SRAC Publication No. 426

Southern
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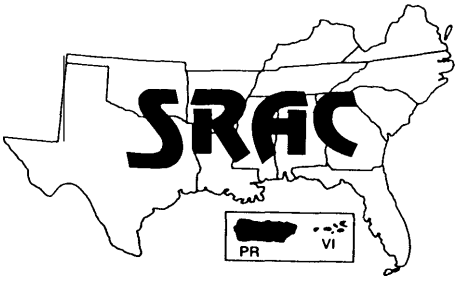

November 1991

Techniques for Taking and Fertilizing the Spawn of Fish

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Following hormone injection, the eggs and milt of fish can be taken by several different methods:

- tank spawning,
- hand stripping, and
- surgically removing the eggs.

The method of choice depends on the fish species, hatchery facilities, experience and skill of the hatchery staff, and the desired manipulations of eggs, sperm or fertilized eggs.

Taking the eggs

Ovulation is the final phase of normal egg development. The time between the final or resolving dose of hormone and ovulation is referred to as the latency period. This is usually dependent on the species of fish, water temperature, and hormone preparation used, It is especially important to know the latency period when hand stripping or surgically removing the eggs. Check the literature for the latency period for the fish species you are spawning.

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During ovulation, the connection between the female fish and the eggs in the ovary is eliminated. In warm-water fishes, egg quality can deteriorate rapidly if eggs are not taken shortly after ovulation; they become "overripe" and can no longer be fertilized. In general, the eggs of tropical and sub-tropical species of fish become overripe more quickly than those that spawn at cooler water temperatures. The eggs of cold-water species remain viable for several days after ovulation. Table 1 presents the reported maximum period between ovulation and the deterioration of egg quality for some species of fish commonly spawned by hormone injection.

Tank spawning

Tank spawning is the simplest method for obtaining a hatchery spawn. Brood fish of both sexes are placed together in the spawning tank following injection(s). Brood fish should not be disturbed and subdued lighting is recommended. The female ovulates when she is physiologically ready. The males stimulate the female to

Table 1. The maximum period between ovulation and deterioration of the egg quality for various species of fish.

Bighead carp *(Hypophthalmichthys nobilis)*	50 to 80 minutes
Common carp *(Cyprinus carpio)*	50 to 80 minutes
Grass carp *(Ctenopharyngodon idella)*	30 to 45 minutes
Rainbow trout *(Salmo gairdneri)*	7 days
Red-tailed black shark *(Labeo bicolor)*	15 to 30 minutes
Snook *(Centropomus* sp.)	15 to 30 minutes
Striped bass *(Morone saxatilis)*	15 to 30 minutes
Sturgeon *(Acipenser sp.)*	2 hours
White bass *(Morone chrysops)*	30 to 45 minutes

release the eggs and fertilize the spawn.

Better fertilization occurs if males are accustomed to the tank, and have been injected with a preparatory dose of hormone several days prior to and again at the same time as the female. Males can be used for several tank spawns, week after week, until their milt flow diminishes. Unless the males are aggressive toward each other, it is advisable to put two or three males for each female in a tank to ensure fertilization.

If the spawning tank is of sufficient size, more than one female may be spawned in the same tank. The presence of other individuals may help stimulate fish that are mass spawners. However, too many breeders in a small tank might be disruptive to the spawning process.

A round tank is advantageous for species with non-sticky, floating or semi-buoyant eggs that spawn in a river or estuary. The circular flow simulates the current in which these fish naturally spawn. The vigorous swimming action of the female in the swift water current is believed to assist in emptying the ovaries. The eggs are carried with the drain water from the spawning tank to a screened collector (Figure 1). Eggs are then transferred to an incubator.

Nest breeders and substrate spawners can also be tank spawned if suitable nesting sites or spawning material are provided. When tank spawning species that scatter sticky eggs, it is advisable to place spawning mats or brushes on the bottom of the tank. The eggs will attach to the substrate. Brood fish are removed from the tank after spawning, unless they provide parental care to the eggs. Fertilized eggs are usually incubated in the spawning tank.

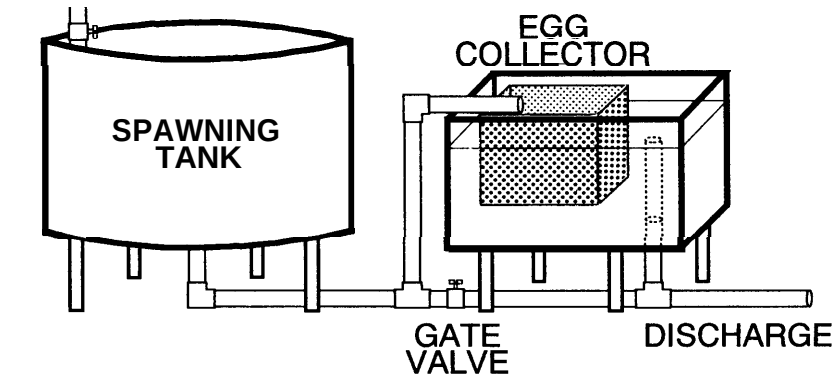

Figure 1. Round spawning tank with screened egg collector.

Tank spawning has both advantages and disadvantages. Advantages are:

- skill on the part of the hatchery staff in predicting the exact time of ovulation or checking females to verify ovulation is unnecessary;
- rapid deterioration of eggs in the ovary after ovulation is not a problem;
- there is less potential of injury to the brood fish because it is unnecessary to check and strip the fish; and
- less labor is required.

Disadvantages are:

- a screened egg collector or suitable spawning substrate is required;
- dirt and debris may be mixed with the eggs, potentially causing problems during incubation;
- some females may not release all their eggs;
- it is more difficult to accurately estimate the number of eggs;
- the surface area of substrate in tanks is insufficient for large species that release large quantities of sticky eggs. The-eggs clump together, resulting in fungus problems and poor hatch; and
- this method cannot be used if induction of polyploidy or other manipulations of eggs, sperm, or fertilized eggs are desired.

Hand stripping

Hand stripping is commonly used for taking the spawn of many species of fish. Brood fish are separated by sex prior to hormone injection to prevent spawning in the holding-tank.

It is important to determine the exact time of ovulation when hand stripping. However, the eggs of cold-water species may remain viable for several days after ovulation; for example, in trout, eggs are usually stripped within 3 to 4 days. The eggs of some species such as striped bass and white bass progressively clear or become transparent as they near ovulation.

This visual cue is used by hatchery workers to estimate the approximate time of ovulation. An egg sample is taken by carefully inserting a tube (catheter) into the urogenital opening and examining the sample under a microscope (See SRAC Publication No. 423, Deter*mining Sexual Maturity of Broodstock for Induced Spawning of Fish).* Eggs taken more than 15 hours before ovulation cannot be accurately staged using this method.

For most species, ovulation can best be verified by checking the female to determine when eggs flow freely from the vent. At least one hour prior to the earliest anticipated time of spawning, female fish are captured and the process of checking to verify ovulation is initiated. Tropical species are usually checked every 45 minutes until ovulation is verified, temperate water species are usually checked every hour. It is not necessary to take the fish out of the water to verify ovulation. The fish is turned belly up and gentle finger pressure is applied to the abdomen starting at the pectoral fins, moving slowly toward the vent. Do not try to squeeze or force the eggs from the fish; this will only injure the female. Frequent or rough handling of females retards ovulation, reduces spawning success and increases fish mortality.

If only a few eggs flow from the vent when slight pressure is applied, partial ovulation has occurred; the fish should be released and checked again later. Attempting to hand strip a female fish that has only partially ovulated will result in few mature eggs and physical damage to the ovaries, preventing a complete spawn. When eggs flow freely from the vent, complete ovulation has occurred. The hatchery worker quickly plugs the flow of eggs by placing a thumb over the vent. Brood fish may be anesthetized with MS-222 for stripping, if necessary, after ovulation is verified.

It is important to insure that no water comes in contact with the eggs until after the milt is added and mixed. Water and slime from the vent and tail area of the female fish are dried with a towel. Water activates the sperm and also causes the opening through which the sperm enters the egg (micropyle) to close. For many fish, this closure takes place within only 45 to 60 seconds.

To strip the eggs, the fish is held slightly on her side, tail down; gentle hand pressure is applied to the abdomen, moving toward the vent (Figure 2). The stream of eggs is directed into a clean, dry bowl positioned so that water from the fish does not drip onto the eggs. The head of small fish can be held by one hand while the eggs are stripped with the other. A cloth glove may be worn to help hold the fish while stripping. Larger species are either wrapped in a towel and held by one or more hatchery workers while another strips the eggs, or the fish maybe restrained on a padded table or stretcher for stripping.

Good quality eggs usually flow readily from the genital opening of the female and have little ovarian fluid. If the ovarian fluid is watery or milky and many of the eggs are cloudy white, this indicates poor quality eggs.

Surgically removing the eggs

Because the internal anatomy of fish varies greatly, hand stripping may be difficult in some species. Sturgeon and paddlefish have no ovarian sac; the eggs are released into the abdominal cavity during ovulation. The best method for taking the spawn in many of these species is to surgically remove the eggs. The first indication of ovulation for sturgeon and paddlefish is the appearance of several eggs stuck to the sides or bottom of the tank. The brood fish are usually left undisturbed for an additional 1 to 2 hours, depending on the size of the female (small females 1 hour and large females 2 hours), to insure complete ovulation.

If the female sturgeon or paddlefish is to be saved, it is first anesthetized. The fish is temporarily placed in an aerated holding tank with MS-222. When opercular movement slows and the fish is unable to right herself when turned over, she-is then placed belly-up on a stretcher. Two hoses are used to ventilate the gills during surgery. One hose delivers aerated hatchery water, and the other delivers water from a recirculating tank containing aerated water with MS-222. During surgery, one

Figure 2. Hand striping the female by applying gentle hand pressure to the abdomen, moving toward the vent; the stream of eggs is directed into a bowl.

of the two hoses is always in the fish's mouth, flushing the gills providing dissolved oxygen. The hoses are exchanged so that the fish remains anesthetized but opercular movement continues. Too much anesthetic will kill the fish. Before surgery, an antibiotic solution is applied to the abdomen. An incision is made along the midline of the abdomen, and the eggs are carefully removed with a spoon (Figure 3). To avoid injuring the internal organs, only the eggs easily accessible are taken. The incision is closed with a half-circle surgical needle and suture material; a length of surgical tubing, split length-ways, may be used to reinforce the abdominal tissue (Figure 4). The incision area is treated with an antibiotic before the fish is returned to the holding tank. A high level of dissolved oxygen is crucial for rapid recovery of the fish.

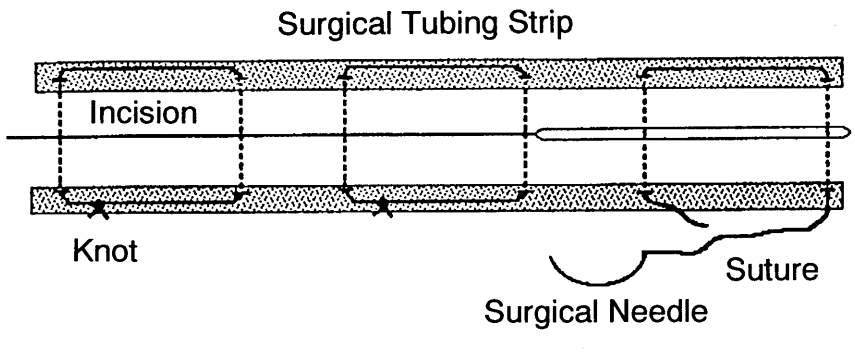

Figure 4. Closing the incision with a half-circle surgical needle and suture material. Note the surgical tubing, split lengthways, used to reinforce the abdominal tissue.

A greater quantity of eggs can be obtained by sacrificing the female sturgeon or paddlefish. If the female is to be sacrificed, it is: 1) held in a net and killed with a blow to the head; 2) hung from a hook; 3) the tail is cut off to bleed the fish, minimizing the contamination of the eggs with blood; 4) an incision is made in the abdomen starting at the vent; and 5) a bowl is placed under the vent, directly below the incision. The eggs flow quickly from the abdomen, pulled by the force of gravity.

Not all the eggs will be free in the abdomen; some remain attached to the folds of the ovaries. These eggs are removed by hand and placed in separate bowls. The proc&s of ovulation will be completed in the bowls. Within 15 to 20 minutes these eggs can be fertilized. Visible amounts of blood, which can inhibit fertilization, may be present with the eggs.

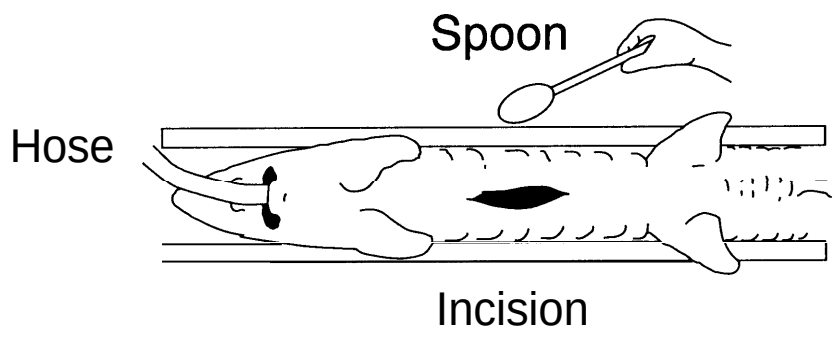

Figure 3. Surgically removing the eggs from the female sturgeon. Note the hose placed in the fish's mouth to ventilate the gills.

After fertilization, these eggs are processed and incubated separately.

For delicate species that seldom survive the rigors of hand stripping, humanely killing them and surgically removing the eggs may be the best option. In addition, more eggs can usually be obtained by this method than by hand stripping. Once ovulation has been verified (see section on "Hand stripping"), the brood fish is held in a net and administered a blow to the head to kill it. The eggs can then be removed from the fish by carefully cutting open the abdomen, pinching off the oviduct, and removing the ovaries individually. The total volume of eggs can then be gently squeezed out of the ovarian sac into a clean dry bowl to be fertilized.

Fertilizing the spawn

Eggs are usually fertilized with fresh milt. Males are captured, wiped off, and held belly down over the bowl containing the eggs. The portion of the abdomen posterior to the pelvic fins is gently massaged to extrude the milt onto the eggs. The number of sperm in a volume of milt is extremely variable, ranging from millions to billions of sperm per milliliter.

Creamy-white milt contains more sperm per volume than grayish-white milt. When available, milt from two or more males is used to insure fertilization of the spawn. Individual males can be used to fertilize more than one spawn.

The fresh milt is spread over the eggs and thoroughly mixed by hand, plastic spatula or feather. Only then is water added to activate the sperm. The sperm remain active in water for a very short time (less than 1 minute to 5 minutes), depending on the species of fish and the temperature of the water. Water also results in closure of the micropyle of the egg in approximately the same amount of time. Water is added to only cover the eggs. Do not add too much water because the sperm may be too diluted to ensure fertilization of the eggs. To fertilize sticky eggs, see section on "Eliminating the stickiness from eggs. "

The bowl containing the eggs and sperm should be gently rocked, swirled or stirred continuously for several minutes to insure uniform distribution of the sperm and to prevent the eggs from settling. Additional water is added as the eggs take up the water and enlarge (water harden). After several minutes, the fertilized eggs are transferred to the hatching apparatus. Fertilized eggs should not be exposed to direct sunlight. Subdued lighting should be used in the spawning and incubation area.

Storage of milt

Milt can be collected from males and stored up to three weeks prior to stripping eggs. Males are captured and anesthetized, if necessary. The fish is then turned belly up, and the vent area dried by blotting with a towel. The area just behind the pelvic fins is gently massaged toward the vent to extrude the milt. The first few drops of milt are wiped away. The milt is collected by inserting a plastic tube attached to a syringe into the urogenital opening. Suction is applied while stripping to draw the milt into the syringe. Care must be taken to insure that water, urine, intestinal contents, slime, or blood is not mixed with the milt. It is best to collect and store milt separately from each male to avoid contamination.

The milt is expelled into a sterile plastic bag, and antibiotic (e.g., 50 micrograms of dry streptomycin sulfate per milliliter of milt) may be added to control bacteria. The bag is filled with oxygen, sealed with a rubber band, and gently swirled to mix the antibiotic. Rough handling and shaking of milt may be detrimental to the sperm; mixing should be done slowly and gently.

Bags of milt should be laid flat to maximize the surface area of milt exposed to the oxygen and immediately stored on ice in a cooler or refrigerator. Do not freeze the milt as this will kill the sperm. Oxygen may be replaced and the milt should be gently swirled in the bag periodically to insure maximum aeration. Milt that has been contaminated with blood, slime, etc., will appear to have congealed and should be discarded. Milt has been held in this manner for up to three weeks; however, the quality of the stored milt may deteriorate with time.

The motility of stored sperm should be checked before it is used to fertilize eggs (See SRAC Publication No. 423, *Determining Sexual Maturity of Broodstock for Induced Spawning of Fish).* Milt with no or low motility should be discarded. When using stored milt to fertilize the spawn, it should be mixed with water first and then gently shaken for five seconds before being added to the bowl with the eggs. Remember, the sperm remain active in water for a very short period of time, so this must be done quickly.

Eliminating the stickiness from eggs

Ovulated eggs of many species such as white bass, sturgeon, paddlefish, common carp, and channel catfish become sticky after coming in contact with water. During natural spawning, this stickiness causes the eggs to become attached to rocks, sticks, or aquatic plants. Catfish eggs are connected by a sticky matrix that holds the eggs together in a mass in the spawning cavern or container. In the hatchery, this stickiness causes problems during incubation.

Silt-clay, bentonite and Fuller's earth have been used to remove the stickiness from the eggs of many species of fish. Do not use diatomaceous earth because the sharp edges of the diatoms will damage the eggs. The dried material is added to hatchery water until a suspension is formed and a residue accumulates on the bottom of the container.

Paddlefish and sturgeon eggs are commonly treated with silt-clay as soon as the first few sticky eggs are noticed after fertilization, usually 1 to 4 minutes. The silt-clay suspension is added to the fertilized eggs at a ratio of 2 to 4 parts suspension to 1 part fertilized eggs. The mixture is gently stirred by hand. Any clumps of eggs on the side of the container are gently broken up. The suspension is poured off the eggs, and fresh suspension should be added every 10 minutes to maintain proper temperature and dissolved oxygen. Continue the process until the eggs do not stick to fingers or each other when removed from the suspension (minimum of 20 minutes).

Urea and salt solution has been used to remove the stickiness from common carp eggs. The addition of water to common carp eggs and milt will result in their sticking together in a clump within a few seconds. By using urea-salt solution instead of water, the spawn can be fertilized without the eggs sticking. A commonly used solution is prepared by dissolving 30 grams of urea and 40 grams of salt in 10 liters of hatchery water. The solution is added

to the eggs and sperm. The initial volume of solution added is approximately 25 percent of the volume of eggs. The mixture is gently stirred continuously with a feather, plastic spatula, or by hand. It has been observed that the motility of common carp sperm lasts much longer in the urea-salt solution (20 to 25 minutes) than in water (1 to 2 minutes). As the eggs water harden, additional solution is added. A portion of the solution with the dissolved sticky material is poured off at intervals and replaced. After about 1 to 1.5 hours the water hardening process is completed. The eggs are then transferred to a tannic acid solution (750 mg/L) for 5 seconds to eliminate any remaining stickiness. To remove the tannic acid, the eggs are thoroughly rinsed with fresh water.

The urea and salt solution has also been used to remove the stickiness from white bass eggs for the production of hybrid striped bass. A solution is prepared in a McDonald jar with 5 liters of hatchery water, 15 grams of urea and 20 grams of salt. The solution is aerated with a weighted air stone at the bottom of the jar until the chemicals are dissolved. A small amount of the solution is added to cover the eggs and sperm, fertilizing the spawn. The mixture is gently stirred. Four minutes after the solution is added to the egg-sperm mixture, 400 milliliters or less of fertilized eggs are placed in each jar with the solution. Air flow is adjusted to keep the eggs in suspension without rupturing them. After 6 minutes, the urea and salt solution is poured off the eggs, and 0.75 grams of tannic acid mixed in 5 liters of hatchery water (150 milligrams/liter) is added and aerated for an additional 6 minutes to eliminate any remaining stickiness. The water inlet valve to the jar is opened, flushing the tannic acid.

Tannic acid alone has also been used to remove the stickiness from white bass, sturgeon, and paddlefish eggs. A tannic acid solution of 150 milligrams/liter is often used for this purpose. This solution is prepared by adding 0.75 grams of tannic acid to 5 liters of hatchery water in a McDonald jar just prior to adding the eggs. The solution is aerated with a weighted air stone at the bottom of the jar. The fertilized eggs (1 minute after the water is added to activate the sperm) are placed in the jar. Aeration is adjusted to just keep the eggs in suspension. The air stone is removed after 10 to 12 minutes, and the water inlet valve is opened to the jar. Although not absolutely necessary, an excess quantity of milt appears to help reduce stickiness of the eggs.

When the alkalinity of the water is above 200 milligrams/ liter, additional tannic acid maybe needed. However, an excessive amount of tannic acid can strengthen the egg shell, resulting in difficulties at hatch. For white bass eggs, an additional disadvantage of this procedure over the use of urea-salt is that some batches of eggs are extremely sticky, especially if only a limited quantity of milt is available. In addition, the resulting egg shell is opaque rather than clear, preventing microscopic examination of the developing embryo.

A sodium sulfite (Na_2SO_3) solution can be used to dissolve the gelatinous matrix of catfish egg masses so they may be incubated in hatching jars, eliminating many problems associated with traditional paddle-wheel-trough incubators. The solution is prepared by mixing 15 grams of sodium sulfite with 1 liter of hatchery water. If the water supply has low alkalinity, the pH must be adjusted back to that of the hatchery water supply using 10 percent hydrochloric acid (HCl). The egg mass is removed from the spawning container at least 24 hours after spawning and placed in a plastic pan. One liter of the sodium sulfite solution is added per 500 grams of eggs. The egg mass is gently kneaded and stirred until the gelatinous matrix is completely dissolved. The entire contents of the pan is poured into a hatching jar and the water flow is adjusted to gently tumble the eggs without washing them from the jar. No more than 1400 grams of eggs should be incubated in a seven-liter hatching jar. Dead eggs, white in color, float near the top of the egg mass and should be removed by siphon to prevent fungus problems.

Conclusions

The spawn can be taken from fish by several different methods, including allowing the fish to spawn in the tank, hand stripping, and surgically removing the eggs. When hand stripping or surgically removing the eggs, either fresh or stored milt can be used to fertilize the spawn. Ovulated eggs of many species of fish have a sticky exterior. Several common preparations can be used to eliminate the sticky layer of fish eggs.

Induced hatchery spawning requires a continuous series of steps. A mistake during any phase of the process can diminish or completely obliterate the success of the project. Consistent performance requires strict attention to detail.

The work reported in this publication was supported in part by the Southern Regional Aquaculture Center through Grant No. 89-38500-4516 from the United States Department of Agriculture.