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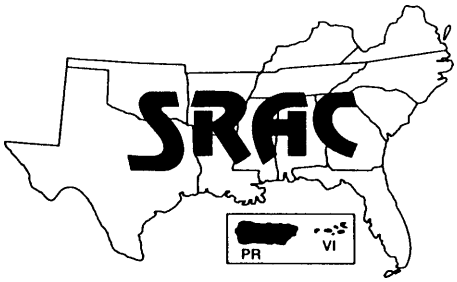

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Hormonal Control of Reproduction in Fish for Induced Spawning

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Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances. The internal mechanisms that regulate spawning are similar for most fishes. The external environmental factors that control reproduction, however, vary considerably among species. For this reason, more is known about the internal regulatory mechanism of fish reproduction than the specific environmental requirements for spawning each species.

Many fish spawn in environments that are nearly impossible to simulate in a hatchery. Hormone-induced spawning is the only reliable method to induce reproduction in these fishes. Hormone-induced spawning of fish has been used for almost 60 years. Surprisingly, the same procedures, with only minor modifications, have been used to spawn an entire range of fishes from the ancient sturgeon and paddlefish to carp, catfish, salmon, sea bass, redfish, snook, and mullet.

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Reproductive control mechanisms

Environmental factors that have been shown to play a significant role in the reproductive cycle are:

- photoperiod;
- water temperature;
- water quality (e.g., dissolved oxygen, pH, hardness, salinity, alkalinity);
- flooding and water current;
- tides and cycles of the moon;
- weather cycles (e.g., atmospheric pressure, rainfall);
- spawning substrate (e.g., aquatic plants, sticks, gravel, spawning mats, spawning caverns);
- nutrition;
- disease and parasites; and
- presence of other fish.

These factors do not function independently of one another, but are interrelated. While proper environmental conditions stimulate the reproductive process, unsuitable conditions can override any attempt at induced spawning.

The internal mechanism that regulates the process of reproduction in fish is the **brain-hypothalamus-pituitary-gonad** chain (Figure 1). This mechanism is complex, and additional scientific information is continually being added. The following is a simplified explanation.

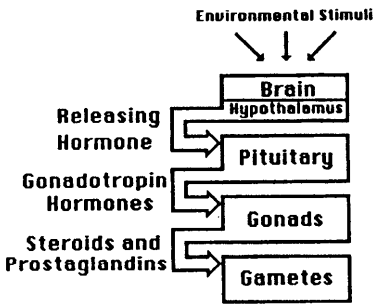

Figure 1. Mechanism that regulates reproduction in fishes.

Environmental stimuli are received and translated by the **brain.** Stimuli of reproductive importance are routed to a portion of the brain called the **hypothalamus.** The hypothalamus produces go-

nadotropin releasing hormone (GnRH) and also gonadotropin release inhibiting factors. Experimental results suggest that **dopamine** is a substance that inhibits the release of gonadotropin.

Gonadotropin releasing hormone (GnRH) is thought to stimulate the **pituitary,** a small gland located beneath the brain, to produce and release **gonadotropin hormones (GtH).** Studies of induced ovulation of many fishes using injected pituitary extract indicate that an increased blood GtH is a prerequisite for ovulation.

Gonadotropin hormones (GtH) act on the ovaries and testes **(gonads). Steroids and prostaglandins** appear to be the local ovarian mediators of GtH action causing release of the eggs. Elevated blood levels of GtH trigger two distinct ovarian processes: 1) final maturation of the egg, which appears to be stimulated by steroids (e.g., progesterone) that are produced by the follicle, and 2) rupture of the follicle (ovulation), which evidently is stimulated by prostaglandins. Steroids also appear to induce spermiation in the male.

Hormones for induced spawning

Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. The primary substances used for hormone-induced spawning have been:

- **pituitary extracts** and
- **purified gonadotropins** to stimulate the ovaries and testes;
- **LHRH analogs (LHRHa)** alone or in combination with
- **dopamine blockers** which enhance the potency of LHRHa to stimulate the pituitary; or
- **steroids** to stimulate the gametes directly.

The appropriate hormone preparation should be selected on the basis of the species to be spawned and the availability of the hormones. Many variables impact the ability of injected hormones to induce spawning, including: 1) condition of the fish; 2) stage of sexual maturity; 3) size of the fish; 4) previous spawning history, 5) water temperature; and 6) season of the year.

Pituitary extract

The pituitary gland produces and stores gonadotropin hormones (GtH), which play a decisive role in ovulation and spermiation. Injected pituitary material bypasses the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood GtH levels that normally precedes spawning (Figure 2).

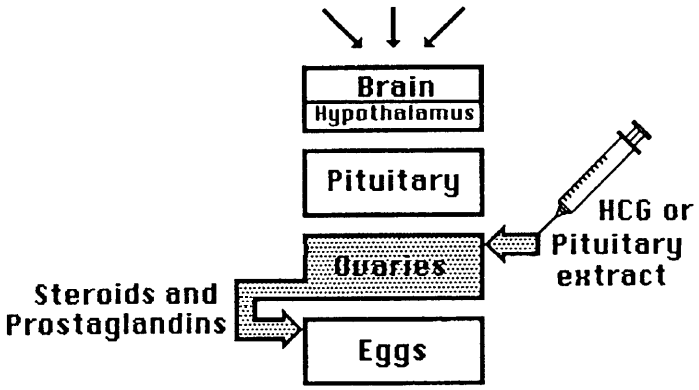

Figure 2. Mode of action of injected pituitary extracts and purified gonadotropin (e.g., HCG).

Fresh pituitaries

The first material used for hormone-induced spawning was pituitary glands collected from fish of the species to be spawned. This method is still widely practiced today. To gain access to the pituitary, the top of the skull is removed with a saw or knife. When the brain is removed from the skull, the pituitary remains connected to the brain in some species, or more commonly, the gland is left behind on the base of the skull. Unfortunately, brood fish must be killed to obtain the pituitaries, because hormone content is greatest in sexually mature fish just prior to spawning. This is a problem when adult fish are scarce.

Fresh pituitary glands should be used immediately or preserved by either freezing or acetone-drying. Glands can simply be placed in a sterile vial or plastic bag and stored in a freezer until needed. To acetone-dry, the glands are immediately placed in a vial with acetone. After collecting the required number, the acetone in which the glands were placed is drained off and replaced with fresh acetone. The acetone is again changed 8 to 12 hours later. After 24 hours in acetone, the glands are air dried on a paper towel. The dried pituitaries are then stored in a sealed clean vial at room temperature or in a desiccator. They c&-t be stored in this manner for 5 to 8 years.

Commercial pituitary extracts

Common carp pituitary or salmon pituitary extracts, available commercially, are widely used for induced spawning. These are crude acetone-dried powdered whole pituitaries. As with fresh pituitaries, these preparations also contain the pituitary tissue and hormones unrelated to reproduction, in addition to GtH. In general, the closer the donor species is related to the recipient fish, the greater the chance of successful induced

spawning. Therefore, carp, goldfish, Chinese carps, catfish, etc., are more likely to spawn successfully when injected with pituitary extracts from carp. Salmon, trout, etc., are more likely to spawn successfully when salmon pituitary is used. However, both are effective on a wide variety of fish species.

There is always uncertainty about the hormone potency of pituitary material. Hormone content necessary for spawning is greatest in sexually mature fish just prior to spawning and lowest in immature fish and mature fish after spawning. The potency of pituitary material can also be destroyed by improper collection, processing, or storage.

Purified gonadotropin

To better quantify the hormone injected, purified gonadotropin hormones are frequently used. Human Chorionic Gonadotropin (HCG) is the most common purified gonadotropin hormone used for induced spawning. In fish, the injected gonadotropin mimics the natural GtH produced by the fish's pituitary. Just as is the case with pituitary extracts, purified hormones such as HCG bypass the brain-pituitary link, acting directly on the ovaries and testes (Figure 2). HCG has been used to spawn fish such as striped bass, white bass, red drum, catfish, and mullet.

HCG + pituitary extract

HCG, however, is not effective on all species. HCG has been used in combination with common carp pituitary extract; for some species, the combination has shown to have improved potency than either preparation used alone. The two hormones can be prepared and injected separately, or the HCG solution can be used when mixing the pituitary extract.

Luteinizing hormone-releasing hormones

Injections of mammalian Luteinizing Hormone-Releasing Hormone (LHRH) have been used experimentally to mimic the fish's GnRH. However, a comparatively large dose and frequent injections were required. Recently, synthetic LHRH analogs, referred to as LHRHa or GnRHa, have been manufactured. These hormones last longer in the fish's system and have potent stimulator effects on ovulation and spermiation in fishes. Therefore, only one or two small doses are needed to induce spawning. LHRHa stimulates the fish's own pituitary to produce and release the GtH necessary for spawning (Figure 3). LHRHa has been used to induce ovulation in a wide range of fishes. One of the synthetic analogs that has been used successfully is **Des-GLY^{10},** [D-Ala^{6}]-LH-RH Ethylamide.

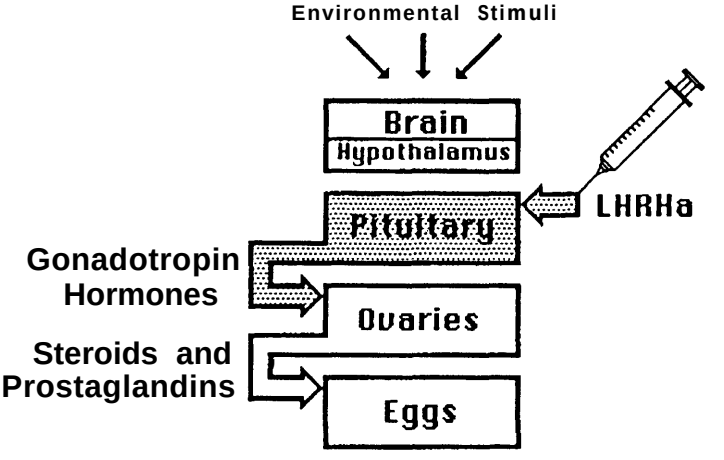

Figure 3. Mode of action of injected synthetic LHRH analogs (LHRHa).

LHRHa + dopamine blockers

Although LHRHa has not been shown to be species specific, some fish do not respond to injections of LHRHa alone (e.g., goldfish, red-tailed black shark, rainbow shark). Dopamine inhibits the release of hormones from the pituitary, effectively blocking the pituitary's positive response to injected LHRHa. There is a family of drugs that act as dopamine blockers, either by preventing the release or by inhibiting the binding of dopamine. Experimental results indicate that the use of dopamine blockers prevents this negative feedback, enhancing the effectiveness of LHRHa for these species (Figure 4).

Because of the tremendous variety of aquarium species and their individual spawning requirements, as compared to food and sport fish, development of hatchery spawning technology has been more difficult. Many ornamental species have had to be imported from wild populations. The use of LHRHa with dopamine blockers has helped change this situation.

Haloperidol {4-[4-(4-chlorophenyl)-4-hydroxy-piperidino] -4'- fluorobutyrophenone} has been used recently as a dopamine blocker in ornamental fishes and tested experimentally for food and sport fish production.

Steroids

Several steroids (e.g., progesterone and testosterone) have been tried experimentally for inducing maturation, ovulation and spermiation in fishes. However, there appears to be little indication of widespread importance of these substances for hormone-induced spawning.

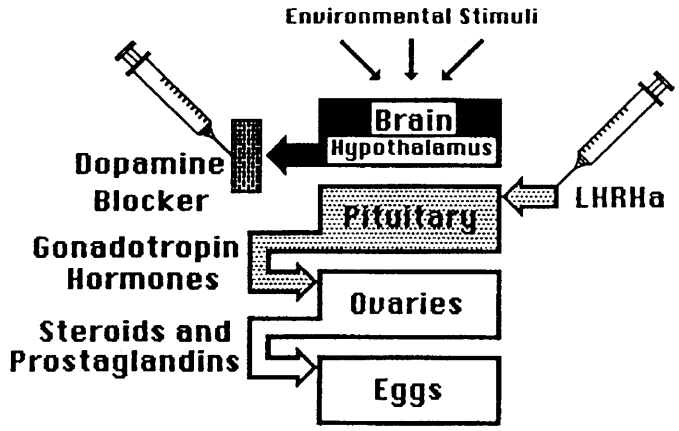

Figure 4. Mode of action of injected dopamine blockers, enhancing the effectiveness of LHRHa.

Conclusions

Reproduction in fishes is regulated by both internal mechanisms within the fish and external environmental factors. The environmental factors trigger the internal mechanisms into action. The internal mechanism that controls the process of reproduction in fish is the brain-hypothalamus-pituitary-gonad chain. Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. The primary substances used for hormone-induced spawning are: (1) pituitary extracts and purified gonadotropin to stimulate the ovaries and testes; or (2) LHRH analogs (LHRHa) alone or in combination with dopamine blockers which enhance the potency of LHRHa to stimulate the pituitary.

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