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Some data on the reproductive system condition of Antarctic toothfish (Dissostichus mawsoni) males and females from the Indian Ocean area in the summer period

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Abstract:
The study deals with reproductive characteristics of the life history of Antarctic toothfish caught in the seamounts of the Indian Ocean area of the Antarctica in the fishing period 2004-2005. This fish species is one of the important deepwater resources of Antarctica. The study is a part of complex investigation of the life history adaptations of this fish species in the different areas of the Antarctica.
The morphological parameters, indices of gonads have been described. The histological criteria of the assessment of the ovary maturity stages and cytological parameters of oocytes and type of the toothfish oogenesis have been determined. Under the maturing of toothfish ovaries from the stages II to IV show a slow increase in oocyte diameter. It was shown that for Antarctic toothfish during the fishing period the individuals with gonads of the III late stage of maturity were dominated. Their ovaries contained three size groups of oocytes: cytoplasmic group and two groups of vitellogenous oocytes. Antarctic toothfish was not matured for spawning in the Indian Ocean area of the Antarctica in the investigation period.

Keywords: Antarctic toothfish, seamounts, reproductive adaptations, gonads, oogenesis

Introduction

The present paper is a part of the histological research of the toothfish reproductive system. Antarctic toothfish is the most valuable target species in the Convention Zone on Conservation of Living Marine Resources of Antarctica and CCAMLR (Shust and Brukhis, 1994; Shust et el., 2005). Previously we have analyzed the ovary condition of Patagonian toothfish (*Dissostichus eleginoides)* caught near by the Island of the S. Georgia (Piyanova, 2006) and the reproductive system characteristics of the Antarctic toothfish from the Ross Sea (Piyanova, Petrov, 2007; Piyanova, Kokorin, 2007).

Antarctic toothfish is the bathyal and pelagic species of the *Nototheniidae* family distributed circumpolar in the high latitude Antarctic waters. Its life cycle is divided into three stages (Yukhov, 1982; Andriyashev, 1985): juveniles inhabit in the subsurface waters near the continent during the spring-summer season; immature grown individuals deepen into the near-bottom layer in the shelf zone by the beginning of winter where they live in the near-bottom and pelagic layer for several years. With maturing they move to the larger depths and inhabit the continental slope and underwater rises. The largest size groups of mature toothfish keep the deepwater areas, and fish of the smaller size spread off the islands and shelf zone (Shust et al., 2005). Toothfish have a high growth and maturity rate and a high fecundity (Kock, 1992; Shust et al., 1990). Antarctic toothfish maturate at the 8^{th} year of life cycle with the length of 95-105 cm (Gon and Heemstra, 1990; Fenaughty, 2004). Toothfish reproductive system is not insufficiently investigated yet, questions on exact localization, time, periodicity and type of its spawning are remain discussion.

This study aiming to a histological analysis of gametogenesis of Antarctic toothfish caught in the Indian Ocean area of the Antarctica.

Materials and methods

The material for histological study of the life history of Antarctic toothfish was collected in the seamounts of the Indian Ocean area of the Antarctica (subareas 58.4.3 and 58.4.1) in December-February 2004-2005 during the fishing cruise of longliner YEON SEONG. Toothfish individuals were caught at a depth of 1 147-2 032.5 m, average fishing depth was 1586 m. In the paper the data of the histological analysis of 44 individuals of Antarctic toothfish with visual determination of sex and stages of maturity were used. We used the standard histological methods (Roskin and Levinson, 1957) modified by ourselves. The histological dehydration of the material fixed in the 4% formalin solution was made through the spin tissue processor. The pouring of the material into paraffin was made with the use of tissue embedding station. The sections with the width of 5 microns were obtained with the sliding microtome and colored with alum hematoxylin by Erlich with coloring by eosin. For photographing we used microscope with the automatic video camera. The histological sections were analyzed with *Image-J* software.

When describing the histological state of the Antarctic toothfish gonads, we used the standard methodology for gonads of *Notothenia* (The methodical guidance…, 1983) and study of toothfish by Yukhnov (1982). When calculation of the oocytes diameters we used the coefficient of cells compression 25% after the histological proceeding (Voronina, 1981).

Photos of histological sections of Antarctic toothfish gonads were compared with the photos of the physiognomy of the same gonads made with the use of camera during the bioanalysis of fish. Index of maturity was determined as a percent ratio of gonad weight to the fish body weight with the inner organs.

Results and Discussion

Morpho-physiological parameters of Antarctic toothfish

The main size-age characteristics and parameters of toothfish maturing are presented in Table 1. During the period of investigations the average length of was about 130-151 cm. The females had the highest parameters of maturity.

The main biological characteristics of Antarctic toothfish analyzed by histology.

Table 1.

Characteristics	Female	Male
	M±m	M±m
Number of fish	24	20
Weight, kg	44.4±3.4	29.4±3.3
Length, cm	151.3±3.4	130.8±4.0
Index of maturity, average, %	3.6±0.5*	2.3±0.4*
Stages of gonad maturity	II, late III, III – IV, IV	III, III – IV
Dominated stage of gonad maturity	III – IV	III – IV

Notes: differences are significant $p<0.5$ (*)

Fish had the low average index of maturity. Variations in the index of maturity for the Antarctic toothfish individuals were affected by the different physiological state of fish (Patchell, 2003).

The histological analysis revealed that gonads of 37.5% females reached the III – IV stage of maturity and gonads of 55% males were on the III stage of maturity.

Oogenesis and stages of ovary maturity

A number of *Notothenia* oogenesis aspects were described earlier (Bogutskaya, 1984; The Methodological guide…, 1983; Silyanova, 1981; Lisovenko, 1987). There are the literature data on the histological structure of Patagonian (Zhakharov and Frolkina, 1976; Chikov and Melnikov, 1990; Zhivov and Krivoruchenko, 1990) and Antarctic toothfish (Yukhov, 1982; Fenaughty, 2004; Prutko, 2004).They were supplemented by the results of S.V. Piyanova for oogenesis of Patagonian toothfish caught in the shelf zone of the South Georgia Island in October-November 1986. The obtained results allow to widen the histological picture of toothfish oogenesis with the cytological parameters of oocytes of Antarctic toothfish from the Indian Ocean Area of Antarctica during the period of vitellogenesis.

Stages of ovary maturity for Antarctic toothfish

The ovaries of analyzed fish occupied from 1/3 to a half of abdominal cavity. They were dense, yellowy and had the thickened rounded ends. For repeatedly spawning fish the envelope of ovaries was dense and thick, and the blood vessels were clearly seen via the envelope. The individuals of Antarctic toothfish caught in December-February had ovaries at the different stage of trophoplasmic growth of oocytes.

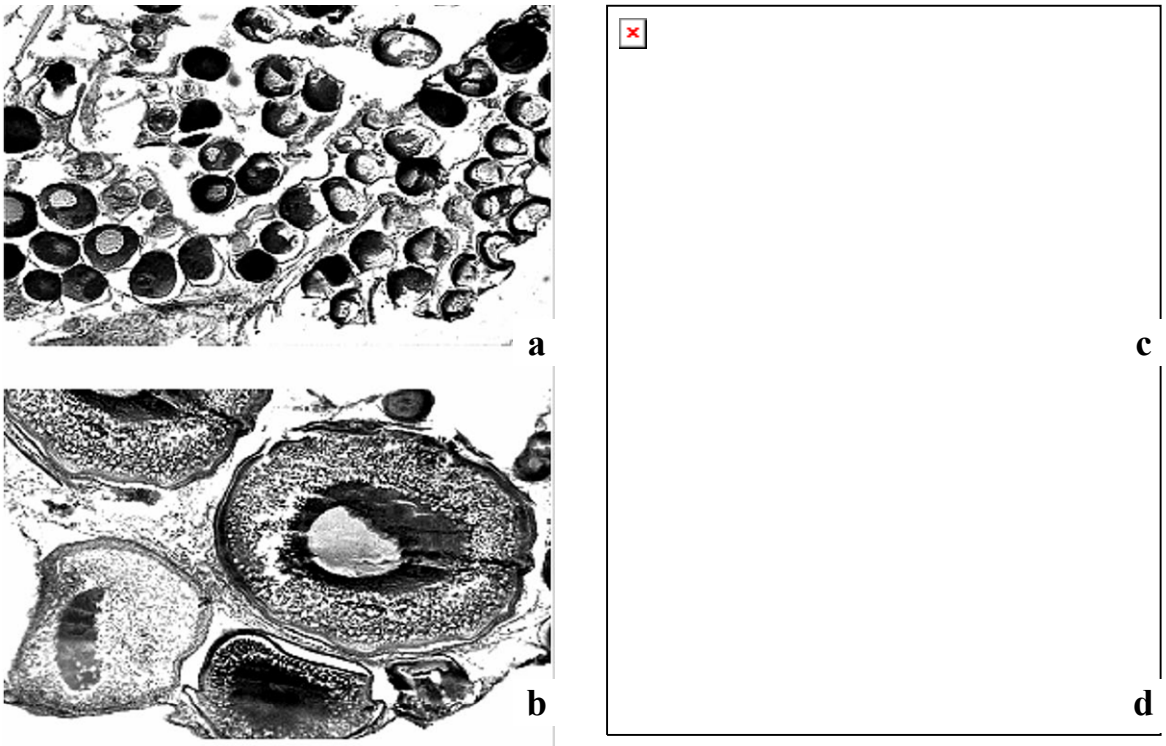

Fig. 1. Microstructure of ovaries at different stages of maturity from Antarctic toothfish caught in the Indian Ocean Area of the Antarctica, magnification 10×10:
a – ovary at the II stage of maturity, b - ovary at the late III stage of maturity,
c - ovary at the III-IV stage of maturity, d - ovary at the IV stage of maturity.

The average diameter of their oocytes and nucleus are presented in the table 2.
The cytomorpholocical parameters of sex cells in ovaries of toothfish at different stages of maturity.

Table 2.

Stage of gonad maturity		II	Late III	III-IV	IV
Large oocytes	Diameter of oocyte, 10^{-6} m	-	995.46± 27.18	1027.48± 25.52	1244.0± 26.68
	Diameter of nucleus, 10^{-6} m	-	223.97± 10.47	249.22± 14.81	257.42± 23.84
Medium oocytes	Diameter of oocyte, 10^{-6} m	-	672.47± 22.03	580.08± 19.04	727.52± 50.61
	Diameter of nucleus, 10^{-6} m	-	211.78± 16.87	181.28± 9.37	167.30± 23.09
Small oocytes	Diameter of oocyte, 10^{-6} m	140.36± 3.47	224.90± 15.90	198.54± 9.48	242.42± 21.25
	Diameter of nucleus, 10^{-6} m	61.32± 2.36	83.33± 10.80	77.75± 4.13	99.22± 13.17

At the II stage of maturity the ovaries have the look of light yellow cords with the thin envelope. At the histological sections the oocytes of different phases of cytoplasm growth and oogenesis were seen. This stage is noted for Antarctic toothfish females (Fig. 1).
At the late III stage of maturity the ovaries were somewhat larger, their coloration varied from the rose-pink to yellowy. At this stage, as at the following stages, there were oocytes of the three stages divided by size into small, medium, and large ones. The presence of two size groups of trophoplasmic oocytes is the characteristic feature of vitellogenesis of *Notothenia* females (Siliyanova, 1981; Lisovenko, 1987). The small oocytes were the ones of the cytoplasmic growth. The medium size group included oocytes of the III stage of the cytoplasmic growth (generation of the reserve fund). In the predominated large size group were the oocytes in the beginning of cytoplasm vacuolization.
At the III-IV stage of maturity the active vitellogenesis was revealed for the more developed oocytes (from phase of intensive vitellogenesis to the phase of completion of the trophoplasmic growth. The medium size group included the oocytes of beginning of vitellogenesis. The smaller cytoplasmic oocytes were a few.
At the IV stage of maturity ovaries reached the maximal size, and their envelope was strongly strained and covered gonad. The most of the section area was occupied by cells in the phase of ending of vitellogenesis and ripening with the confluent yolk and formed envelopes. This was the generation of oocytes of the latest spawning (Table 2).

The results allow to expand the histological picture of toothfish oogenesis with the cytological parameters of oocytes (Table 3).

Histological parameters for identification of stages of ovary maturity for Antarctic toothfish.
Table 3

Stages of maturity	Oocytes microscopic sections	Fish biological parameters	Morphological characteristics of oocytes
II	figure 1a	weight=15.8 kg, length=113 sm, index of maturity = 0.6 %	cytoplasmic oocytes and rare oogoniums
Late III	figure 1b	weight=53.2 kg, length=160 sm, index of maturity = 1.2 %	yolk granules are fill up oocyte cytoplasm, fatty vacuoles are small, noncoalesce , nuclear has irregular shape
III-IV	figure 1c	weight=42.5 kg, length=142 sm, index of maturity = 4.0 %	yolk granules are interflow into homogeneous aggregations, fatty vacuoles are numerous, nuclear is considerably decreases
IV	figure 1d	weight=81.8 kg, length=188 sm, index of maturity = 6.5 %	yolk is fully homogeneous, nuclear is not reveal itself

We interpret these ovaries as resting because some sections contained a very infrequent atretic oocytes (Fig. 1b), indicating that fish had previously spawned. Atretic cell in the Antarctic

toothfish ovaries was revealed for the females from Ross Sea (Eastman, DeVries, 2000; Vanella et al, 2005).

Size composition of cells in ovaries of Antarctic toothfish

As seen from figure 2, at the development of toothfish ovaries from the II to IV stage a slow increase in oocyte diameter occurred.

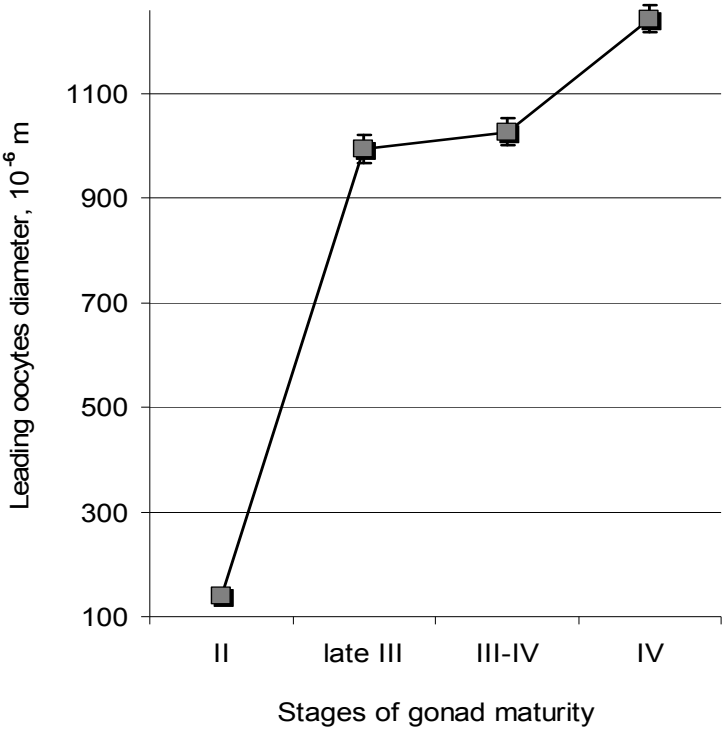

Fig. 2. Dynamics of changes in leading oocytes diameter from ovaries at the different stages of maturity of Antarctic toothfish females caught in the Indian Ocean Area of the Antarctica.

The size composition of oocytes in ovaries is the main parameter of the oogenesis type (Oven, 2004). As seen from Fig. 3, variation curve of oocytes composition in the ovaries on IV stage of maturity is demonstrated the presence of two separated groups of vitellogenic oocytes with diameter 400 - $500 \cdot 10^{-6}$ m (10.9 %) and 1100 - 1200 $\cdot 10^{-6}$ m (8.9%) on the average. Two size groups of vitellogenius oocytes were isolated, and thus the portion of the next spawning has been already formed. The peak of the curve shifted to the left reflected the size group of cytoplasmic oocytes predominated in ovaries (19.3% from the total number of cells).For the leading group of vitellogenius oocytes only the processes of the further growth, ripening and dehydration will occur.

Thus, our data evidence on the discrete type of Antarctic toothfish oogenesis, when in ovaries at the III stage of maturity the large oocytes of the following spawning season are isolated from the reserve group of trophoplasmic oocytes of the next year. It is believed that spawning period for Antarctic toothfish is starting in May and over by October in the Ross Sea to the north of the continental slope (Hanchet et al., 2003). Probably, the studied fish ovaries are at the prespawning period, because it is known that final maturation with rapid increase in gonad and oocytes sizes takes place in March – April (Everson, 2002; Knox, 2006).

Fig. 3. Size composition of oocytes from ovaries at the IV stage of maturity of Antarctic toothfish females.

The size of mature eggs is large, and thus it takes for them an essential time to accumulate a large volume of the trophic inclusions at the low temperature conditions of Antarctica under the limited spawning season. Consequently, toothfish gonads, as for the other high-latitude Antarctic species (Lisovenko and Svetlov, 1980), after the single spawning reach the VI-III stage of maturity and then return to the III stage of maturity continued from 3-5 to 5 months, according to the literature data (The methodological guidelines…, 1983). There is an evidence that some females of Antarctic toothfish do not spawn in the current year, and their ovaries are at the stage of yolk accumulation in oocytes (Prutko, 2006).

Spermatogenesis and stages of testis maturity

The development of the Antarctic toothfish testes was studied incompletely because the researches paid the main attention to the problems of oogenesis and fecundity of the species. We considered the features of composition and development of testes for males. For toothfish as representatives of *Perciformes* order, the radial type is characteristic. Gonads have the triangular form in the cross section, slow gyrose ductules are congregate the radial from the periphery to the excurrent duct located in the gland closer to the dorsal surface. The testes are separated by the gyrose streaks into 8-10 parts that agreed with our data and material on Patagonian toothfish (Zakharov and Frolkina, 1976). It is known that for this high-latitude species gonads are at the III stage of maturity.

In December-February, the testes of examined fish were mainly at the III stage of maturity. The III stage of maturity. Testes had the rounded dense chords of the pinkish and grayish or yellowish color, and their envelope was non-transparent. The testis ductules were located dispersed, especially in the central parts of the gland, their lacunas were elongated and chords of the between-tissue were narrow. The gleams of chords were filled by cysts. At the

histological prepares the spermogonians were seen, as well as the spermocytes of the I and II stages and spermatids (Fig.4a, c).

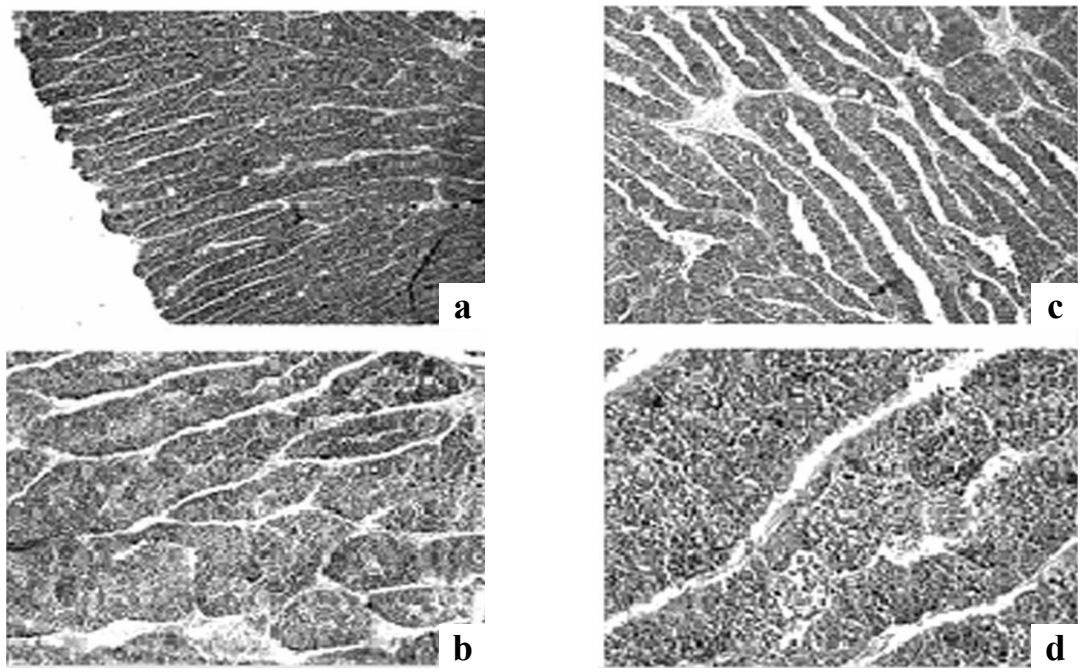

Fig. 4. Microstructure of testes at the different stages of maturity from Antarctic toothfish caught in the Indian Ocean Area of the Antarctica:
a, c – testis at the II stage of maturity, magnification 10×5 (a) and 10×20(c),
b, d – testis at the III-IV stage of maturity, magnification 10×10 (b) and 10×40 (d).

III-IV stage of maturity. The testes increased in their volume, lightened. At the touching they were the softer and crept lightly on the cut. At the histological level the characteristic parameter of this stage is the appearance of spermatozoids in the gleams of testis ductules (Fig. 4b, d). By the end of this stage cysts with spermagonians of the earlier stages of maturity were met solitary near the testis edge, and the prepares were dominated by spermatozoids. The process of spermatogenesis for toothfish occurs non-synchronously. The ampoules showed cells at the different stages of differentiation. It is important to note that in the different parts of testis of Antarctic toothfish the different processes may be revealed.
Thus, in the part located closer to genital hole the development of spermatogenesis advances the process in the head part of gonad.
This results in the long spawning period of males compared with females that is the characteristics of *Notothenia* fishes (The methodological guideline.., 1983).

Conclusion

We have analyzed morphological and physiological parameters of fish, gametogenesis, and stages of gonad maturity and size composition of cells in the ovaries. In summer period the average length of Antarctic toothfish caught in Indian Ocean Area and studied by histology was 151 sm for females and for 130 sm for males. The females had the highest parameters of maturity. The histological analysis revealed that ovaries reached mainly the III-IV stage of maturity (37.5 %) and testes – the III stage (55%). It was shown that in the Antarctic toothfish

ovaries starting with III stage of maturity there were cytoplasmic oocytes and two size groups of trophoplasmic oocytes. At the development of toothfish ovaries from the II to IV stage a slow increase in oocytes diameter occurred. Variation curve of oocytes composition in the ovaries on IV stage of maturity is demonstrated the presence of two separated groups of vitellogenic oocytes. There were no Antarctic toothfish females with completed maturation and definitive sizes of oocytes in the ovaries. In the December – February the oocytes in the ovaries of investigated fish has not reached the definitive size, therefore, Antarctic toothfish was not ready to spawn in the Indian Ocean Area of the Antarctica.

It was revealed the radial type of structure for the Antarctic toothfish testes. The process of spermatogenesis for toothfish occurs non-synchronously. This result in the long spawning period of males compared with females as the characteristics of *Nototheniidae* fishes.

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