Aquafarmer Information Sheet

Spawning the tinfoil barb, *Barbodes schwanenfeldi* in Hawaii

CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

by Richard Bailey and Brian Cole
Aquaculture Extension Agents, Sea Grant Extension Service
School of Ocean and Earth Science and Technology, University of Hawaii

Introduction

The tinfoil barb, *Barbodes schwanenfeldi*, is a common fish found in the international aquarium trade and food markets in Southeast Asia. Tinfoil barbs have a dorsal, ventrally flattened body covered with moderately large, silver, chrome-like scales and red fins with a red leading fin ray (Figure 1). Tinfoil barbs are a peaceful fish that can reach a length greater than 40 centimeters or 16 inches, and like other, larger barbs, can often live more than 10 years in captivity. In nature, tinfoil barbs are primarily macrophages; that is, they feed on vascular aquatic plants. However, they will eat most commercially prepared diets consisting of vegetable and fish meal proteins.

Figure 1. A female tinfoil barb.

The genus Barbodes is closely related to the genera *Capoeta* and *Puntius* found in the subfamily Cyprininae. The taxonomy of these three genera are often treated as being synonymous. These closely related species inhabit the freshwater lakes, rivers and tributaries of Southeast Asia. Water quality parameters of the tinfoil barb's natural habitat ranges from soft to moderately hard water (German scale dH 5- 12°, or 100 to 200 milligrams per liter [equivalent to parts per million] calcium carbonate), pH of 6.5 to 7.0, and a temperature of 22° to 25°C [72° to 78°F]). Tinfoil barbs are a hardy fish that can do well in conditions exceeding the water quality parameters of their natural habitat.

In Hawaii, tinfoil barbs become sexually mature from May through July during the onset of rising water temperature, 25° to 27°C (77° to 81°F). Many fish naturally spawn during seasonal changes, such as an increase in temperature or availability of natural feeds (phytoplankton and zooplankton) for the developing fry. The tinfoil barb, like other related species, is an egg scatterer, laying eggs over a substrate such as plants or gravel to help hide and protect the eggs and larvae from predators.

Unfortunately, the tinfoil barb will not reproduce naturally in a captive environment. Certain environmental cues, such as lunar phase, photoperiod, temperature, feeds, substrate, etc., stimulate a complex of hormonal interactions that control reproduction in fish. These environmental cues must be present for most fish or the fish species will not mature and spawn in captivity. Fortunately, the endocrine system of many fish is relatively well understood. The complex hormonal mechanisms controlling maturation of oocytes (oocytes are maturing eggs inside the female ovaries; once spawned, they are called eggs) and ovulation in fish can be bypassed through induction with hormones. If hormones are administered to the fish by injection at the right time and dose, the fish can be deceived into responding as though the right environmental stimuli are present. This will initiate a sequence of natural hormonal changes that will lead to final oocyte maturation, ovulation and spawning. Some fish will naturally spawn eggs in captivity after hormone induction, while others must be "hand stripped" of eggs and milt.

Facilities

Success in hormonally induced spawning in the tinfoil barb and other related species results from a good working knowledge of hormone induction as well as adequate support facilities. Broodfish, holding, hatching and larval rearing tanks are important to the success of producing larvae.

Broodstock tanks or ponds should be large enough (>1,000 gallons) to hold two to three times the number of broodstock required to produce the desired number of eggs.

Two separate, static 50- to 150-gallon holding tanks

Center for Tropical and Subtropical Aquaculture Publication Number 136
January 1999

equipped with thermostatic controlled heater(s) are needed to maintain a constant temperature to hold sexed broodstock fish during the induction process. Fish are not fed while they are in the holding tanks. Static tank systems work well if the density is kept low, for example, 1 kilogram per 189 liters or 4 to 5 fish per 50 gallons. High water quality should be maintained during spawning induction.

An egg hatching system that consists of a hatching jar and a larval rearing tank is needed. Use of a flow-through system is recommended to eliminate water quality problems. Recirculating systems are not recommended because they are not often biologically established and require additional management skills. Hatching jar systems require, on the average, 1 to 2 liters per minute of water flow, based on the egg density in the jar.

The larval rearing tank should be large enough to handle the potential amount of larvae from a spawn. For example, a 30-gallon glass aquarium can hold two spawns of tinfoil barb larvae (10,000 or 88 per liter) for a period of one week with a water exchange rate of 2 liters per minute (760 gallons per day per tank) before requiring a reduction in density.

Conical fiberglass or acrylic tanks are now becoming more commonly used due to their improved water circulation and efficient removal of wastes, which improves water quality and larval management.

Broodstock

One-year-old fish should be isolated from growout facilities and placed into separate broodstock rearing ponds or large tanks at a stocking density of 2 kilograms per cubic meter (1 fish per 25 gallons). The fish are kept at this density for a year to achieve fully mature size, to reduce stress and disease problems, and to ensure proper nutrition and water quality. Two broodstock ponds or tanks should be maintained for each sex to ensure quality stock for spawning at a sex ratio of at least two males to one female. Broodfish should be given a proper commercial diet consisting of at least 30-plus percent protein in two or more rations at a rate of 1 to 2 percent of the total biomass weight (TBW) per day. Most commercial catfish and trout production diets are suitable. Floating feeds are often used; these allow a farmer to observe the animals' feeding response and gauge the population health.

Sexing Broodstock

Mature two-year-old fish, 200 to 300 grams in size, can be visually sexed using external characteristics. Ripe female tinfoil barbs can be identified by their swollen abdominal region and smooth scales. Male fish are more slender in body shape and have distinctive rough tubercles (pimple or sandpaper-like bumps) on their scales. One can extrude milt from the males by gently pinching the fish between one's fingers and sliding down the abdominal region towards the anterior genital opening or papillae. Whitish clear fluid or milt will come out of the male's genital papillae when it is sexually mature and ripe.

Tinfoil barbs can achieve sexual maturity by their first year. However, two-year-old fish are most commonly used for induced spawning because they produce a larger number of eggs per spawn and often a greater fertilization rate. One-year-old tinfoil barbs can be used for spawning if the females have fully developed oocytes and if the males show milt. Female tinfoil barbs 200 to 300 grams in size can produce 5,000-plus eggs per spawn.

Determining Female Maturity

In Hawaii, female tinfoil barbs begin to develop oocytes in March or April and mature in May through July. Farmers should harvest broodstock from the broodstock ponds and select a number of large females that have visibly well rounded abdominal region or ovaries and weigh within approximately 10 percent of each other. The broodfish should be handled gently, and their time out of water should be limited by placing them into bucket(s) with quinaldine (for quinaldine mixture see Table 1). Sedating brood fish helps limit stress and injury during handling at this critical stage prior to spawning.

Determining oocyte maturity requires removing a sample of oocytes (~30) from the ovaries of the fish using 1.14 millimeter I.D. cannula tubing (also called "microbore tubing" in chemical supply catalogs). The tubing is made from flexible tygon, silicon, or polyethylene material that can be gently inserted into the genital vent and moved up the oviduct to the ovaries of the fish without causing injury.

After the fish are completely sedated, which they exhibit by being upside down and not reactive to touch, they can be staged for maturity. The cannula tubing should be inserted into the genital vent while gently twisting and working the tube down the oviduct 2 to 3 inches to the ovary (Figure 2). Once in, the cannula should be placed in the farmer's mouth and gentle suction should be applied. The end of the tubing should be held with one's thumb while retracting the tube containing the oocyte sample, which should be 30-plus oocytes, from the fish. The fish should be placed into a numbered, aerated bucket that is marked "#1." The fish will revive fully from the quinaldine sedation within 2 to 5 minutes.

Place the dry oocyte sample on a graduated glass microscope slide to determine the oocyte's diameter. Ripe tinfoil barb oocytes are mature at a mean size of 1.12 millimeters. If the oocytes are 1.12 millimeters in size, they should first be checked for overripe oocytes, which can be distinguished by the presence of space between the oocytes' internal content and the membrane (Figure 3), indicated by a withdrawal of the oocyte's internal contents from the outer wall. If the oocytes are not overripe and have a good density, a couple of drops of "oocyte/egg clearing solution" (Table 2) should be added to the oocytes; which will allow the farmer to better judge migration of the nucleus. Within 10

Figure 2. Extracting oocytes from a tinfoil barb.

minutes, the oocytes will clear, and their maturity can be staged. The nucleus in the oocyte, which is seen as a dense spherical mass within the oocyte, should be located. Oocytes with centered nuclei are not yet ripe. Ripe oocytes can be identified by nuclei that have migrated off-center toward the outer cell wall of the oocyte (Figure 3). Fish that have centered nuclei should be returned to the broodstock pond and rechecked in two weeks.

The best fish with highest degree of nuclei migration should be selected from the sampled fish. From that subset, fish that weigh within 10 percent of each other should be selected so that one hormone mixture can be made for the entire set of fish, thus simplifying the induction steps.

Several publications describe oocyte staging and other aspects of hormone induced spawning of fish. These highly recommended publications are listed at the end of this document.

Selected ripe fish are sedated, towel dried and weighed to the nearest gram. An average weight is calculated by totaling the weight and dividing by the number of fish. Fish that weigh 10 percent more or less than the average body weight should be returned to the broodstock tank and used for the next spawning event.

The identified ripe female fish are then placed into a static holding tank equipped with aeration and thermostatically controlled submersible heaters set at 27.2°C (82°F). Fish should be stocked at a density not greater than 1 kilogram per 189 liters (50 gallons). The fish should not be fed at this time.

A number of fish should be sampled because oocyte maturity of fish within a population can vary slightly during the spawning season. The population will follow a typical bell-shaped curve, with a small percentage of fish maturing early in the spawning season and the greater percentage of the population maturing during the middle or peak of the season and then tapering off.

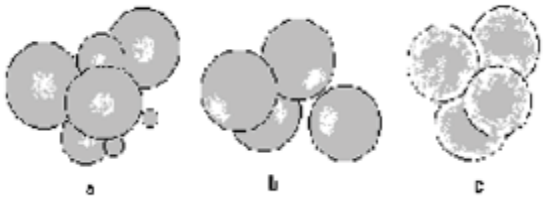

Figure 3. Oocyte staging
a. Centered nuclei (immature);
b. Off-center nucleus (ripe);
c. Yolk material pulled away from oocyte wall (overripe).

Male Brood Fish Selection

A sex ratio of one female to two males will be needed for artificial spawning. The farmer should select large males exhibiting rough tubercles or scales from the broodstock tank. The males should be checked for milt by the procedure stated previously. The average weight for the male fish should be determined, then the fish should be placed into a holding tank at a density not greater than 1 kilogram per 189 liters (50 gallons) at ambient temperature. The fish should not be fed at this time.

Hormone Mixture Preparation

Tinfoil barbs can be hormonally induced to ovulate by using a mixture of Human Chorionic Gonadotropin (HCG) and carp pituitary extract (CPE) at a dose of 60 International Units (IU) HCG and 4 milligrams CPE per kilogram of body weight of fish. Human chorionic gonadtropin usually is available in vials of 10,000 I.U., and CPE is often sold by the gram in whole or ground form.

The total volume injected for male and female tinfoil barbs is 0.5 cubic centimeters or 0.5 milliliters. The total injection volume is determined by body weight. The total injection volume should not exceed 0.5 cubic centimeters for fish weighing an average 250 grams.

Female fish require two injections. The first, or priming injection, is given at 2 AM, and the second, or resolving injection, is given at 8 AM. However, male tinfoil barbs require only 20 percent the dosage of that given the females and receive one injection at 8 AM.

Preparing a Stock Mixture of HCG

Used, empty HCG bottles work well for storing and holding mixtures of hormones. The bottle is opened, washed, dried and sealed with the rubber stopper secured with elastic tape (electrical tape works well). Microliter syringes (1 cubic centimeter = 1milliliter = 1,000 microliters) work well for taking small amounts of hormone from stock solution bottles. When spawning a few fish, the amount of hormone needed from the stock solutions of hormone is often quite small. The stock solution can be further diluted to allow for greater volumes to be used to gain more accuracy in mixing hormone amounts when spawning just a few fish. However, be aware of the total volume of hormone to inject.

Farmers should make a stock mixture of HCG so that small amounts of hormone can be removed at a time, while not risking contamination or spoilage of the remaining bulk material. The hormone comes in dry form in a sealed vial and is often accompanied with bacteriostatic water. Ten cubic centimeters of sterile, bacteriostatic water should be added to the dry 10,000 I.U. vial of HCG. This will result in a concentration in the vial of 1 cubic centimeter equaling 1,000 I.U. HCG or 0.1 cubic centimeter equaling 100 I.U. The vial should be marked "HCG 1cc/1,000 IU" with a grease pencil.

Preparing a Stock Mixture of CPE

A stock mixture of carp pituitary extract (CPE) can be made by placing 250 milligrams of finely ground CPE into a clean vial. A tissue grinder can be used to grind CPE into a fine powder. Care must be taken to prevent the tissue from getting warm while grinding with a tissue grinder. Overheated tissue will result in a loss in potency. Five cubic centimeters of bacteriostatic water should then be added to the vial and mixed thoroughly. This will make a stock solution with a concentration of 1cubic centimeter equaling 50 milligrams CPE. The vial should be marked "CPE 1cc/50 mg" with a grease pencil.

Both stock mixture and bulk materials should be placed in the freezer to retain potency and to prevent spoilage. Bulk CPE should also be placed into a bottle containing an anhydride, that is calcium sulfate, to keep the hormone dry.

Calculating Dosage

Hormones are made up from the stock mixtures for individual spawning events. Female and male dosages are calculated separately based on the average weight of the fish. When calculating the total volume required, an additional amount will be needed to make up for loss of hormone in the syringe, the needle, and the vial. To compensate for this loss, an additional one or two "ghost" fish (average weight of fish selected for induction) are added to the total weight for every ten fish. The following example provides step-by-step calculations to prepare the amount of hormone needed to induce ovulation in tinfoil barb females and primer for the male tinfoil barbs.

Example:

Eight mature female broodstock that weigh within 10 percent of each other and have fully migrated nuclei have been placed in a holding tank fitted with aeration and thermostatically controlled heaters set at 27.2°C (82°F). Sixteen mature males have been selected and placed into a holding tank at ambient temperature.

Calculating Female Hormone

Average weight of each female fish

- 1,680 grams total weight *divided* by 8 fish = 210 grams average weight per fish
- One "ghost fish" is added to make up for hormone loss in syringe and bottle
- Thus, 8 + 1 = 9 fish = 1,680 + 210 = 1,890 grams or 1.89 kilograms total weight used in calculating hormone volume

Amount of HCG Needed

- 1.89 kilograms *multiplied* by 60 International Units (I.U.) per kilogram = 113.4 I.U. HCG (stock HCG vial concentration = 1cc = 1,000 I.U.)
- 113.4 I.U. HCG *divided* by 1,000 I.U./1 cubic centimeters = 0.1134 cubic centimeters
- Take 0.1134 cubic centimeters, or 0.11 cubic centimeters of stock HCG solution with a syringe and place it into a new clean vial marked "female"

Amount CPE Needed

- 4 milligrams per kilogram CPE *multiplied* by 1.89 kilogram = 7.56 milligrams CPE (stock CPE vial concentration = 1 cubic centimeter = 50 milligrams CPE)
- 7.56 milligrams CPE *divided* by 50 milligrams CPE / 1cubic centimeter = 0.1512 cubic centimeters
- Take 0.1512 cubic centimeters, or 0.15 cubic centimeters of stock CPE solution with a syringe and place it into the vial marked "female"

Volume Needed for Females

(Total injection volume per female = 0.50 cubic (centimeters)

- 0.11 cubic centimeters HCG + 0.15 cubic centimeters CPE = 0.26 cubic centimeters
- 9 fish multiplied by 0.5 (injection volume per fish) = 4.5 cubic centimeters total hormone solution needed
- 4.5 cubic centimeters - 0.26 cubic centimeters (hormone volume in vial) = 4.24 cubic centimeters
- Add 4.24 cubic centimeters bacteriostatic water to the vial

marked “female”

- Place the vial in the refrigerator until first injection at 2 AM

Calculating Male Hormone

Male broodstock fish require only 20 percent of the female hormone dosage and one injection. The procedures to obtain the male dosage are the same for calculating the dosage for the females and multiplied by 0.2 (20 percent). Hormones are used to stimulate an increase in the total volume of milt to increase fertilization success and sychronize final maturation in both male and female fish.

Using the example above, 16 male fish were selected by gently applying pressure anterior toward the vent which produced milt. The fish were weighed to the nearest tenth of a gram.

- 16 males = 3,680 grams = 230 grams average weight each
- Two “ghost fish” (average weight of males used) are added to the make up for hormone loss during volume transfers
- 18 males = 4,140 grams (= 3680 + 230 + 230)
- 4,140 grams = 4.14 kilograms

Amount of HCG Needed

- 60 I.U. HCG per kilogram multiplied by 4.14 kilograms = 248.4 I.U.
- (stock HCG vial concentration = 1cc = 1,000 I.U.)
- 248.4 I.U. HCG *divided* by 1,000 I.U. per 1 cubic centimeters = 0.24 cubic centimeters
- Take 0.24 cubic centimeters of stock HCG solution with a syringe and place it into a new, clean vial marked “male”

Amount CPE Needed

- 4 milligrams CPE per kilogram multiplied by 4.14 kilograms = 16.56 milligrams CPE (stock CPE vial concentration = 1 cubic centimeters = 50 milligrams CPE)
- 16.56 milligrams CPE divided by 50 milligrams / 1cubic centimeter = 0.33 cubic centimeters CPE
- Take 0.33 cubic centimeters of stock CPE solution with a syringe and place it into the vial marked “male”

Volume Needed for Males

(Total injection volume per male = 0.50 cubic centimeters)

- 0.25 cubic centimeters HCG + 0.33 cubic centimeters CPE = 0.58 cubic centimeters
- 18 fish multiplied by 0.5 (injection volume per fish) = 9.0 cubic centimeters total hormone solution needed
- 9.0 cubic centimeters - 0.58 cubic centimeters (hormone in vial) = 8.42 cubic centimeters

Figure 4. Injecting the tinfoil barb with hormones.

- Add 8.42 cubic centimeters bacteriostatic water to the male vial to bring the total volume to 9.0 cubic centimeters. Place the vial in the refrigerator until needed at 8 AM.

When using very small volumes in setting up mixtures and for administering small dosages, use of a micro-syringe of 100 microliter (0.1cc) to 500 microliters is recommended to more accurately withdraw the proper amount of hormone from stock bottles. A standard, 1-cubic-centimeter insulin syringe with a one-half-inch #22-gauge needle works well for injecting tinfoil barbs.

Hormone Injection

At 2 AM, the female brood fish should be netted from the temperature-controlled holding tank and placed into a bucket containing water from the holding tank with quinaldine (100 to 150 milliliters of stock quinaldine solution per 2 to 3 gallons water) to sedate the fish. Once sedated, the first injection of 0.1 cubic centimeters (20 percent of 0.5 cubic centimeters) should be administered by inserting the needle under a scale near the muscular area just below the dorsal fin ray about 0.5 inches in (Figure 4). The syringe plunger should be slowly depressed to inject the hormone. The needle should be withdrawn and a finger should be placed over the injection puncture hole. The spot should then be massaged for a couple of seconds to close the hole, after which the fish should be

Figure 5. Hand stripping eggs from a tinfoil barb.

placed back into the holding tank.

Female fish are given a second injection of the remaining 80 percent volume or 0.4 cubic centimeters of hormone at 8 AM following the procedure outlined above.

Male fish are injected at 8 AM with 0.5 cubic centimeters using the method stated for the female fish.

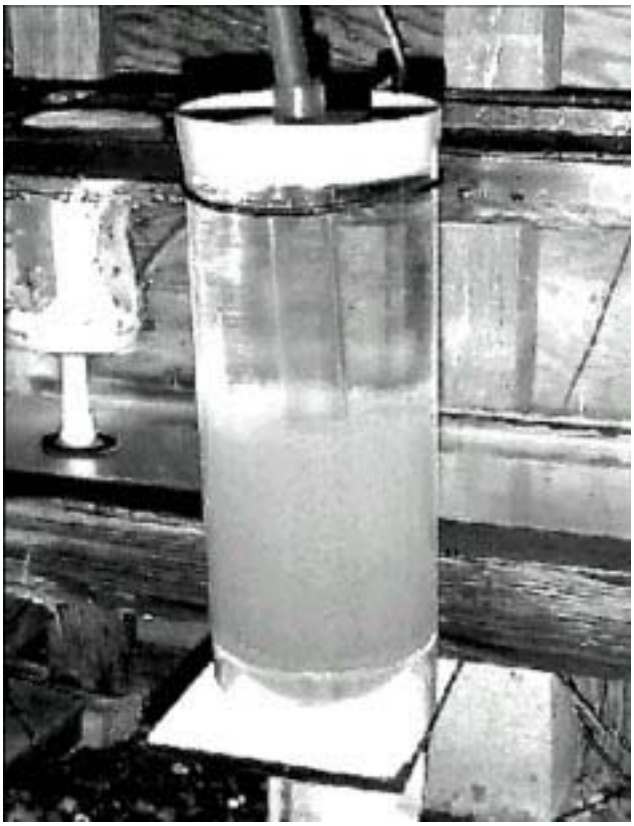

Figure 6. An egg hatching jar.

Artificial Spawning

Six hours after the resolving injection, the female fish should be checked for ovulation. The fish should be placed upside down in a net and their abdominal area gently squeezed toward the genital vent. If an ovulated egg (oocytes spawned are now called eggs) does not come out freely, the fish is not ready. If eggs freely flow from the vent, the fish should be placed in a bucket with water from the holding tank and sedated with quinaldine. Then two male fish are netted for every female fish that is ovulating. The remainder of the female fish should be checked every half hour for ovulation.

Once the female and male fish are sedated, one female should be removed from the quinaldine bath and dried. Her eggs should be hand-stripped by placing her over a clean, dry, 10-inch-diameter glass bowl. (Glass is preferred, but a plastic bowl with a smooth surface will work). The abdominal area should be gently squeezed starting near the pectoral fins and moving slowly toward the vent (Figure 5). Stripping eggs should be stopped if blood or tissue are expressed. It is important not to let water touch the eggs during hand stripping. Water on the eggs prior to adding milt will reduce fertilization greatly.

The eggs should flow evenly without much effort. If the fish seems unwilling to release her eggs, she has not fully ovulated and should be returned to the holding tank and rechecked for ovulation in 15 to 30 minutes.

Next a sedated male fish should be towel dried and placed so that his vent is over the eggs in the bowl. The male fish should be gently squeezed starting at the abdominal region toward the vent, so that the milt drips into the bowl. It is important to avoid allowing the milt to travel down the side of the fish's body, which could pick up water off the fish. Repeat the sample procedure with a second male fish.

The bowl with eggs and sperm is then swirled or mixed with a soft feather to evenly distribute the milt over the eggs. Enough clean water from the egg incubation system should be added to the bowl to just cover the eggs by 1 centimeter. The eggs must then be swirled again to evenly distribute sperm over the eggs. At this time, fertilization will take place. The mixture should be swirled slowly for an additional 1 to 2 minutes, then the sperm-laden water should be decanted. Then enough water should be added to completely cover the eggs by 2 to 4 centimeters, the mixture should be swirled and the water decanted. Rinsing and decanting should be contin-

ued until the eggs are clean of blood or the water is clear. A portion of the water from the egg hatching jar (sometimes called a "McDonald's jar," Figure 6) should be removed by placing a hand into the jar and displacing some water. Then the eggs should be poured into the jar.

The water flow into the jar should be adjusted so the eggs are gently tumbling and not forced near the top of the jar. The eggs will expand up to ten times their volume after fertilization. As the eggs swell, they become less dense, so the water flow rate into the jar will require fine adjustment to keep the eggs from overflowing into the larval tank.

Determining Fertilization Rate

First cell division, or cleavage, can be seen one hour after fertilization. At that time, between 20 and 30 eggs should be taken from the hatching jar and placed on a microscope slide. A couple of drops of egg clearing solution should be added. After waiting a few minutes for the eggs to clear, they can be checked for the percentage of fertilization. Dead eggs will appear opaque white in color as compared to viable eggs, which will be translucent. Divide the number of viable eggs by the number of dead eggs in the sample to estimate percentage of fertilization. Eggs will hatch in 24 hours at 28°C (82°F). A large number of dead eggs will greatly affect the viability of the remaining few.

Conclusions

Techniques using hormones to induce spawning of fish have been around for more than 40 years. Common fish species such as koi, goldfish, kuli loaches, redtail black and rainbow sharks are routinely induced to spawn using hormones. However, no one standard protocol works for all species. Fish reproduction is regulated by both external environmental cues and internal endocrine mechanisms. Hormones are used to intervene in a chain of internal events that control reproduction in fish.

Although the preparation of hormones, screening egg quality in broodfish, establishing holding tanks and a hatchery require extra effort and resources, the basic techniques are not overly involved. Using hormones to induce spawning in fish facilitates the production of a large number of eggs simultaneously, which can increase hatchery efficiency and profit.

Table 1. Quinaldine Sedative Stock Solution

3.78 milliliters Quinaldine

10 milliliters acetone

1 US gallon water

Note: Some fish may require more or less quinaldine for complete sedation. Water temperature, fish weight and species all can affect dosage rate. Start by adding 50 milliliters of stock solution to fish in two gallons of water. Wait five minutes; if fish are not sedated, add 50 milliliters more quinaldine solution.

Table 2. Formula for Egg Clearing Solution

60% ethanol

30% formalin

10% glacial acetic acid

Note: Clearing eggs with this solution will make it difficult to determine if an egg is overripe.

Table 3. Useful Conversions

1 cubic centimeter (cc) = 1 milliliter (ml)

1 gram (g) = 1,000 milligrams (mg)

1 mg = 1,000 micrograms (μg)

1 kilogram (kg) = 1,000 g

1 ml = 1 gram of water

Recommended Reading

Rottmann, R., Shireman, J., & Chapman, F., 1991. Introduction to Hormone-Induced Spawning of Fish. USDA SRAC pub. #421

Rottmann, R., Shireman, J., & Chapman, F., 1991. Capturing, Handling, Transporting, Injecting and Holding Brood Fish for Induced Spawning. USDA SRAC pub. #422

Rottmann, R., Shireman, J., & Chapman, F., 1991. Determining Sexual Maturity of Broodstock for Induced Spawning of Fish. USDA SRAC pub. #423

Rottmann, R., Shireman, J., & Chapman, F., 1991. Hormonal Control of Reproduction in Fish for Induced Spawning. USDA SRAC pub.# 424

Rottmann, R., Shireman, J., & Chapman, F., 1991. Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning in Fish. USDA SARC pub. # 425

Rottmann, R., Shireman, J., & Chapman, F., 1991.Techniques for Taking and Fertilizing the Spawn of Fish. USDA SRAC pub.# 426.

Acknowledgments

All photos were taken by Rich Bailey and Clyde Tamaru, University of Hawaii Sea Grant Extension Service.

This work was part of a project titled “Expansion and Diversification of Freshwater Tropical Fish Culture in Hawaii,” which was funded by the Center for Tropical and Subtropical Aquaculture through a grant from the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service (USDA grant #96-38500-2743).

This publication was funded in part by a grant/cooperative agreement from the National Oceanic and Atmospheric Administration, project #A/AS-1, which is sponsored by the University of Hawaii Sea Grant College Program, SOEST, under Institutional Grant No. NA36RG0507 from NOAA Office of Sea Grant, Department of Commerce.

This publication was funded in part by the Aquaculture Development Program, Hawaii Department of Agriculture, as part of the Aquaculture Extension Project with the University of Hawaii Sea Grant Extension Service.

The views expressed in this publication are those of the authors and do not necessarily reflect the views of CTSA, USDA, NOAA, ADP or any of their sub-agencies (UNIHI-SEAGRANT-TR-98-04).

Sea Grant Extension Service
University of Hawaii
School of Ocean and Earth
Science and Technology
2525 Correa Rd., HIG 237
Honolulu, HI 96822