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Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning: Baitfish and Ornamental Fish

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It is common for many species of fish to fail to spawn in captivity. This is usually due to some environmental inadequacy and failure of the fish to successfully initiate or fully carry out the internal hormonal cascade required for spawning (see SRAC Publication No. 0424, *Hormonal Control of Reproduction in Fish for Induced Spawning*). To overcome this, hormonal therapies can be applied to fish by producers to ensure successful completion of the hormonal cascade and ultimately ovulation and/or spawning.

Species of freshwater baitfish including golden shiners (*Notemigonus crysoleucas*) and fathead minnows (*Pimephales promelas*), and brackish water species such as mud minnows (*Fundulus* spp.) will spawn reliably in production systems without the need for hormonal therapy. Common freshwater ornamental species such as guppies (*Poecilia reticulata*) and marine species such as clownfish (*Amphiprion* spp.) also spawn reliably without the need for hormonal therapy in their production protocols. For other species that do not readily spawn in captivity, controlled spawning using hormonal therapies ensures successful captive propagation and allows producers to develop more stringent and reliable production protocols to meet hatchery goals. This publication will review methods that can be used for hormone induced spawning of ornamental and baitfish species.

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Available hormones

Many hormonal aids have been used for induced spawning of many fishes. Currently, Chorulon® (human chorionic gonadotropin) is the only hormonal spawning aid cleared by the FDA for use in aquaculture. One important regulation is that an administered dose should not exceed 25,000 international units (IU) per fish if intended for human consumption. Required dosages for many of the ornamental and baitfish species are highly unlikely to exceed 25,000 IU per fish. Hormone treated fish or resulting progeny are not to be consumed by humans. Chorulon® is a potential option for ornamental and baitfish producers, and a prescription from a veterinarian is required for its use. Another FDA reviewed spawning aid categorized as "Legally Marketed Unapproved New Animal drug for Minor Species" for use in spawning of ornamental fishes is Ovaprim® [salmon gonadotropin releasing hormone analogue (sGnRHa) 20 ppb (20 µg/mL); domperidone 10 ppm (10 mg/mL)]. Ovaprim® can be obtained over-the-counter and is available through national aquaculture product retailers and sometimes through local co-op stores.

Ovaprim® and Chorulon® (Fig. 1) have been used effectively to induce spawning in a variety of ornamental and baitfish species. Marine baitfish including pinfish (*Lagodon rhomboides*), pigfish (*Orthopristis chrysoptera*), spot (*Leiostomus xanthurus*), and Atlantic croaker (*Micropogonias undulatus*) can be successfully spawned using Ovaprim® and/or Chorulon®. Ovaprim® has been tested on a

Figure 1. Pre-packaged vials of Chorulon® powder and diluents (left) and vial of Ovaprim® (right).

wide range of ornamental fishes and has been successful for induced spawning of species in families such as Characidae (pacu) and Cyprinidae (koi, goldfish, barbs, and freshwater sharks), and several different species of catfish in multiple families.

Ovaprim® has a general recommended dose of 0.22 milliliters per pound (0.5 mL/kg) fish body weight, but the exact dose given of either hormone will vary depending on its effectiveness on different species. Ovaprim® comes pre-packaged in 10 milliliter self-sealing vials with a sGnRHa concentration of 20 ppb (20 µg/mL). Chorulon® also comes in 10 milliliter self-sealing vials packaged as a set of five vials each filled with 10,000 IU of chorionic gonadotropin powder and another set of five vials filled with 10 milliliters of sterile diluent. A single 10,000 IU vial can be reconstituted with the supplied sterile diluents to produce a stock concentration as low as 1,000 IU per milliliter if a full 10 milliliters of diluent were added. The exact dose of Chorulon® used will vary by species, but should fall within the range of 45 to 1,820 IU per pound (100 to 4,000 IU/kg). Spermiation is rarely the limiting factor during captive spawning. However in many cases where a dosage is not reported for males, they are generally administered half of the dosage used on female conspecifics.

Although many other hormones and products have been historically used for spawning of ornamental and baitfish species, Ovaprim® and Chorulon® are the only products with completed Investigational New Animal Drug (INAD) applications or alternative review processes. Other spawning aids with ongoing INADs include LH-RHa, Ovaplant® (sGnRHa), OvaRH® (sGnRHa), Channel catfish (*Ictalurus punctatus*) pituitary, and common carp (*Cyprinus carpio*) pituitary. Use of any of these later products could require a $700 U.S. annual fee per facility depending on how they were used. Because of their legal use, effectiveness, and obtainability, the example calculations provided will focus on either Chorulon® or Ovaprim®.

Hormone administration

Minimizing stress on broodfish is critical to ensure they are more likely to respond to hormone administration. Using sedatives and anesthetics on the fish during handling can reduce the stress on the fish, and also make some species easier and safer to work with for producers. One of the most commonly known anesthetics in aquaculture is tricaine methanesulfonate (MS-222) and is effective on many ornamental and baitfish species. Effective dosages vary from 20 to 200 ppm (20 to 200 mg/L) MS-222 but species specific dosages can often be found in the production manuals for the species of interest. Detailed methods for using MS-222 and other effective anesthetics such as clove oil and quinaldine for ornamental and baitfish species can be found in SRAC Publication No. 3900, *Anesthetics in Aquaculture*. Soft, knotless nets with small mesh sizes and made of polyester, nylon, or even rubberized are often used by ornamental and baitfish producers when collecting and moving fish to avoid bodily injury and stress on broodfish. Care should be taken to minimize crowding, the time out of water, and to ensure optimal water quality is maintained within the containers broodfish are held in during hormone administration.

The effectiveness of hormones is largely dependent on the reproductive readiness of the fish. Only fish showing the appropriate signs of spawning readiness should have hormones administered to them. Fish should be within their natural spawning season and/or under appropriate captive conditions. Signs of readiness when the conditions are appropriate vary by species but can generally be distinguished by soft, swollen, and distended abdomens in females, and potentially free flowing milt in males during handling or after gently applying pressure to the abdomen. Catheterization can be a more accurate means to determine spawning readiness in females. While the fish is under anesthesia, eggs can be suctioned out by gently inserting a small, catheter tubing in the genital pore, passing through the oviduct into the ovary, and then staged under a microscope to ensure the egg nucleus has migrated and the female is more likely to ovulate or spawn after injection. Catheterization requires much care not to damage the female internally. It is always best to use the smallest size tubing as possible to be as minimally invasive as possible. The small size of some ornamental species inhibits the potential for catheterization. More detail on determining the developmental stages of egg and sperm can be found in SRAC Publication No. 0423, *Determining Sexual Maturity of Broodstock for Induced Spawning of Fish*.

Many hormones including Ovaprim® and Chorulon® are liquid in form and are administered to fish by injection via syringes (Fig. 2). The injection can be intra-coelomic or given intramuscularly, and the location is usually based on prior effectiveness for a given species. For coelomic injections (Fig. 3), the needle is inserted bevel side down, under and not through any scales, and just behind either pelvic fin, while being careful not to go deeper past the first point of no resistance after initial insertion. For intramuscular injections (Fig. 4), the needle of the syringe is inserted bevel side down, under and not through any scales, and just behind the dorsal fin on either side of body. Due to the small bodies of many ornamental and baitfish species, small gauge needles in the range of 26 to 30 are recommended, and intramuscular injections are often preferred. Although it would be optimal for a single injection to result in ovulation and spawning, some hormones for females of some species have to be administered in more than one injection. In these cases, a smaller priming dose (10 to 50 percent of the total) may be administered 6 to 24 hours prior to final injection when the resolving dose (50 to 90 percent) is administered. For males, one single injection will usually suffice and is often given at the same time as a single female injection or the same time as the resolving dose if priming/resolving doses are used. Some individuals of species with asynchronous egg development can be repeatedly injected throughout a spawning season and spawn on a regular basis. It is important to always use new needles or whole syringes for each fish to avoid potential infections and disease transfer. Used needles should also never be inserted into stock solutions, even to replace unused hormone back in to vials/containers.

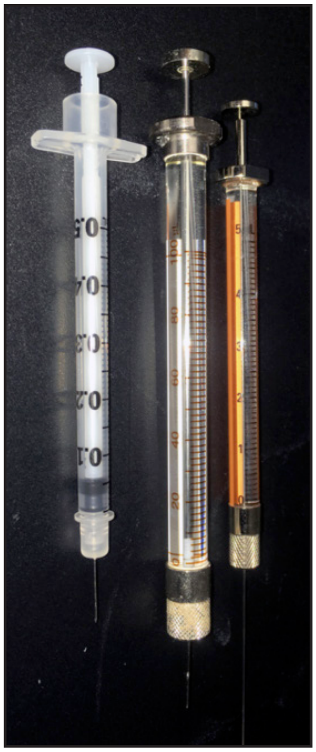

Figure 2. Syringes varying in capacities of 500, 100, and 5 microliters from left to right.

Figure 4. Intra-muscular injection administered to a female grass carp (*Ctenopharyngodon idella*).

Figure 3. Intra-coelomic injection administered to a flowing, male pinfish.

After hormone administration, fish should be placed into spawning tanks and observed closely to ensure they have fully recovered from anesthesia. The time until ovulation and/or spawning will vary, but many warm-water species will likely respond within 48 hours. The exact timing is sometimes found in production manuals for different species, and often reported as "degree-hour", which is the time from injection until ovulation and/or spawning at a given tempearture. This allows a producer to predict how soon a fish should respond based on the tank water temperature.

Hormones should be stored according to label instructions when not in use. Both Ovaprim® and Chorulon® are recommended to be stored below 77°F (25°C) and out of sunlight. Hormones should be used by their labeled expiration date. Once a vial is opened, it should be used as quickly as possible and not stored long term.

Dosage calculations

When administering hormonal aids via injections, there are four factors that are needed: 1) desired injection volume or range of acceptable volumes, 2) recommended dose for the species and sex of fish being injected, 3) weight of the fish being injected, and 4) concentration of the hormone solution being used. The concentration of the solutions used for injections is critical as it determines the volume of hormone to be injected into broodfish. Historically, a total injection volume of 0.2 to 1.0 milliliters has been recommended for many species, but is less applicable to ornamental and baitfish species. Many broodfish of the ornamental and baitfish species are often relatively small (≤ 1.1 pounds; ≤ 500 g) and large injection volumes up to 1.0 milliliter could be overwhelming to small-bodied species. The availability now of small volume capacity syringes (≤ 300 µL) has aided in being able to use stronger stock concentrations to achieve smaller injection volumes more precisely than standard 1.0 milliliter capacity syringes. With such syringes available, a safeguard for ornamental and baitfish species might be to not exceed an injection volume equivalent to 57 microliters per ounce (2 µL/g) fish body weight [i.e. do not inject more than 0.5 milliliter into an 8.8 ounce (250 g) broodfish]. Calculating needed stock concentrations so that a targeted injection volume is not exceeded can be done by multiplying the recommended dosage to induce spawning by each individual fish weight, dividing by the targeted injection volume, and multiplying by any appropriate conversion factors.

Example: A 2.8 ounce (80 g) fish is to be injected with a dosage of 228 IU per pound (500 IU/kg) and it is desired that no more than 50 µL be injected into the fish. What is the required hormone stock concentration?

((228 IU per pound × 0.175 pounds) / 50 microliters) × 1,000 microliters per milliliter conversion factor = 800 IU hormone per milliliter

or

((500 IU/ per kilogram × 0.08 kilograms) / 50 microliters) × 1,000 microliters per milliliter conversion factor = 800 IU hormone per milliliter

Chorulon® comes pre-packaged as 10,000 IU powder per 10 milliliter vial, so its concentration in a single vial can be initially produced to be as low as 1,000 IU per milliliter simply by adding a full 10 milliliter vial of sterile diluents to a single 10 milliliter vial of powder. From that vial, a known volume can be taken out and further diluted in sterile closed vacutainer tubes or similar vials by adding a required quantity of sterile diluent to produce the desired concentration. This can be calculated by dividing the amount of hormone taken out of the original stock solution, dividing by the desired stock concentration, and subtracting the volume of solution taking out of the original stock solution.

Example: A hormone stock concentration contains 1,000 IU per milliliter. A hormone concentration of 800 IU per milliliter is required for a fish species. One milliliter of stock solution is removed for dilution to create the new concentration. What is the volume of sterile diluent that must be added to achieve the final concentration of 800 IU per milliliter?

(1,000 IU per milliliter / 800 IU) – 1 milliliter stock volume = 0.25 milliliters of diluent

Similar calculations would be used for Ovaprim® to estimate a needed stock concentration to achieve a target injection volume and diluent to add to achieve a specific stock concentration. Ovaprim® comes pre-mixed at a concentration of 20 ppb (20 µg/mL), so it is impossible to start with a higher concentration to minimize the volume of hormone injected. Fortunately, the recommended dosage of Ovaprim® for many species is 0.23 milliliters per pound or 14 microliters per ounce (0.5 mL/kg or 0.5 µL/g), well under the potential safeguard of 60 microliters per ounce (2 µL/g) to protect from over-injecting broodfish with too much volume of hormone. And with the precision of many small volume syringes, dilutions for Ovaprim® are rarely needed.

On a large scale, it would not be practical to do multiple dilutions for every injection so that only a single targeted injection volume was used for every fish. When dealing with large groups of fish, determining the weight of the largest fish to be injected and using that weight to determine the needed stock concentration would allow a producer to not exceed the targeted injection volume required for all individuals in the group of fish.

Calculating the amount of hormone solution to be injected is straightforward and only requires knowing the 1) recommended dose for the species and sex of fish being injected, 2) weight of the fish being injected, and 3) concentration of the hormone solution being used. All that is needed is to multiply the recommended dosage to induce spawning by each individual fish weight, dividing by the stock concentration, and multiplying by appropriate conversion factors.

Example–Chorulon®: A 2.8 ounce (80 g) fish is to be injected with a dosage of 228 IU per pound (500 IU/kg) using a stock solution of 5,000 IU per milliliter. What is the amount of hormone solution the fish should be injected with?

((228 IU per pound × 0.175 pounds) / 5,000 IU) × 1,000 microliters per milliliter conversion factor = 8 microliter hormone solution injection

or

((500 IU per kilogram × 0.08 kilogram) / 5,000 IU) × 1,000 microliters per milliliter conversion factor = 8 microliter hormone solution injection

Example–Ovaprim®: A 2.8 ounce (80 g) fish is to be injected with a dosage of 2.27 micrograms per pound (5 μg/kg) using an undiluted stock solution of Ovaprim® at 20 μg/mL. What is the amount of hormone solution the fish should be injected with?

((2.27 micrograms per pound × 0.175 pounds) / 20 micrograms) × 1,000 microliters per milliliter conversion factor = 20 microliter hormone solution injection

or

((5 micrograms per kilogram × 0.08 kilograms) / 20 micrograms) × 1,000 microliters per milliliter conversion factor = 20 microliter hormone solution injection

For many species, the recommended dosage for Ovaprim® is often reported on a hormone volume/fish mass basis and not a hormone mass/fish mass basis. This makes the calculation even easier.

Example–Ovaprim®: A 2.8 ounce (80 g) fish is to be injected with a dosage of 0.23 milliliters per pound (0.5 mL/kg) of an undiluted stock solution of Ovaprim®.

0.23 milliliters per pound × 0.175 pounds × 1,000 microliters per milliliter conversion factor = 40 microliter hormone solution injection

or

0.5 milliliters per kilogram × 0.08 kilograms × 1,000 microliters per milliliter conversion factor = 40 microliter hormone solution injection

Calculating the amount of hormone needed to achieve a production goal is also straightforward and only requires knowing the 1) recommended dose for the species and sex of fish being injected, 2) fecundity of females when given the recommended dosage, and 3) desired egg quantity. All that is needed is to divide the desired egg quantity by the fecundity of females and then to multiply by the dosage used.

Example: A producer needs 1,000,000 eggs to achieve his yearly production goal. He plans to use Ovaprim® injections at 0.23 milliliters per pound (0.5 mL/kg) on females which typically yield 2,273 eggs per pound (5,000 eggs/kg) female body weight. What is the amount of Ovaprim® required to inject all females (440 pounds; 200kg)?

Step 1: Determine the total weight of females required to produce 1,000,000 eggs.

1,000,000 eggs / 2,273 eggs per pound female body weight = 440 pounds of females

or

1,000,000 eggs / 5,000 eggs per kilogram female body weight = 200 kilograms of females

Step 2: Determine the volume of Ovaprim® needed for total weight of females required.

(1,000,000 eggs / 2,273 eggs per pound) × 0.23 milliliters per pound = 100 milliliters of Ovaprim® per 440 pounds of females

or

(1,000,000 eggs / 5,000 eggs per kilogram) × 0.5 milliliters per kilogram = 100 milliliters of Ovaprim® per 200 kilograms of females

Conclusion

This document has reviewed the methods that can be used for spawning ornamental and baitfish species using two commonly available hormonal aids. The calculations within can also be used for other hormone products. Reviewing any production manuals available for species of interest should provide exact dosages and injection schedules to be used for that species.

Suggested readings

Coyle, S.D., R.M. Durborow, and J.H. Tidwell. 2004. Anesthetics in aquaculture. SRAC Publication No. 3900. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Hill, J.E., K.H. Kilgore, D.B. Pouder, J.F.F. Powell, C.A. Watson, and R.P.E. Yanong. 2009. Survey of Ovaprim use as a spawning aid in ornamental fishes in the United States as administered through the University of Florida Tropical Aquaculture Laboratory. North American Journal of Aquaculture 71:206-209.

Oesterling, M.J., D.G. Sennett, and D.P. Kilduff. 2005. Production of spot as live bait for recreational angling. William and Mary, Virginia Institute of Marine Science.

Ohs, C.L., M.A. DiMaggio, and S.W. Grabe. 2011. Species profile: Pigfish, *Orthoprisits chrysoptera*. SRAC Publication No. 7209. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Ohs, C.L., M.A. DiMaggio, and S.W. Grabe. 2011. Species profile: Pinfish, *Lagodon rhomboides*. SRAC Publication No. 7210. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Rottmann, R.W., J.V. Shireman, and F.A. Chapman. 1991. Hormonal control of reproduction in fish for induced spawning. SRAC Publication No. 0424. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Rottmann, R.W., Shireman, J.V., and F.A. Chapman. 1991. Determining sexual maturity of broodstock for induced spawning of fish. SRAC Publication No. 0423. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Sink, T.D. 2011. Species profile: Atlantic croaker. SRAC Publication No. 7208 Southern Regional Aquaculture Center, Stoneville, Mississippi.

United States Fish and Wildlife Service. 2016. INADS available. (accessed May 1, 2017) https://www.fws.gov/fisheries/aadap/inads-available/index.html

United States Food and Drug Administration. 2009. Freedom of information summary-Ovaprim®. MIF 900-001. (accessed May 1, 2017) https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/MinorUseMinorSpecies/ucm125475.htm

United States Food and Drug Administration. 1999. Freedom of information summary- Chorulon®. NADA 140-927. (accessed May 1, 2017) https://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM115909.pdf

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