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The development of sexual glands, maturation, and spawning in fish are regulated by strict periodicity. This results from a long time adaptation to the environment. Once maturity is reached, this periodicity is repeated annually or every other year in all fish except those that spawn once in a lifetime, such as chum salmon. For artificial propagation in fish culture, it is important that the sex periodicity and breeding behavior of the cultured species be understood.

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20. ABSTRACT (Continued)

The development of gonads in fish is controlled by hormones secreted by the pituitary glands. Maturity and sexual behavior are also affected by the hypophysis, thyroid, adrenal cortex, epiphysis and sex glands.

Generally speaking, ecological factors, such as high water temperature, long daylight, ample food, and good water quality (DO, pH, hardness, and salts), can cause fish to mature earlier. In addition, water movement, the nature of the substratum, material for egg attachment, and the presence of the opposite sex, all play a part in fish maturation and spawning.

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Chapter 6

BIOLOGICAL BASIS OF FISH PROPAGATION

Each fish species reaches maturity at a certain age. Even when maturity is reached, sexual glands show cyclic changes. Within a single year, the ovaries of most species of fish mature only once; however, they mature several times in some species and, in still others they mature only once in two years. During the spawning season, some fish spawn their eggs all at once; others spawn in several installments. The development of the sexual glands is controlled by hormones, which in turn, is controlled by the nervous system. External factors also exert some influence.

Sexual Maturity and Sexual Cycles in Fishes

Fish gonads are developed from two dorsal (reproductive) folds in the coelom. During the stage when the epithelial cells transform into primordial sexual cells, sexes are undistinguishable. It is only after the formation of ongonium and spermatogonium, that the sexual cells follow different paths in becoming sperm and eggs.

1. Development, maturity, ovulation, spawning and degeneration-resorption of teleost eggs. According to Meyeh, three stages are passed from oogonium to mature eggs. (a) Cleavage stage -- from oogonium to oocyte; (b) growth stage of oocyte -- pairing of chromosomes and minor and major growing periods; (c) maturing stage -- eggs fully matured. The latter two stages are especially important from the standpoint of artificial propagation.

During the minor growing periods of the oocytes, because both the nucleus and cytoplasm enlarge in size, oocytes increase in volume. This period can be subdivided into early and late stages according to cell morphology. In the early stage, egg membrane is very thin, and its outer surface is scattered with elongated nucleus-like substances; cytoplasm granular; nucleus oval-shaped, large, occupying the major portion of the oocyte, its inner wall lined with many nucleoli, and the central portion is occuped by chromatin. In the late stage, a layer of follicle cells is added to the egg membrane; it is formed from single layer of epithelial cells containing elongated nuclei. Otherwise there is no further change from the early stage.

Oocytes during the minor growing period are in Phase II maturation. Ovaries containing mainly Phase II oocytes are said to be in Stage II. This is a short period in some fishes, but can last a few years in other fishes. The major cultured species such as the four cyprini ds in China belong to the latter category.

The major growing period is the stage of nutritional substances. Due to the accumulation of yolk and fat, oocytes greatly increase in volume. Vacuoles appear along the periphery. Yolk at first accumulates in the vacuole area, later on gradually extends into the center (sometimes starting from around the nucleus). The nucleus is centrally located, irregular or oval shaped. There are many round or oval nucleoli along the inner egg membrane. These nucleoli may number to several hundred in oocytes of the carp and catfishes; and they are usually arranged in two concentric circles. The precipitation of yolk may be distinguished in two stages: the initial precipitation stage and yolk filling stage. During the former stage, egg membrane thickens and shows radiating striations. The epithelial cells in the follicle membrane are stratified into two layers; the inner layer possesses large, oval nuclei, and outer layer consists of flat elongated cells. Inter-yolk-granular cells become reticular in structure. Oocytes in this stage are in maturation Phase III, and ovaries consisting primarily of Phase III oocytes are said to be in Stage III. Spring spawners reach Stage III during the previous autumn. Toward the end of autumn when temperature drops, egg cells enter into yolk filling stage. The characteristics of oocytes in this stage are that while there are still two layers if follicle membranes there appears a layer of funnel-shaped cells between the follicle and egg membranes, and that yolk granules fill up the entire cytoplasm. Only a very thin layer inside the egg membrane is devoid of yolk granules. Yolk granules are varied in shape and react differently to stains. Generally three kinds can be distinguished after using Mallory stain: (1) those close to the nucleus are dark red; (2) middle ones are orange; (3) outer ones are blue. Based on recent histochemical studies, the outer blue globules are not yolk but are polysaccharides. Their function is not as nutritional or body building material for the embryo or larval fish, but rather is used to absorb water. At this time the nuclei have a wavy membrane, become irregular in shape or elongated. In buoyant eggs, there appear many oil globules, which vary in shape and size according to species. Yolk is of phosphoprotein, and when it fills up the entire oocyte, vegetative growth is completed, and the eggs have reached their final size. In the grass carp, black Chinese roach, and the domestic bigheads, they are about 1.5 mm. Oocytes at this time are in maturation Phase IV, and ovaries consisting mainly of Phase IV oocytes are said to be in Stage IV. Many spring spawners enter into Stage IV during the previous winter.

After completing vegetative growth, oocytes undergo maturation of the nucleus. This is called maturation stage. Characteristics of this stage are: polarization of the nucleus and the transformation from tight to loose eggs. It usually takes only a few hours or up to a day or two to complete the maturation. Initially yolk granules coalesce into a mass, and the outer surface of the egg becomes semitransparent. The nucleus and surrounding cytoplasm migrate toward the micropyle, and polarization appears. At the same time, nucleoli move from the periphery toward the center, and there start to dissolve in the nucleoplasm.

Then, the nuclear membrane dissolves, and chromosomes undergo first maturation division, liberating the first polar body. Immediately after that, second maturation division takes place. It is during the middle part of this division that the eggs are cast outside of the follicles and become mature and loose and are ready to be deposited and fertilized. Ovaries are said to be in Stage V.

During the maturation of the eggs, the epithelial cells of the follicles secret a substance which dissolves and absorbs the tissue between follicle and egg membranes, thereby liberating the eggs from the follicles. At this time, what is previously called radiating membrane is now the egg membrane.

If conditions are such as to prevent the parent fish from spawning, eggs that are in their Stages III and IV will degenerate and be absorbed, and the ovary will revert to Stage II or III. The processes of degeneration and resorption of eggs are: radiating membrane starts to deteriorate due to the effect of secretion from epithelial cells of the follicle; nucleus enlarges in size and deforms due to accumulation of liquid; nucleoli dissolve; nuclear plasm merges into cytoplasm; epithelial cells in the follicles start engulfing yolk masses; this engulfing activity is joined by the surrounding connective tissue and lymph cells. The entire resorption process consumes a very short time. When resorption is completed, the ovary returns to Stage II. Oocytes in Stage II are strongly resistent to unfavorable conditions.

Ovulation induced by artificial means does not always result in normal egg deposition. This is because ovulation and egg deposition are two different things; they come one after the other and are under different controls physiologically. It is therefore incorrect to think that once artificial ovulation is succeeded, egg deposition will always follow.

2. Maturation stages of ovary. Six stages can be distinguished according to the morphology of egg cells and the characteristics of the ovary itself.

Stage I ovary: The pair of ovaries are transparent, fine filament looking. Oocytes are irregular in shape, 10-15 in diameter. Nucleus is large, over half the size of the oocyte. The connective tissue and blood vessels are very feebly developed. The grass carp, black Chinese roach, and the two bigheads remain in this stage during their second summer.

Stage II ovary: Still transparent, but slightly pinkish due to the presence of a number of fine blood vessels. The egg cells are in the monor growth period, and are 100-200 in diameter. In ovaries that pass through Stage II for the first time, blood vessels and connective tissue are not well developed. However, after spawning, ovaries that degenerate to Stage II have very well developed blood vessels and connective tissue. Furthermore, not all oocytes are in Stage II, some are in the transitional stage between II and IV.

Stage III ovary: Egg cells in this stage are visible to the naked eye. In the white bighead, eggs in this stage are 189-240 in diameter. Blood vessels are relatively well developed; they are branched and are distributed along the septa in the ovary. Eggs close to the septum are larger. Egg cells start to accumulate yolk, but may still contain cells that belong to earlier stages. This is a relatively short stage. In the labrids, for instance, this stage occurs only from mid-August to the end of September. In the white bighead, it occurs during autumn and winter.

Stage IV ovary: Connective tissue and blood vessels are very well developed; eggs large. Eggs of the white bighead measure 801-1128 in diameter, round or polyhedral. The entire ovaries are so large as to fill up the abdominal cavity. The ovarian membrane is elastic. The development of oocytes in this stage is of two kinds: One in which the eggs are spawned in one installment. Here the yolk is already precipitating or is even already filling up. The other in which the eggs are spawned in separate installments and the oocytes are in various stages of development. Because of this, some authors suggest to subdivide this stage into several substages such as IVa, IVb, IVc, etc. This is important to artificial breeding work. Experiments have proved that with the sturgeon, one cannot succeed in inducing the female to produce mature eggs during early stage IV (nucleus centrally located unchanged). IN our work with the cyprinids the same was found true.

Stage V ovary: Egg cells mature and separated. A light pressure on the abdomen will cause the eggs to come off the genital pore. The color of the eggs varies according to species: yellow, green, blue, etc. Ovaries in this stage may also be classified into two kinds: In those that spawn their eggs all at once, the undeposited eggs that remain in the follicles are in the pre-minor growth period and will not mature until the next year. Examples are grass carp, bigheads, etc. In those that spawn their eggs in separate installments, oogonium cells are in various stages of growth. These eggs may mature and be deposited at different times of the year.

Stage VI ovary: This is the post spawning stage. There are also two kinds:

(1) Characteristics of Stage VI ovary of those that deposit their eggs all at once: i. Ovary greatly reduced in size, ovarian membrane soft and loose and deep red in color; ii. There are a small number of unspawned degenerated eggs that are semitransparent and reddish yellow; iii. There are oogonia in minor growth period and chromosome synapsis; iv. Many empty egg follicles which may form a corpus luteum or become resorbed; v. After a certain time the ovary becomes Stage III again and undergoes further development.

(2) In ovaries that deposit their eggs in separate installments, conditions are similar to the above only after the last

batch of eggs are deposited. Otherwise they are characterized by: i. Some empty follicle cells are present but not numerous; ii. There are many transitory oogonia; iii. Stage IV oogonia degenerate to Stage II and develop toward Stage IV; Iv. Ovaries stay in maturing stage for a long time.

(3) The development, maturation, and deposition of sperm cells. The important feature of the development of fish sperm cells lies in the large productive capacity of spermatogonia. This is a safeguard for fertilization in water. Each primordial spermatogonium is wrapped in a tubule formed from a vegetative follicle cell. Within this, it divides and multiplies continuously like somatic cells until a large number of spermatogonia in various stages are produced.

Each spermatogonium passes into a primary spermatocyte during the prephase of maturation division. After the first maturation division, each primary spermatocyte becomes two secondary spermatocytes, which in turn become four spermatids after one more division. Hereafter, no more division takes place and the spermatids grow into spermatozoa. The numerous spermatozoa within one tubule are produced from a single primordial spermatogonium.

Among species that spawn several times, the maturation of sperm cells in the males also takes place at different times. The order of maturation is first, the posterior end; then the anterior end.

(4) Sexual periodicity in fishes. In fishes, the development of sexual glands, maturation, and spawning all are regulated by a strict periodicity. This is a result of long time adaptation to the environment. After maturity is reached, this periodicity is repeated annually or every other year in all fishes other than those that spawn only once during their life time, such as the chum salmon. In artificial propagation, it is important that we understand sex periodicity and breeding behavior of the species that we are dealing with.

The Physiology of Pituitary Glands in Fishes

The development of gonads in fishes is directly controlled by the hormones secreted by pituitary glands. Even many environmental factors that may affect the maturity of gonads are controlled through the pituitary glands. In order to understand this very important problem, some fundamental knowledge on the pituitary gland is discussed below.

1. Morphology and histology of fish pituitary glands. As in other vertebrates, the pituitary glands of fishes are situated on the ventral side of the diencephalon and are connected with the gray ganglion. They differ from those of higher vertebrates in external morphology. Their stem part is very poorly developed and is therefore much more closely associated with diencephalon. The entire pituitary body can be divided into neurohypophysis and adenohypophysis. These two parts are markedly different in

histology. The neurohypophysis is directly connected with the diencephalon and its nerve fibres enter deeply into the adenohypophysis. The adenohypophysis is subdivided into fore, mid, and hind lobes. The forelobe, or proadeno-hypophysis, is the one that is closest to the junction between the hypophysis and diencephalon, not really at the anterior end of the hypophysis. Although located at the basal part of the nerve bundle, few nerve fibres enter into it or go in very far. Cellular structure in the fore lobe is quite uniform and remains unchanged all year round. Its acidiophylic cells do not show secretive activity and the entire lobe is poorly supplied with blood vessels.

Meso-adenohypophysis is ventral to and often anterior to proadenohypophysis, to which it is connected. The secretive activity of its acidiophylic cells is related to the development of gonads and ovulation. In recently spent females, the secretive cells are highly vacuolar. Nerve and blood vessels are richly supplied. It is separated from the hind lobe by the branching-off of nerve fibres. The hind lobe is easily separated from the other lobes after the hypophysis is dehydrated with alcohol.

Meta-adenohypophysis is the farthermost away from the diencephalon junction. In the carp, it is actually the most anteriorly located and occupies more than 1/3 of the entire hypophysis. The cells are slightly acidiophylic, and show secretive activity the year around. Its function has been proven to be related to the contraction of pigment cells. The hind lobe is highly vascular; the blood vessels form a network. After dehydration with alcohol, it turns deepest in color, and is therefore easily separated out from the other lobes.

Kinds and Physiological Function of Pituitary Hormones in Fishes

Since the 30's, there has been prominent accomplishment in the induction of ovulation in fishes by the use of pituitary hormones. However, much is yet to be understood theoretically. Briefly, the following points are presented.

(1) Kinds and application of sex-stimulating hormones in the pituitary glands of fishes. In 1930, Houssay used pituitary extracts from various teleosts to stimulate sexual maturity. Thereafter, many workers experimented with fishes by injecting the body with pituitary material or its hormone extracts, urine from pregnant women, or sex-stimulating hormone extracted from serum of pregnant horses. By injecting into gobies with the tissue fluid or extracts of shark's hypophysis, it is possible to induce sperm or yolk production, but not ovulation. Based on experiments in which purified sex-stimulating hormones obtained from higher vertebrates are injected into teleosts from which hypophysis had been removed, Butler (1940) and Hoar (1955) consider that both FSH and LH are present in teleosts; Pickford (1957),

however, maintains that only hormone equivalent to LH is present. The drawback of this kind of experiment is that hormone effect may be host specific, and therefore a negative result does not necessarily prove the absence of that particular hormone in the fish.

On the other hand, positive results have been obtained by injecting into fats sex-stimulating hormones extracted from fish hypophysis. On this basis, it is considered that both FSH and LH are present in fish hypophysis.

Witschi (1955) points out that fish are richer in LH and poorer in FSH than higher vertebrates. He also maintains that LH can be accumulated in secretive cells, but FSH must be secreted as soon as it is produced. Still, whether both hormones are present in all species of fish is not determined.

The question of host specificity in sex hormone is of prime importance in maturity-inducing work. Generally speaking, hypophysis can be effective to species within the same genus, although it can be effective only from one species to the other but not vice versa. It has been reported that long term use of sex-stimulating hormone may produce antihormone in the serum of the receiver fish and nullify any effect. It is also maintained by some that antihormone is present in the hypophysis itself. All of these are, however, speculations based on findings in mammals and not in fishes themselves.

It is shown that in some fishes sex-stimulating hormone is not produced until the fish approach maturity. Others have shown that a certain amount of sex hormone is present in parent fish that are not mature. To ensure success one should use only hypophyses of mature fish.

(2) Physiological function of sex-stimulating hormone in fishes. Since the kinds of sex hormones are not definitely known, we can only make some general statements regarding their physiological function.

i. Effect on sperm development, egg development and ovulation. Vivien (1939, 1941) finds that sex hormones from hypophysis introduced into gobies can stimulate yolk accumulation in early stages of oogonia, hasten maturation in late stage of ovaries and induce ovulation. Many similar experiments have shown that fish hypophyses from the same or different species can stimulate the development and maturation of testes or ovaries and induce spawning in both sexes.

The Fisheries Research Institute of Academia Sinica in 1958 succeeded in inducing carp to spawn and hatch into a large quantity of fry during the middle of winter. It proved that hormone technique would work if the ovaries have reached maturation Stage IV.

ii. Effect on the formation of corpus leutem. In fishes, yellow bodies are formed in both oviparous and ovoviviparous species. In oviparous species, yellow bodies produced before ovulation and those produced after ovulation are of two different kinds. In some fishes, oviductin is produced from yellow bodies before ovulation and it stimulates the development of the oviduct. The function of post ovulation yellow bodies is not well known.

iii. Other functions. In anadromous and Catadromous species, hypophysis hormone can regulate salt content in the blood to cope with salinity changes in the environment. It has some effect too on the production of secondary sexual characters and on the oxygen consumption by the parent fish.

C. Effect of other ductless glands on the reproduction of fishes. The hypophysis is the king of all ductless glands. This is also true in fishes. The ductless glands discussed below are in one way or another associated with the hypophysis.

1. Thyroid. Normally, thyroid in fishes does not play a role in the maturation process. However, during spawning, especially in low temperatures, thyroid secretion becomes more active so that the metabolic rate is sufficient during the process of spawning.

2. Adrenal cortex. It is generally recognized that the adrenal cortex has no direct bearing on reproduction. It can, however, interfere with reproduction indirectly through the hypophysis and sexual glands.

3. Epiphysis. According to Thieblot (1954), epiphysis has an opposing fuction to hypophysis. Injection of epiphysis extract and sex stimulating hormone simultaneously can nullify the function of the latter. Male fish from

which epiphysis has been removed will still exhibit secondary sexual characteristics and sexual behavior.

4. Sex glands. Hormones secreted from sex glands have a direct effect on reproductive function, especially on sex characteristics, sex behavior, and parental care.

D. Ecological factors affecting sexual maturity and sexual periodicity in fishes. Fish of the same species, when grown under different temperature regimens, or under the same temperature regimen but with different food or water quality, may reach initial maturity at a different age. Generally speaking, under high water temperature, long day light, ample food, and good water quality (DO, hardness, pH, and certain salts), fish will mature earlier. Fish in southern China, for instance, generally mature one to two years earlier than those in central China. The quantity and quality of food can affect the development and maturation of sex glands.

Temperature is an important factor affecting metabolic rate. Temperature requirement for spawning in each species of fish is quite definite. Fish will not spawn if the water temperature is lower than that required.

Many species of fish spawn during dawn or twilight. Darkness can depress the secretion of the hypophysis and reduce the function of gonads, a condition similar to that when the hypophysis is removed. When light resumes, the hypophysis restores its function very rapidly and actually increases its activity more than normal, which, in turn, induces the gonads to develop more rapidly. It is apparent, therefore, that the effect of light on the function of sex glands is through the secretion of hypophysis.

It is not a single factor but a combination of factors that affects maturation and spawning. Aside from the factors mentioned above, water movement, the nature of the substratum, material for egg attachment, and the presence of the opposite sex all play a part. Flowing water is particularly important for the spawning of certain species of fish. Water flow not only provides a mechanical stimulus but also increases dissolved oxygen. The carp which spawns in still water also demands some water movement.

Despite the effect of environmental conditions on the maturation and spawning of fishes, artificial propagation has made great strides by the use of pituitary technique.