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Nature of Spermatogenesis, Type of Spawning and Maturity Scale for Testes of Maurolicus muelleri

by

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ABSTRACT

Sex glands of male pearlside at different stages of their reproductive cycle are investigated. Proceeding from the histo-physiological analysis the nature of spermatogenesis, duration and a seasonal basis for separate maturity stages, type of spawning are determined, the maturity scale for gonads is developed. The one year cyclicity of reproductive activity with a long spawning period is revealed. Functional mechanisms ensuring a prolonged engagement of males in reproduction and promoting the rise in their fecundity are analysed.

INTRODUCTION

Our study is the part of complex investigations into the ecosystem of the pelagial in the open part of the North Atlantic carried out by the Polar Research Institute (PINRO).

Pearlside (Maurolicus muelleri) , the representative of the Sternoptychidae family, widely distributed in the epi- and mesopelagial of the World Ocean including the North Atlantic is examined closely.

The task of investigations was reduced to histo-physiological analysis of sex glands of male pearlside. The aim of the study is to reveal the regularities in biology of the species reproduction and to construct the maturity scale

for determining the degree of gonad development under field conditions.

The peculiarities of reproductive biology of male pearlside given in the paper have no reference in literature.

MATERIAL AND METHODS

The materials on pearlside were mainly collected in the central part of the North Atlantic between 48° and 58°N (one sample was taken at 40°N over the continental slope of the Pyrenean Peninsula). The survey covered the greater part of the year cycle (spring, summer, autumn).

Fish were sampled from trawl catches taken 30 to 800 m deep.

The samples were analysed visually at sea. Then fish fixed in Bouin's fluid or 5-10% formalin were examined in the laboratory. Fish were measured and weighed, the weight of gonads and liver being determined by means of the torsion balance. Sections of gonads 4-6 μm thick prepared according to ordinary histological methods were stained after Heidenhain - by azan and haematoxylin (Roskin and Levinson, 1957). Testes of 125 individuals were analysed microscopically.

Micromeasurements were made by ocular micrometers: by the ordinary one with the scale factor 0.7 μm and the MOB - 1 - 15 spiral micrometer with the scale factor 0.04 μm. The cytological analysis of glands and microphotographing were made by means of the MBI-6 microscope.

While developing the maturity scale for testes general principles of 6-point scales accepted in native ichthyology were followed (Kulaev, 1944; Sorokin 1960; Sakun and Butskaya, 1968; Lisovenko, 1970 etc.).

To facilitate the studies of such a small object as pearlside, the following criteria, besides the traditionally used macroscopic features (maturity coefficient, colour, density of gonads etc.), were introduced, namely, the relative length of testes (the ratio of their length to fish body length in %) and position of gonads in the body cavity relatively the intestines.

RESULTS AND DISCUSSION

Anatomical features of the testes.

The testes of pearlside are represented by the twin glands of a lancet-like shape placed in the dorsal part of the body cavity. Excretory ducts are on the dorso-medial side of each gland stretching as far as their central parts. Here the ducts divide and enter the tissue of testes (Fig. 1).

As to the anatomical structure, sex glands of male pearlside belong to the radial, or percoid, type (Fig. 2). Germinative tissue is represented by the system of seminal ampullae on the walls of which sex cells of different formations are located. The ampullae are fixed to the membrane of the testis with their basal parts, their apices being radially directed towards the zone adjacent to the excretory duct. In the cross section of mature testes this zone is represented by the spacious cavity of an irregular shape (now this structural system will be termed "delta" for short). Microscopical analysis of different states of the testes showed that the "delta" performs mainly the sperm storing, or accumulating, function. The degree of its filling may serve as an indicator of functional activity of gonads.

Nature of spermatogenesis and type of spawning.

Sex cells in pearlside gonads undergo certain stages of development similar to those described for other fish species (Sorokin and Grigoryev, 1968; Oganesyan and Grigoryev, 1983). The processes of reproduction, growth, maturation and formation of sex cells take place in testes.

The microscopical analysis of pearlside gonads showed that the spermatogenesis is of asynchronous nature typical of the species with a prolonged spawning period. Asynchronous development of sex cells is especially prominent from the middle of StageIIIand goes on into StagesIV and V. The latter is especially important for understanding variations of spermatogenesis because namely during spawning,

i.e. at Stage V, the peculiar nature of functional activity of testes is most prominent. While in fish with a brief spawning period the production of spermatozoa in fact finishes by the moment of transition into the spawning state (Kulaev, 1927, 1944; Butskaya, 1955, 1959) this process remains very intensive in male pearlside. In the testes of fish captured during the peak of spawning, the whole spectrum of transformation of sex cells is observed - from production of spermatogonia to the formation of mature spermatozoa. The sperm amount reduced during reproduction is, thus, replenished due to additional waves of spermatogenesis accomplished by the formation of new portions of spermatozoa. By the end of the spawning period in each specimen this process gets down gradually, males extrude their spawning products and conclude the spawning. Spermato- and spermiogenesis of pearlside have much in common with the processes described for other fish species with a similar (prolonged) type of spawning (Sakun, 1955; Chertov and Bolkvadze, 1971; Moiseeva and Ponomareva, 1975; Oven, 1974, 1977; Talikina, 1975).

Characteristics of maturity stages (maturity scale). The following stages of maturity are outlined for the testes of pearlside: Stage II* - preparation of immature specimens to maturation; Stage III - maturation; Stage IV - potential (pre-spawning) maturity; Stage V - functional maturity; Stage VI - extrusion (postspawning emptiness of the gland); Stage II_r - recreation (relative quiescence of the gland before the start of the next year cycle). The maturity stages throughout the reproductive cycle of pearlside reflect qualitative changes in gonads at different stages of their development.

Stage II. Histological features. As to the set of sex cells and the anatomo-histological structure, the testes are like those of immature specimens. Sex cells are represented

*There were no fish with gonads at earlier stages of development in the samples.

by spermatogonia of different generations. Frequent are mitoses of spermatogonial cells. Seminal ampullae as a histological structure are not completed yet, and have no gaps in them. The "delta" of the testis is not outlined. The walls of the excretory ducts are squeezed, gaps are empty.

Visual and meristic features. Lobules of testes are flat, translucent or opaque, hardly discernible, fit tightly to the intestines dorsally without approaching the stomach, as a rule. Only testes of fixed fish may be prepared for measurement and weighing.

The maturity coefficient is no higher than 0.5-0.7%. The relative length of testes does not exceed 15-17%.

The maximum length of immature males (Stage II) in the open areas of the North Atlantic, according to our data, is no more than 38 mm, and their weight - 460 mg.

Stage III. Histological features. The start of physiological maturation of testes is determined by the appearance of spermatocytes of the 1st order. Mass mayotic division of spermatocytes and formation of spermatids which in their turn gradually transform into spermatozoa is observed (Fig. 3).

During the stage, as the testes are getting ripe, the ratio of sex cells changes: first spermatocytes prevail, then - spermatids and spermatozoa. By the end of Stage III central gaps are formed in seminal ampullae where spermatozoa joined in bunches go out from ruptured cysts. At the same time apices of separate ampullae start to rupture and the part of spermatozoa go out from gaps into the "delta" and excretory ducts. The system "delta" + duct starts to store the sperm.

Visual and meristic features. During Stage III the appearance and meristic features of the testes change greatly.

At the beginning of the stage lobules of the testes are still rather flat and stretch along the intestines from the anus to the stomach. Glands are opaque-grey, translucent.

As the testes get mature they become opaque, their colour changes to white, they become thicker (especially in the middle) and longer, upper parts of the glands envelope the stomach tightly from the sides and at the top, approaching

the gullet. By the end of the stage excretory ducts start resembling whitish chordae and are visible against a light grey background of the gland.

The maturity coefficient within the stage grows from 0.5-0.7% to 12-14%, the relative length of testes - from 15-17% to 39-41%.

The highest frequency of occurrence of males at Stage III is observed in winter-spring (from January to May). The duration of the stage is nearly 3-4 months.

The minimum length of the first maturing males is 38 mm, the weight - 490 mg.

Stage IV. Histological features. This stage stands for the prespawning state of sex cells. Proceeding from cytological criteria and the nature of hametogenesis, gonads in this period are similar to those at the end of Stage III: the same set of sex cells and their asynchronous development are observed. The main difference lies in the fact that at Stage IV accumulating and excretory systems are developed much better than at the previous stage (Fig. 4). Intra-ampullar cavities (gaps) are well seen and filled with freely located spermatozoa which enter the "delta" through the ruptures of apices of ampullae. The "delta" is expanded, excretory ducts and tubules are overfilled with the sperm.

Visual and meristic features. The size of the testes, their position in the body cavity relatively the intestines and their coloration are the most indicative macroscopic features of the testes at Stage IV.

Lobules of the testes are maximally developed, greatly thickened in the middle, expand notably the abdomen of the fish. They envelope tightly the intestines and the greater part of the stomach, overlapping its front limit with their upper parts. The glands are greyish-white with a creamy hue.

A highly swollen excretory system may be seen on the dorsal side of glands, the colouration of tubules and ducts is notably lighter than the total background of lobules.

On the cross section of the testes the edges become

swollen, drops of pasty sperm become obvious in the region of ducts.

The maturity coefficient of gonads at this stage of development reaches maximum values in the reproductive cycle and amounts to 13-15%. The relative length of glands is also maximum - 40-42%.

Males in the prespawning state are found mainly in April-May; the duration of Stage IV is less than one month.

Stage V. Histological features. The functional maturity is characterized by running testes, i.e. by a gradual discharge of ripe spawning products. With respect to cytology, the first half of the stage is marked by the asynchronous spermatogenesis still going on and, correspondingly, by the availability of sex cells both of late and early generations (Fig. 5).

In the second half of the stage sex cells of concluding stages of hematogenesis - spermatids and spermatozoa - start dominating and, partly, spermatocytes are also present. The activity of production of both initial and medium categories of sex cells (spermatogonia and spermacytes) gradually declines. By the end of the stage only a thin layer or separate nests of spermatogonia from the reserve stock remain as well as rare cysts with spermatocytes and spermatids delayed in development (Fig. 6).

With respect to morpho-histology, the inner structure of the testis within the stage is characterized by the availability of wide gaps in ampullae, by the full opening of their apices, an expanded "delta" and considerable cavities formed by ruptured intra-ampullar membranes. The excretory system and "delta" are filled densely with mature spermatozoa.

At concluding stages of spawning, when the stock of expandable spermatozoa is already not replenished, the amount of half-empty ampullae and cavities in the testis sharply rises. At the same time their area reduces greatly owing to weakening of the tone of intra-ampullar septa and the

membrane of the testis.

Visual and meristic features. In the first half of Stage V lobules of testes are thickened greatly in the central part, envelope tightly the intestines and a greater part of the stomach. Their coloration is white, with a creamy hue. The excretory system is well developed, swollen, purely white against the background of lobules. Drops of fluid sperm are secreting on the cross section of the testes.

As the sperm is being discharged, the glands are getting smaller, reddish,flabby.

At the end of the stage the front edge of lobules slightly overlaps the stomach, and the lobules adjoin only to the dorsal-lateral side of the intestines. The glands are brownish, the intensity of colouration is irregular, the consistency is sluggish.

The maturity coefficient during the stage decreases from 12-14% to 0.7-1%, the relative length - from 39-42% to 17-19%.

The highest frequency of occurrence of males with running testes is observed in May to September. The duration of Stage V is 3-4 months.

Stage VI. The transition of testes to Stage VI manifests the complete fading of spermatogenesis and the cessation of a functional activity of the gland.

Histological features. Out of the sex cells only spermatogonia from the reserve stock remain in the generative tissue, and a small amount of non-extruded spermatozoa - in the gaps of ampullae. No reproduction of spermatogonia is observed in this period.

The inner structure of the testis is represented by the system of reduced seminal ampullae (Fig. 7). The "delta" is poorly outlined, but excretory ducts are still rather densely filled with residual sperm.

Visual and meristic features. The lobules of extruded testes are flat, brownish, flabby; they adjoin closely to the dorso-lateral side of the intestines and slightly overlap the stomach with their apices.

The maturity coefficient of gonads varies from 0.7 to 2% averaging 1.3%; the relative length of the testes is no higher than 20%.

The highest frequency of occurrence of extruded males is observed in September-October. The stage lasts for less than a month.

Stage II_r. For this stage the state of relative quiescence of the germinative tissue is typical. In the testes two simultaneous processes are in progress: removing the consequences of spawning (restoration of inner supporting structures and broken blood vessels, phagocytosis and elimination of residual spermatozoa), and the first stage of spermatogenesis - reproduction of spermatogonia - starts.

Histological features. During the whole stage the residual spermatozoa are present in the excretory system (and sometimes in the ampullar gaps). This is typical of the testes in fish engaged in spawning in contrast to immature fish at Stage II.

Visual and meristic features. In the process of elimination of spawning consequences glands become of lighter colouration, thicken and slightly enlarge (some thickness becomes apparent in their middle part).

The maturity coefficient varies insignificantly within the limits of the stage and usually it does not exceed 1%; the relative length of the testes is no more than 19%.

The highest frequency of occurrence is probably observed in the period from October to January, the total duration of the stage approximating 3-4 months.

Gonads transit from the state of relative quiescence to Stage III.

CONCLUSIONS

Thus, histophysiological analysis of changes in the sex glands of male pearlside permitted to reveal the peculiarities in development of their reproductive system, to determine the approximate duration and the seasonal basis of maturity stages, type and nature of spawning.

The one-year cyclicity is typical of the reproductive function of the testes. Stages III, V and II_r are the longest in the cycle, each of them lasting for 3-4 months. Stages IV and VI are comparatively short - less than one month.

The transition of gonads to the state of functional maturity (running testes) is performed under incompleted spermatogenesis. In the spawning period, alongside with the discharge of milt, the processes of development and maturation of new generations of spermatozoa are in progress in the testes. Owing to this, the stock of mature spawning products is constantly replenished. Such nature of spermatogenesis and extrusion provides the engagement of males in reproduction for a long spawning period (3-4 months) and promotes the growth of their fecundity.

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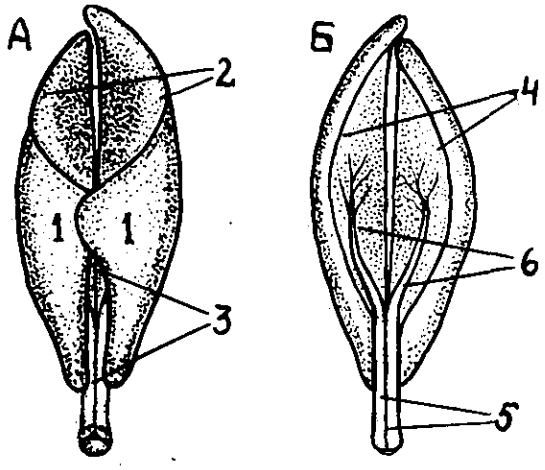

Fig. 1. Scheme of pearlside testes:

А - ventral view; В - dorsal view;
1 - lobules;
2 - stomach hollow;
3 - intestine hollow;
4 - swim bladder hollow;
5 - spermatic tubules;
6 - excretory ducts.

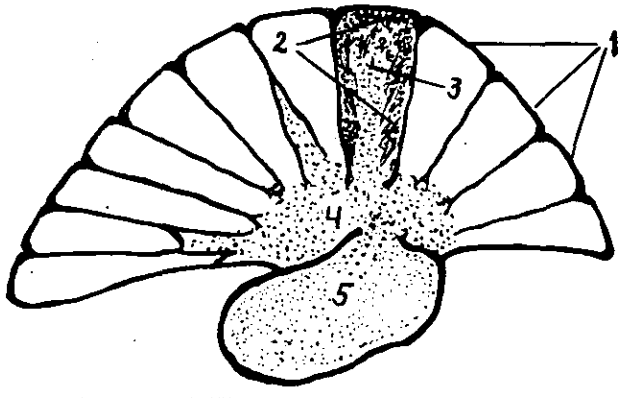

Fig. 2. Scheme of middle part of the lobule in mature testis (cross section):

1 - seminal ampullae;
2 - sex cells of different formations;
3 - gap;
4 - "delta";
5 - excretory duct.

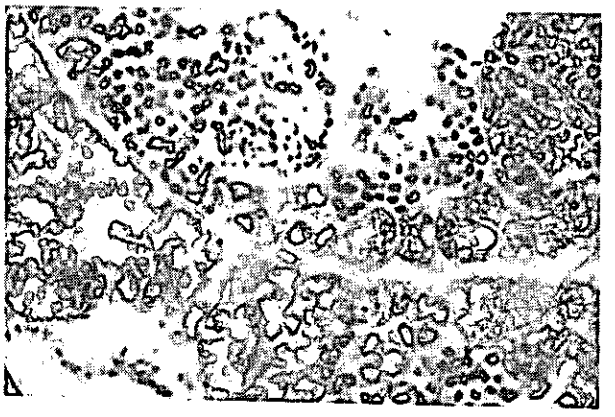

Fig. 3. Asynchronism of spermatogenesis in the middle of Stage III. All categories of sex cells are visible: spermatogonia, spermatocytes, spermatids and apermatozoa. X630 magnification

Fig. 4. "Delta" + duct system in testes at Stage IV. X56 magnification

Fig. 5. Fragment of the testis at the middle of Stage V. All categories of sex cells from dividing spermatogonia to mature spermatozoa are visible. X280 magnification.

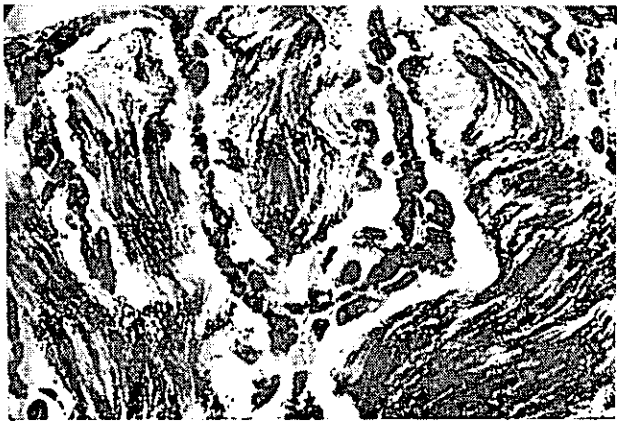

Fig. 6. Fragment of the testis at the end of Stage V. Ampullar gaps are filled with mature spermatozoa. X140 magnification.

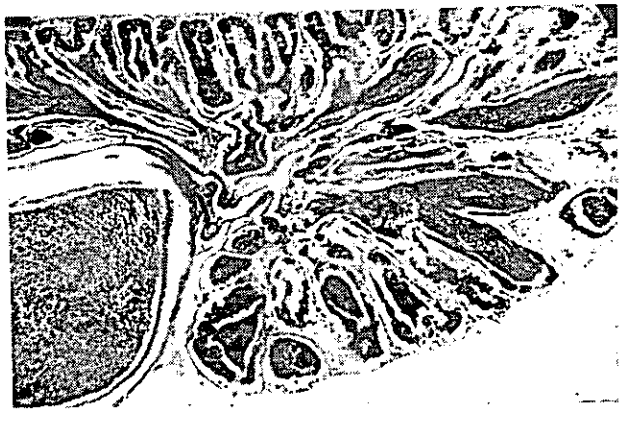

Fig. 7. Structure of the testis at the end of spawning. Half-empty seminal ampullae, excretory duct with residual sperm are visible.
X56 magnification.