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The Reproductive Cycle of Holothuria *Cucumaria frondosa* of the Barents Sea

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Abstract

The study considers the morphological features of the gonad structure and the seasonal changes in the reproductive activity of the gonads of holothuria *Cucumaria frondosa* of the Barents Sea. The sex of individuals can be identified using morphometric analysis with the average mass of gonads of 1.63 g, with the average mass of the skin-muscle sac of 53.00 g and the length of 80.00-83.00 mm whereas the diameter of the genital tubes is not less than 0.50 mm. The spawning of cucumaria, producing plankton progeny, occurs from March to May, but the spawning period can be shifted, since the beginning of the spawning period is associated with the seasonal increase of the surface water temperature and the intensive phytoplankton blooming. The character of spawning is intermittent. Individual fertility is low, in individuals with the average mass of SMS 150.00-200.00 g ranged from 9 to 32 thousand cells.

Keywords: reproduction, gonad, holothuria, reproductive cycle, oocytes, the Barents sea.

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1. Introduction

The abundant species of holothuria *Cucumaria frondosa* inhabits the Barents Sea. The species is included in the register of commercial invertebrates. These animals are inactive and long-living (at least 20-24 years), they become sexually mature in the third or fourth year of their lifecycle, therefore, the rapid irreversible depletion of such biological resources can occur as a result of unsustainable use.

The reproductive cycle of commercial holothuria has been studied by many researchers (15, 14, 5, 7, 13, 3, 8, 9, 10, 11, 12, etc.), but in this regard the cucumaria of the Barents Sea is poorly investigated. In the study of S. A. Oganesyan [4], oogenesis was examined only in the large genital ducts of the gonad; in the work of E. N. Gudimova [2] seasonal changes in the ovaries were described without tackling the histological analysis. To avoid the resources depletion and not to violate the integrity of benthic

communities, the study of the reproductive biology of the species is an essential aspect [1].

The aim of the study was to examine the reproductive biology of females of *C. frondosa* of the Barents Sea.

To achieve the goal, the following tasks were set:

- to reveal the gonad structure and their cellular composition;
- to monitor the seasonal changes in the reproductive activity;
- to define the nature and the period of spawning.

2. Methods and Equipment

The study is based on the materials collected during the research fishing trips organized by PINRO and MMBI from 2000 to 2008. The stations chosen for the survey were selected due to the areas of holothuria accumulation in the Barents Sea (Fig. 1). Animals were harvested by trawling at depths of 76-180 meters. The sampling material was fixed in 4% formalin solution.

During the cameral processing, the length and weight of the skin-muscle sac (SMS) were determined for each individual; the structure of the germinal glands in terms of color, mass, length were described, the total number of sex tubules (thin, medium and thick) was counted.

The gonadal index (GI, in %) was determined as the ratio of the gonad mass *g* to the mass of the skin-muscle sac (SMS) *P* according to the formula: $GI = g / P \times 100$ [2].

Morphometric and histological analyzes were used to study the reproductive biology and the females' gonad maturity stages.

From each individual, 10-20 tubules of the gonads were taken; three sections (1 cm) -- terminal, middle, and the base of the tubule -- were examined in each tubule. Oocytes of different types were counted and their diameter measuring was carried out under a binocular using an eyepiece micrometer. The values if oocyte diameter (d) were grouped into twelve dimensional classes with the span of 200 μm.

The base of the gonad tubule, its middle and terminal segments were used for histological analysis. Microscopic sections were prepared according to the standard technique, staining with hematoxylin-eosin. The preparations were examined under the microscope at magnification of 10 × 40; the diameters of oocytes of various generations were measured using an eyepiece micrometer.

To assess the individual absolute fertility, the average number of germ cells of different diameters in the segment (10.00 mm) was determined, then in one tubule according to the formula: $N = n \times l$, where n is the average number of all oocytes in the segment, l is the length of the tubule. Then, the average value of oocytes per 10 tubules was found and the individual absolute fertility (F) of the gonad as a whole was calculated: $F = L \times M$, where L is the average number of oocytes in 10 tubules, M is the number of all tubules in the gonad.

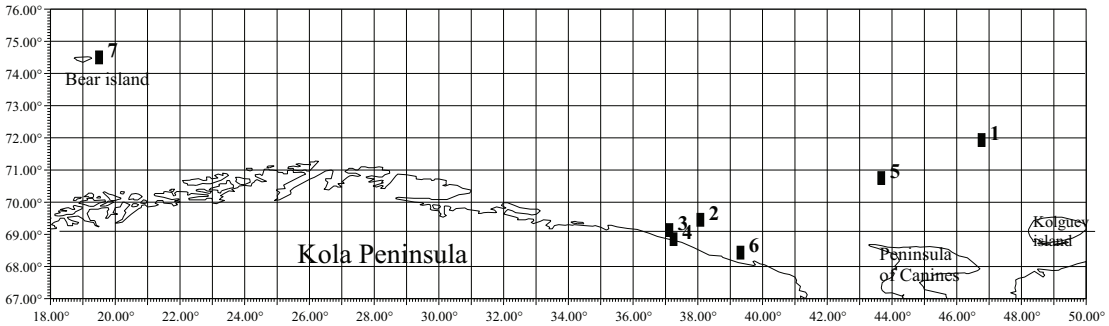

Figure 1: Sampling stations of *Cucumaria frondosa*: 1. The northern slope of the Gus Bank; 2. Murmansk shallow water; 3. The West Coast region; 4. Semiostrovsky district; 5. North Kaninskaya Bank; 6. Svyatonososky district; 7. The eastern slope of the Medvezhinsky bank.

3. Results

Cucumaria frondosa is a diclinous animal. The sex gland is unpaired, it consists of two bundles of numerous branched and unbranched tubules lying on both sides of the dorsal epithelium. Females and males are impossible to distinguish. They can be distinguished from one another by the color of the gonads only after dissection. The color of the female gonads is dark brown, in males it is pink, or pale purple. Male gonads frequently have thickenings and constrictions of various sizes and shapes. Over the period of the sample material preparing, only one hermaphroditic specimen was found, in which male reproductive ducts make up the majority of the gonad. When analyzing the literature sources, hermaphroditism in *C. frondosa* was mentioned only by Jordan [5].

The number of the tubules in the gonads varies (length of the skin-muscle sac -- 65.00 to 183.00 mm, weight -- 61.80-308.14 g) -- from 67 to 544 pieces. Gonad tubules vary in diameter as well [6]. Three categories of germinal tubules were distinguished: thin -- d -- 0.15-0.50 mm; medium -- 0.60-1.00 mm and thick more than 1.00 mm. In the thickened segments, the diameter of the tubules can reach 2.00-3.00 mm [15]. The length of the tubules also varies. The length of the short tubules is 4.7-91.00 mm, and

the long ones are 100.00-345.00 mm. Some germinal tubules of the gonads have two or three offsets, branching either from the middle or the terminal parts.

The cell composition in different segments of the same duct is not significantly different. The oogenesis phases were found to be different in the tubules of various diameter. Thin tubules are filled with parietal oocytes at the stage of growth. In the middle tubules, pre-vitellogenic oocytes and a small amount of vitellogenic ones are present, and thick tubules contain gametes of all types.

To determine the sex, we used holothuria with the gonad mass 0.39-2.47 g, SMS length from 50.00 to 90.00 mm and the mass ranging from 26.54 to 88.62 g. In 75% of the individuals, it was impossible to determine the sex, since the germinal tubules have d less than 0.50 mm. The ovaries of young animals are composed of either by thin genital ducts with the diameter of 0.50 mm or thin and medium tubules -- d 0.60-0.88 mm. The germinal tubules are from 5.00 to 150.00 mm long. Thin tubules contain parietal oocytes of d 25-225 μm; in the middle tubules, oocytes of the medium size from 225 to 450 μm at the growth stage are found. The results obtained allow us to assume that the sex of holothuria individuals can be identified using morphometric analysis with the average mass of gonads 1.63 g, with an average mass of SMS 53.00 g and the length from 80.00 to 83.00 mm, when the diameter of the genital tubules is not less than 0.50 mm.

When examining the reproductive glands of female holothuria of the Barents Sea in the reproductive cycle, 5 stages of gonad development were identified [1]: restoration (I), growth (II), maturation (III), maturity (IV), and post-spawning (V). It should be noted that the boundaries of the stages are determined conditionally and overlap one another [3].

Figure 2: Part of the ovary. V stage of maturity. June. Mult. 10 × 40.

In early summer, most holothuria females have ovaries at stage V (post-spawning). The determination of this stage of gonadal maturity is doubtless, since after spawning, the genital ducts of the glands dissipate. GI is 12.58%. The wall of the genital tubules is

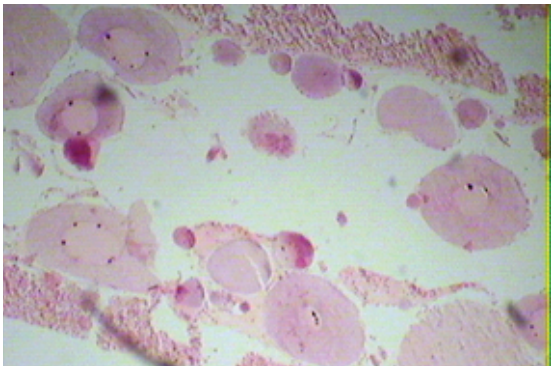

Figure 3: Part of the ovary. I stage of maturity. July. Mult. 10 × 40.

uneven (Fig. 2); they contain gametes of different phases of development. Oocytes of different diameters are resorbed.

In the summer period, gonads are restored, the process of the development and formation of germ cells begins. GI reaches 18.36-18.43%. The wall of the tubules is thickened -- d 190-200 microns (260-280 microns), in some sections of the genital tubules GI can reach 345-350 microns, there is some folding of the walls, along the folding oogonia are odserved. The genital tubules are filled with oocytes of cytoplasmic growth (Fig. 3). They are of a rounded shape and have a basophilically stained cytoplasm, in their nuclei there are from 3 to 6 nucleoli. The oocytes at the stage of intensive growth are found in a small amount. The developing gametes are present in a small amount as well. They are surrounded by follicular cells. The pre-vitellogenic oocytes are resorbed.

In the autumn period, the processes of restoration continue in the gonads, and the stage of accumulation and differentiation of gametes begins. The value of GI ranges from 12.57 to 30.64%. The thickness of the genital tubule wall reaches its maximum 250-350 microns. In September (Fig. 4) and October, along the walls of the germinal ducts of the gonad, oogonia of cytoplasmic growth, ranging from 30 to 210 microns in size, are located. Pre-vitellogenic oocytes of intermediate size were found in the openings of the tubules. Their cytoplasm has granular structure, the nucleus is round with a small number of nucleoli. Resorption of and vitellogenic oocytes is observed. In late autumn, in November, the diameter of the gonadal tubules increases (Fig. 5), there are no oogonia, and the number of vitellogenic oocytes with the diameter of 475--675 μm increases, vitellogenic oocytes in the initial stages of growth decrease in number. In the thicker tubules there are large oocytes, but they are significantly resorbed.

The state of females gonads described for the autumn months is generally characteristic of the winter period (Fig. 6). GI is 12.44-21.09%. There are practically no changes in the cellular composition of the genital tubules. Germinal cells are surrounded by

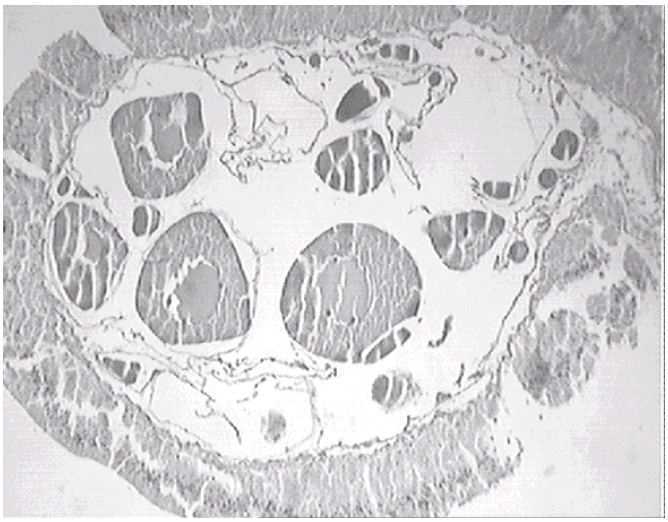

Figure 4: Part of the ovary.III stage of maturity. September. Mult. 10 × 40.

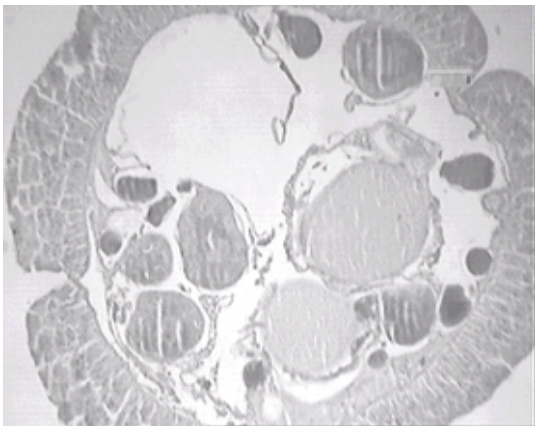

Figure 5: Part of the ovary.II stage of maturity. November. Mult. 10 × 40.

follicular cells, gamete resorption processes are observed; in February, the diameter of the walls of the genital tubules does not exceed 170-215 μm, there are no oocytes d 25-75 μm. Numerous nucleoli (5-10) were found in the nuclea of oocytes of cytoplasmic growth.

In spring, GI is 19.94-24.82%, the gonads of the glands have a minimum wall thickness of 50-80 μm and a maximum diameter (Fig. 7), oocytes d 475-675 μm are abundant. In late spring, the walls of the germinal ducts become thinner; in the openings of the tubules vitellogenic oocytes d 700-900 μm were observed, their amount ranged from 40 to 60%. Germinal cells of previous phases of development are also present.

The nature of spawning is intermittent [1, 2, 4], in the spawning season no specimens with the fully depleted gonads were found. The spawning of holothuria of the Barents Sea occurs from March to May, which is confirmed by earlier studies [1], but the spawning period can be shifted, since the beginning of the spawning period is dependent on

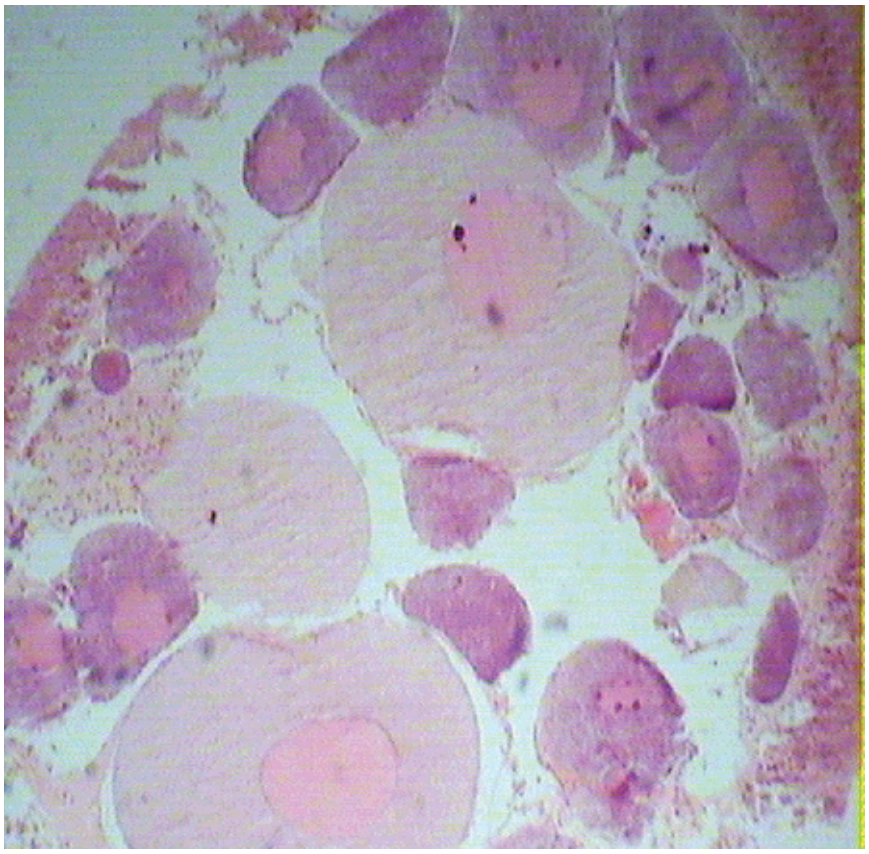

Figure 6: Part of the ovary. III stage of maturity. February. Mult. 10 × 40.

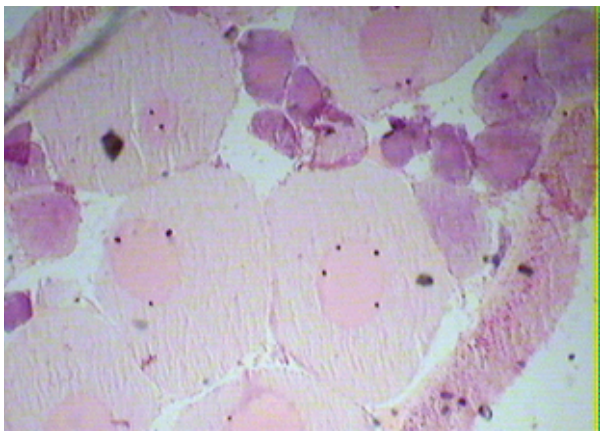

Figure 7: Part of the ovary. IIII stage of maturity. March. Mult. 10 × 40.

the seasonal increase of surface water temperature and the massive development of phytoplankton.

Individual fertility is low, in individuals with the average mass of SMS 150.00-200.00 g it ranged from 9 to 32 thousand cells.

4. Conclusion

The ovaries of holothuria *Cucumaria frondosa* of the Barents sea are featured by heterogeneity of germinal tubules, which is expressed in size and the stages of maturity. In the thin tubules of the gonads mostly oocytes of cytoplasmic growth are found. The medium-scale tubule, 0.60-1.00 mm in diameter and thick tubule, more than 1.00 mm in diameter contain all categories of gametes.

In the reproductive cycle of female holothuria *C. frondosa* of the Barents sea the post-spawning stage is the shortest period. In most individuals it starts in May and ends in June or in the beginning of July, then the gonads are restored. In middle summer and early autumn the number of oogonia and vitellogenic oocytes increases. The growth stage is the longest in the annual cycle, it usually begins in September and continues till February. Females in pre-spawning condition are found from February to April and the spawning lasts from April to May. It should be noted that after the spawning the gonads of holothuria of the Barents sea still contain the gametes of different stages of development, the processes of resorption of vitellogenic and pre-vitellogenic oocytes last in the ovaries throughout the year.

The spawning of cucumaria populations of the Barents sea lasts from March to May, but the period of spawning can vary, since the beginning of spawning dependst on the seasonal warming of the surface waters and the massive development of phytoplankton. The nature of spawning is intermittent.

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