy. The haloperidol solution is injected in the muscle at 0.1 to 1 mg/kg of fish body weight. The dopamine blocker is usually administered with the first of two injections of LHRHa.

Quantity of hormone injected

The total volume of hormone to be injected can be calculated by multiplying the recommended dose by the weight of individual fish and then dividing by concentration of the hormone mixture prepared.

$$\text{Volume of Injection} = \frac{\text{Recommended Dosage} \times \text{Fish Weight}}{\text{Hormone Concentration}}$$

1) Example (large fish)

For example, if the recommended dose is 4 mg/kg, the individual fish weighs approximately 9 kg, and the concentration of hormone is 30 mg/cc. Then the fish is injected with 1.2 cc,

$$\text{Volume of Injection} = \frac{4 \text{ mg/kg} \times 9 \text{ kg}}{30 \text{ mg/cc}} = 1.2 \text{ cc}$$

2) Example (small fish)

For example, if the recommended dose is 10 µg/kg, individual fish weighs 80 g or 0.08 kg, and the hormone concentration is 5 µg/cc. Then the volume of hormone injected is equal to 0.16 cc.

$$\text{Volume of Injection} = \frac{10 \text{ @kg} \times 0.08 \text{ kg}}{5 \text{ µg/cc}} = 0.16 \text{ cc}$$

Hormone injection schedules

The number of injections required, is dependent on the response of each species to the selected hormone. Some ovulate following a single injection of the total dose. However, multiple injections are usually more successful.

For pituitary extracts or purified gonadotropins, first an injection of 10 to 33 percent volume of the total dose is administered, followed by a final or resolving injection. A rule-of-thumb is do not make the initial dose too large or the resolving dose too small.

Two injections of LHRHa, either 20 percent followed by 80 percent or 50 percent initial and 50 percent final, will usually give a better spawning response than a single injection of the same total dose. The dopamine blocker (e.g., halo-peridol) is usually injected at the same time as the first LHRHa injection.

The time interval between multiple injections is usually 48 to 72 hours for cold-water species (e.g., trout), 12 to 18 hours for warm-water species (e.g., sturgeon, paddlefish, striped bass, white bass, and grass carp) and 6 hours for tropical species (e.g., red-tailed black shark and rainbow shark). Males are usually given only a single injection when the female is given the resolving dose.

Injecting brood fish

The hormone is injected into the fish with a sterile syringe and hypodermic needle. Large food fish or sport fish should be injected with no more than a total of 2 cc of solution. No more than 1 cc should be administered at any one injection site. A 19-23 gauge needle may be used for these larger species. Small ornamental fish species should be injected with no more than 0.2 cc of material using a 26-30 gauge needle.

Hormones are either injected into the muscle (intramuscular) or the body cavity (intraperitoneal). Intramuscular injections are usually preferred because they result in a more constant delivery of hormone and there is less chance of injuring the fish. The preferred site for an intramuscular injection is into the thick muscle of the back. For scaled fish, the needle is inserted directly behind or along side the dorsal or back fin, where there are no scales. For intraperitoneal injections, the needle is inserted at the base of a pelvic fin, where there are no scales, and the mixture is injected into the body cavity (Figure 1).

Conclusions

In this publication we have described common techniques to prepare the hormone mixture, calculate the hormone concentration to be mixed, determine the amount of hormone to be injected, and inject the hormone into the fish for induced spawning. The concentration of hormone to be mixed is calculated from the recommended dose, approximate weight of the brood fish, and the desired volume of the injection. Quantity of hormone to be injected can then be calculated from the estimated weight of each individual brood fish. Always use sterile syringes, needles, vials, and utensils when mixing, injecting, or storing hormones.

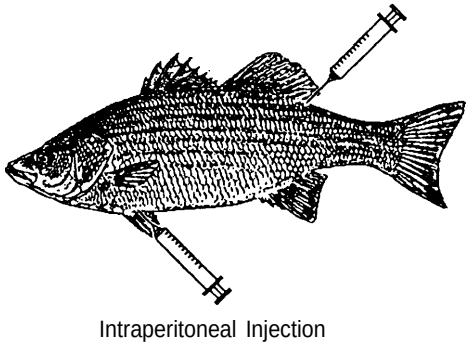

Figure 1. Site of intramuscular and intraperitoneal injection.

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