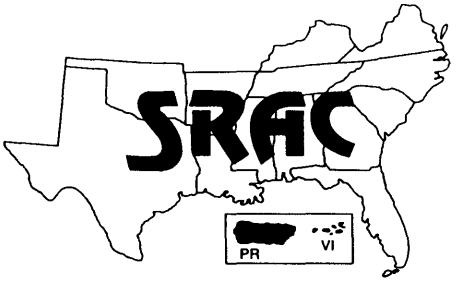
SRAC Publication No. 423

Southern
Regional
Aquaculture
Center



November 1991

Determining Sexual Maturity of Broodstock for Induced Spawning of Fish

R.W. Rottmann, J.V. Shireman, and F.A. Chapman*

Hormones injected for induced spawning of fish do not produce eggs and sperm (gametes); they only trigger the release of fully developed gametes. Fish must not only be sexually mature but also in the advanced stage of sexual development before induced spawning will be successful.

The external appearance of brood fish has long been used to assess the stage of sexual development. In some species, males change in appearance during the spawning season (e.g., vivid breeding colors in cichlids, hooked jaw in salmon, and roughened texture of the pectoral fins or head of cyprinids). These physical changes make it relatively easy to identify sexually mature males. However, secondary female sex characteristics such as plumpness of the abdomen and redness of the vent are extremely subjective and can be misleading.

Taking brood fish from the spawning grounds at the height of the spawning season eliminates most of the guesswork. However, the capture of broodstock from non-spawning areas or use of hatchery reared fish may be preferable or the only option available.

* Institute of Food and Agricultural Services, University of Florida

Sampling eggs and sperm

Sampling the eggs and sperm of the brood fish eliminates the guesswork in determining the stage of sexual development. Brood fish must be sampled quickly and carefully to minimize physical injury and stress. The importance of proper handling cannot be overemphasized. Before sampling the testes or ovaries, the fish maybe quieted, if necessary, with anaesthetic such as MS-222. It is best to keep the fish in the water when sampling. Time spent with the fish out of the water can mean the difference between a perfect spawn, no spawn, or death of the fish.

Sampling the testes

Milt can usually be stripped from males of most species when they are ready for spawning by applying gentle pressure to the abdomen between the pelvic fins and the vent. The color of the milt is usually creamy-white to grayish-white. 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.

Sperm viability usually can be determined by observing motility with a microscope (e.g., 60x). The male is turned belly up, and the vent area is dried by blotting with a towel. The area just behind the pelvic fins is gently massaged toward the vent to strip the milt. The first few drops of milt are wiped away. A sample of milt is collected by inserting the tip of a eyedropper into the urogenital opening. Suction is applied while stripping to draw milt into the eyedropper. Care must be taken to insure that water, urine, intestinal contents, slime, or blood are not mixed with the milt. A drop of milt is placed on a cover slip, and a drop of water is placed on a glass slide. The cover slip with the milt is placed on the drop of water, gently pressed over the slide, and immediately observed under the microscope. The sperm remain active in water for a very short period of time, usually less than 1 to 5 minutes, depending on the species of fish and the temperature of the water. Males with milt that have no or low motility should be discarded.

Sampling the ovaries

Several tests are available to determine the developmental stage of the eggs in the fish's ovary. Two common methods are: 1) egg appearance; and 2) physiological

state of the egg. Both require that an egg sample be taken from the fish. For species that reproduce during a precise spawning season, only a small number of females need be sampled to get an indication of their stage of development. However, if there is a wide variability in egg development, it is best to sample each female.

The ovaries can be sampled with either a rigid or flexible tube (catheter). Rigid catheters are usually made from lengths of glass or hard plastic tubing. Flexible catheters are prepared from lengths of polyethylene or vinyl tubing. The catheters must have an outer diameter small enough to be inserted through the genital opening and sufficient inner diameter to accommodate the eggs. The leading edge of the catheter should also be smoothed or rounded to prevent damage to the fish.

To collect an egg sample, the catheter is inserted through the genital opening and rotated, while gently threading it down the oviduct into the ovary (Figure 1). Forceful pressure will puncture the oviduct or ovarian wall, Sampling with a flexible catheter minimizes damage to the oviduct, and it will not break off in the fish if she struggles when the tube is inserted. If resistance is felt, the tube should be removed and reintroduced at a slightly different angle. If necessary, suction may be applied to the catheter by mouth or a syringe to draw a small number of eggs into the tube.

In China, a metal or plastic rod with a rounded-conical end and a cavity cut in the rod (Figure 2) is used to sample the ovaries of Chinese carps. The rounded tip is inserted in the genital opening, and because of its shape, does not puncture the curved oviduct. Once the cavity of the rod is in the ovary, it is rotated one full turn and withdrawn. The eggs are retained in the cavity.

An egg sample can also be taken from sturgeon and paddlefish by making a small (20 mm) incision

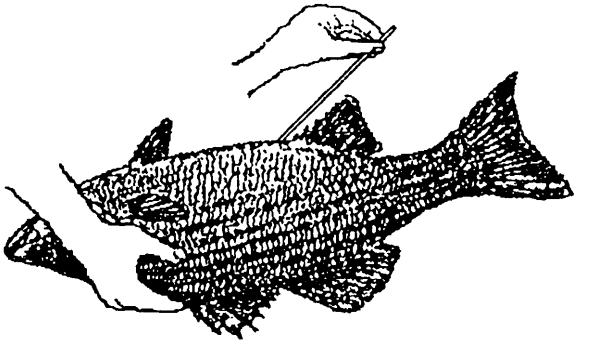


Figure 1. Collect an egg sample by inserting a catheter through the genital opening, into the ovary.

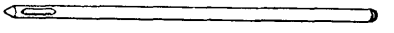


Figure 2. A metal or plastic rod with a rounded-conical end and a cavity cut in the rod used to sample the ovaries of Chinese carps.

along the belly of the fish. First, a small amount of physiological saline solution is drawn into a flexible tube. The tube is inserted through the incision into the ovary, and the saline solution is released. Suction is applied to draw a small number of eggs into the tube. The incision is closed with a half-circle surgical needle and suture material (Figure 3); the area is then treated with an antibiotic.

Determining egg maturity

Visual examination

The diameter and general appearance of the egg are indicators of development. Approximate diameters of ripe eggs of different species of fish are presented in Table 1. The color of ripe eggs also varies with the species of fish. Immature eggs are much smaller than ripe eggs and are usually nearly clear or opaque white or yellow, depending on the fish species. Eggs that have begun to break down (resorb) in the ovary appear whitish in color. Under

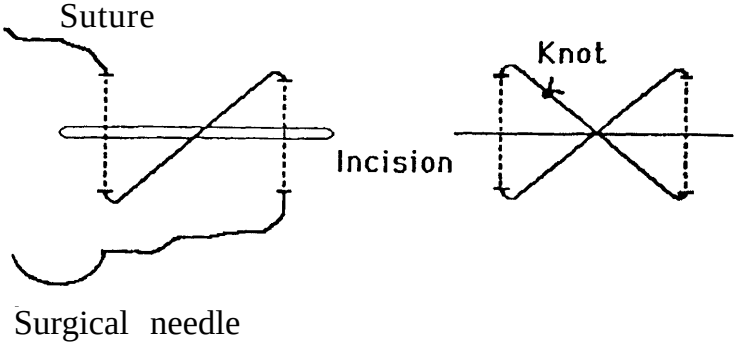


Figure 3. Use of a half-circle surgical needle and suture material to close the incision used to sample the ovaries of paddlefish and sturgeon.

Table 1. Approximate diameter of mature eggs for various species of fish.

Species	Diameter
Bighead carp *(Hypophthalmichthys nobilis)*	0.9-1.2 mm
Channel catfish *(Ictalurus punctatus)*	2.3-2.8 mm
Common carp *(Cyprinus carpio)*	0.9-1.2 mm
Grass carp *(Ctenopharyngodon idella)*	0.9-1.2 mm
Gray mullet *(Mugil cephalus)*	0.6-0.8 mm
Red-tailed black shark *(Labeo bicolor)*	1.0-1.4 mm
Snook *(Centropomus* sp.)	0.6-0.7 mm
Striped bass *(Morone saxatilis)*	1.0-1.2 mm
Sturgeon *(Acipenser sp.)*	3.5-4.0 mm
White bass *(Morone chrysops)*	0.6-0.7 mm

the microscope, eggs that have begun to resorb appear irregular in composition, and the egg contents appear to have pulled away from the cell membrane (Figure 4). Eggs that have begun to resorb often appear soft or partially deflated rather than firm and round.

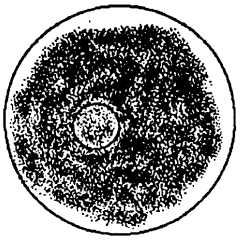


Figure 4. Under the microscope, irregular appearance in composition of eggs that have begun to resorb. Note that the egg contents appear to have pulled away from the membrane.

The eggs of some species of fish (e.g. striped bass, white bass, snook) progressively clear or become transparent as they near ovulation. This process is the result of coalescence of the oil droplets into several large droplets and then into a single oil globule. This clearing of the egg is used to estimate the time of ovulation. Only eggs of fish that are within 15 hours of ovulation can be accurately staged using this method. Immature eggs appear much smaller and remain opaque following hormone injection.

Position of the nucleus in the egg

Movement of the nucleus (germinal vesicle) from the center of the egg to the edge (germinal vesicle migration) is a preliminary step to ovulation. Observing the position of the nucleus is a good method of determining egg development. The nucleus of an egg in the resting phase is located in the center (Figure 5). As the egg matures the nucleus moves to the end (animal pole) that contains the opening(s) (micropyle) through which the sperm enters. When the nucleus is near one edge of the egg (Figure 6), the eggs are considered ripe and the fish should be injected for

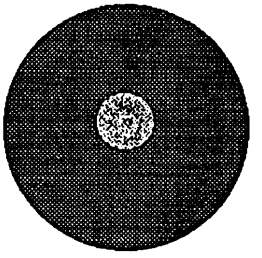


Figure 5. Appearance of an egg under the microscope in the resting phase, with the nucleus located in the center.

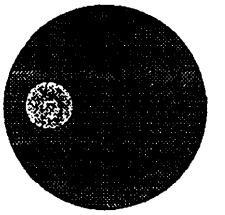


Figure 6. Appearance of mature egg under the microscope. Note that the nucleus is near one edge of the egg.

hormone-induced spawning. The time interval for migration of the nucleus of the egg varies between species and is affected by environmental parameters, especially water temperature.

The nucleus can be observed in some species (e.g., goldfish, common carp, grass carp, bighead carp, snook, red drum, pompano, red-tailed shark, rainbow shark) by placing a cover slip over the eggs on a glass slide. The weight of the cover slip slightly compresses the egg. When lighted from below, the nucleus appears as an translucent circle in the opaque egg when viewed under a microscope or hand lens. The orientation of the egg on the slide will determine the observed position of the nucleus (i.e., if the animal pole is toward the cover slip or slide, the nucleus will appear to be in the center). However, if the nuclei of a great majority of the eggs in the sample are near the edge or off-center, there is a high probability that the eggs are ripe.

Clearing solutions have been used to facilitate viewing of the nucleus, especially in species with eggs that are too opaque to use the "cover slip" technique. A solution of 60 percent ethanol, 30 percent formalin, and 10 percent glacial acetic acid by volume has shown to be effective. Within 5 to 20 minutes in the solution, the nucleus of the egg can be seen.

To determine the position of the nucleus in sturgeon and paddle-

fish eggs, the eggs are boiled in water for 5 to 8 minutes and then cooled. A razor blade is used to cut the egg in half along its length, bisecting the animal pole. The position of the nucleus can then be determined using a hand lens or microscope. The nucleus appears as a dark sphere (Figure 7).

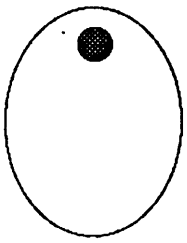


Figure 7. Appearance of mature sturgeon and paddlefish egg under the microscope. Note that the nucleus is near the animal pole of the egg.

Unfortunately, techniques have yet to be developed to observe the position of the nuclei in the eggs of all species of fish (e.g., catfish). The diameter and general appearance of the egg are the best indicators of development for these species.

Hormone assay

Changes during final egg maturation such as breakdown of the nuclear membrane of the egg (germinal vesicle breakdown) occur just prior to ovulation as a result of the first meiotic division. Eggs in the advanced stage of development will undergo germinal vesicle breakdown and sometimes ovulation outside the body *(in vitro)* when placed in a solution containing steroids or gonadotropin. This may be used as a test to determine if egg development is sufficiently advanced to respond successfully to injected hormones.

The eggs are incubated in a solution containing a maturation-inducing substance (e.g., progesterone). Progesterone (4-Pregnone-3,20-dione) is insoluble in water and must first be dissolved in alcohol. An effective solution for hormone assay is prepared by mixing 10 mg of progesterone in 10 ml of 100 percent ethyl alcohol; the final concentration is 1.0 mg / ml. Then 0.2 ml of the progesterone solution is added to 20.0 ml of tissue culture media (e.g., L-15 Medium Leibovitz, physiological saline) in a sterile disposable petri dish and gently swirled to mix. The eggs are added to the petri dish with the solution and are allowed to incubate at the spawning temperature of the species for at least 12 hours. The progesterone artificially stimulates egg maturation.

At the end of the incubation period, the eggs are examined for the presence or absence of the nucleus. The nucleus is observed in the egg using the procedures outlined in the section titled "Visual examination." If no nucleus can be detected (germinal vesicle breakdown) in a high percentage (80 to 100 percent) of the eggs, the females are considered to be suitable for hormone-induced spawning.

Conclusions

External appearance of brood fish has long been used to assess the stage of sexual maturity. In some species of fish, the males change in appearance during the spawning season. Milt can usually be stripped from males of most species when they are ready for spawning. However, secondary female sex characteristics are extremely subjective and can be misleading. Several methods are available to determine the stage of development of the eggs in the fish's ovary. These require that a sample of eggs be taken from the fish. The diameter and appearance of the egg and the position of the nucleus in the egg are visual indicators of development. The steroid assay procedure determines the physiological response of the eggs to hormones. An understanding of sperm viability and egg stage development will greatly improve the success of hormone-induced spawning of fish.

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.