ON REPRODUCTION OF *ELECTRONA CARLSBERGI* (TÅNING)

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Abstract

This paper presents results from histological examinations of gonads of *Electrona carlsbergi* from the Atlantic sector of the Southern Ocean. From materials available it has been ascertained that *E. carlsbergi* spawns in the notal waters. Maturation of females begins when body length reaches 76 to 78 mm. Vitellogenesis is asynchronous. Vitelline eggs have a diameter from 150 to 650 μm; in hydrated oocytes it is up to 900 μm. Maturation of ovaries is continuous, and spawning is serial. The spawning season is long and coincides with summer/autumn in the Southern Hemisphere.

Résumé

Ce document présente les résultats de l'examen histologique des gonades d'*Electrona carlsbergi* du secteur Atlantique de l'océan Austral. A partir du matériel disponible, il a été confirmé qu'*E. carlsbergi* se reproduit dans les eaux subantarctiques. La maturité des femelles commence lorsque la longueur du corps atteint 76 à 78 mm. La vitellogénèse est asynchrone. Les œufs vitellins ont un diamètre de 150 à 650 μm atteignant parfois 900 μm dans les ovocytes hydratés. La maturation des ovaires est continue et la ponte périodique. La saison de ponte est étendue et correspond à la période été-automne de l'hémisphère sud.

Резюме

В настоящем труде представлены результаты гистологического исследования гонад *Electrona carlsbergi* из атлантического сектора Южного океана. По имеющимся материалам было установлено, что нерест *E. carlsbergi* происходит в субантарктических водах. Созревание самок начинается при достижениии ими длины в 76-78 мм. Вителлогенез асинхронный. Диаметр яиц с вителлиновой оболочкой равняется 150-650 микромиллиметрам, он достигает 900 микромиллиметров на стадии гидратированного ооцита. Созревание яичников непрерывное, нерест периодический. Нерестовый сезон продолжительный и совпадает с летне-осенним периодом в южном полушарии.

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Resumen

En este documento se exponen los resultados de los exámenes histológicos de las gónadas de *Electrona carlsbergi* del sector atlántico del océano Austral. De la información disponible, se ha descubierto que *E. carlsbergi* se reproduce en la zona subantártica. La maduración de las hembras se inicia cuando la longitud total es de 76-78 m. y la vitelogénesis es asincrónica. Los huevos vitelinos tienen un diámetro de entre 150 a 650 µm y pueden alcanzar los 900 µm en los oocitos hidratados. La maduración de los ovarios es continua mientras que el desove es seriado. La época de freza es prolongada y coincide con el período verano/otoño del hemisferio sur.

1. INTRODUCTION

Electrona carlsbergi (Tåning, 1932) is a notal-Antarctic species and its distribution is circumpolar. The species inhabits waters of the Antarctic Convergence, wherein it forms concentrations during the major part of the year (Hulley, 1981; Naumov *et al.*, 1981; Bekker, 1983; Kornilova, 1987; Zaselsky, 1988 and others). According to Hully (1981) *E. carlsbergi* spawns beyond the Antarctic region. From the occurrence of larvae it has been inferred by V.N. Efremenko (Lisovenko and Efremenko, 1983) that the reproductive area of the species coincides with the notal zone.

A study of gonads in female *E. carlsbergi* from the Antarctic Convergence and the Argentine Basin areas has been undertaken with the aim of determining where and when this species spawns, specifying the type of area in which it reproduces and delineating zones of its expatriation. Results of the study are presented in this paper.

2. MATERIALS AND METHODS

Observations were carried out and materials collected during surveys by the RV *Artemida* in December-February 1987-1988 and January-April 1989. Samples were collected over the area from 40° to 54°S and between 8° and 59°W. Samples were collected from trawl catches taken within the 50 to 1 500 m depth range. Length of fish (**SL**) was measured to the nearest 1 mm from the tip of the snout to the base of median rays of the caudal fin.

A total of 4 380 females were examined. 160 fish were preserved in Bouin's fluid for further analysis.

Preparation and histological treatment was conducted in accordance with standard methods: treatment with alcohol, gradually increasing its concentration, followed by treatment with xylene and embedding in paraffin. Sections of samples (5-6 µm thick) were stained with haemozyline according to Heidengain's method (Roskin and Levinson, 1957).

The growth of egg cells was described using terminology suggested by V.A. Meien (1927).

The number of eggs in a batch was determined by counting ripe and vitelline eggs by size. The number of batches of ripening eggs was estimated from weights of hydrated oocytes.

The physiological condition of ovaries was evaluated by a 6-point scale (Sakun and Butskaya, 1963).

3. RESULTS

3.1 Development of Egg Cells

The process of egg development may be divided into three periods: nuclear transformations, protoplasmatic and trophoplasmatic growth.

By the beginning of protoplasmatic growth nuclear transformations have been completed and a thin layer of cytoplasm develops in oocytes. Later, the growth of oocytes occurs at the expense of an increase in the cytoplasm volume. We distinguish two phases in the period of trophoplasmatic growth: young oocytes and monolayer follicle.

At the beginning of slow growth oocytes are usually of regular shape and have a diameter from 10 to 30 μm. The membrane has no structure. The cytoplasm is heterogeneous; the intensity of colour in its different parts varies.

At the end of the protoplasmatic growth period, the diameter of the oocyte reaches 90 to 120 μm, and that of the nucleus reaches 56 to 69 μm. The membrane consists of two layers: an internal one - a membrane in itself - and an external one, formed by follicular epithelium cells. The cytoplasm is pale and uniform in colour.

The further development of oocytes and the beginning of trophoplasmatic growth are associated with the ripening of fish gonads. This period may be divided into the following phases: 1 - vacuolization and accumulation of fat, 2 - initial accumulation of yolk, 3 - intensive trophoplasmatic growth, 4 - yolk-filled oocyte, 5 - mature oocyte.

Vacuolization of the cytoplasm occurs before the yolk accumulation phase.

In oocytes of 100 to 500 μm in diameter small vacuoles appear in a peripheral zone and later form a cortex. Thereafter, vacuoles develop in an area near the nucleus. The contents of vacuoles is lipoid which is washed away during treatment of preparations. The external surface of the oocytes is made up of membranes of follicular and connective tissue.

In the phase of initial yolk accumulation, tiny insertions of yolk are observed to appear in oocytes of 180 to 2 020 μm in diameter. Granules of yolk gradually fill the cytoplasm and fat vacuoles are pushed towards the nucleus.

In oocytes undergoing intensive trophoplasmatic growth, the amount of trophic substance increases at the expense of accumulation of yolk granules of increased size. The number of fat balls reduces as they coalesce. The diameter of the oocytes reaches 500 μm.

In the vitelline oocyte phase the diameter of the cells is up to 500-650 μm. The only fat ball of 180-200 μm in diameter is found in the centre of the egg cell. The nucleus is displaced towards the animal pole of the egg.

The phase of maturation is associated with yolk hydration and homogenization. The oocyte grows to 850-900 μm in diameter. The follicular membrane is thin and is loosely attached to the oocyte membrane proper. During this phase, egg cells are released from follicles and are ready for spawning.

3.2 Maturity Stages

Developmental stages are defined by gonad functional status, which is characterized by the presence of a specific set of egg cells in one the of the phases described above.

The following maturity stages were distinguished by observation of the fine structure and variations in weight of the gonads (Table 1).

Field observations and cytological examination provided materials for comparative analysis of reproductive system condition in female *E. carlsbergi* from two zoogeographical zones: notal and Antarctic.

To the south of the Antarctic Convergence (Antarctic zone) catches were made up of specimens of 65 to 96 mm in length. In the Argentine Basin (notal zone) fish were from 70 to 103 mm in length. Males were smaller than females: they did not exceed 95 mm in length, whereas females were up to 103 mm long. In the Antarctic zone the male/female ratio was close to 1:1.1; in the notal zone males prevailed, the sex ratio being 1:0.9. In the notal zone the maturity coefficient for females of less than 78 mm length was low - from 0.5 to 1.0%. The diameter of more fully developed oocytes did not exceed 100 μm. A histological analysis showed that the more fully developed generation of egg cells were in the monolayer follicle phase. Females with such oocytes were regarded as immature (maturity stage II). Females larger than 78 mm were considered as mature because the diameter of egg cells and weight of their ovaries were still increasing.

In January the maturity coefficient for mature females varied from 1.1 to 2.7%. More fully developed egg cells were found to be in the initial yolk accumulation phase (maturity stage III).

By mid-February the vitellogenesis was almost complete in the majority of fish and empty follicular membranes - an indication of partial spawning - were found in some of them. The maturity coefficient increased to 11.4% (maturity stages IV, IV-V, see Figure 1).

In April all females larger than 78 mm had yolk-filled eggs, many contained traces of partial spawning. The maturity coefficient varied from 4.8 to 14.7%. Ovaries were in maturity stages IV-V and VI-VIII (Figure 2).

In prespawning fish the diameter of yolk-filled eggs varied from 150 to 650 μm, the diameter of hydrated oocytes - from 700 to 800 μm. There are several successive peaks (waves) on the plot of oocyte size frequency distribution (Figure 3). Each is likely to correspond with a group of cells of synchronous development. The number of oocytes gradually decreased as their size increased. Oocytes of 100 to 150 μm diameter (beginning of trophoplasmatic growth) comprised more than 50% of the total number of eggs measured. According to the classification suggested by Lisovenko (1985), oogenesis of this type is very similar to a serial fluctuated type. A serial oogenesis implies that there is a possibility of replenishment of spawned eggs by reserve oocytes which may be developed into successive batches of eggs for later spawning.

According to Oven (1976), a serial spawning is typical for the majority of species with continuous oogenesis. Unfortunately it was not possible in experimental conditions to count for *E. carlsbergi* the number of eggs in one batch. An attempt was therefore made to estimate the possible number of egg batches from the weight of hydrated oocytes (Table 2).

Our data suggest that the number of batches of ripening egg cells calculated as a ratio of the theoretical maturity coefficient to observed maturity coefficient, is not less than 7.

The maturity coefficient of female *E. carlsbergi* from the Antarctic zone did not exceed 1.4%. In most fish, more fully developed egg cells were found to be in the monolayer follicle

phase (Figure 4). In some fish, egg cells commencing trophoplasmatic growth were found. These eggs developed faster than the majority of cells and were usually being reabsorbed (Figure 5). This phenomenon may be interpreted in two ways: (1) resorption of "excessive" oocytes during early stages of ontogenesis (Persov, 1963), and (2) resorption of older oocytes due to unfavourable ambient conditions for reproduction.

In our opinion, low water temperature is one of the most important factors. During our observations water temperature in the Antarctic zone in the 50 to 360 m depth layer varied from 0.8° to 2.5°C. In the notal zone at depths of 380 to 460 m where spawning females were found, water temperature was from 3.8° to 10.3°C. In view of this variation in water temperature it may be hypothesized that the fish cannot spawn in the Antarctic zone at all or that no spawning might have occurred in the period in which the observations were made.

Failure to spawn and the dependence of spawning on ecological factors have been noted for myctophids many times in the past. Expatriation of subtropical species *Lobianchia dofleini* and *Lobianchia gemellarii* into colder waters of the Continental Shelf of North America results in inhibition of growth and resorption of sex cells (O'Day and Nafpaktitis, 1967). The Norwegian researcher, I. Gjøsaeter (1981) came to the conclusion that *Notoscopelus kroeyerii*, a boreal species in the North Atlantic, is capable of forming aggregations of non-breeding fish in Norwegian waters.

4. CONCLUSIONS

Results obtained show that *E. carlsbergi* breeds in the notal zone. The spawning season of this species is extended and covers summer and autumn. Ripening of ovaries is continuous and this species is a serial spawner. Expatriation of *E. carlsbergi* into colder waters of the Antarctic zone south of the Antarctic Convergence results in inhibition of growth and resorption of egg cells. It is unlikely that individuals at this stage of gonad development will spawn.

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Table 1: Maturity stages of ovaries.

Maturity Stage	Weight (mg)	Maturity Coefficient (%)	Composition of Sex Cells	Functional Condition
I	12-30	0.1-0.2	Oogonia	Juvenile
II	40-90	0.5-1.1	Oogonia, oocytes in protoplasmatic growth	Not ripe
III	108-550	1.1-7.8	Oogonia, oocytes in protoplasmatic and trophoplasmatic growth (phase of vacuolization, initial vitellogenesis, intensive trophoplasmatic growth)	Ripening Intensive accumulation of yolk and fat in ovaries
IV	660-1 000	7.3-10.8	Oogonia, oocytes in protoplasmatic and trophoplasmatic growth (phase of vacuolization, intensive trophoplasmatic growth, vitelline oocytes)	Prespawning condition Vitellogenesis close to completion
IV-V	1 010-2 050	11.5-14.7	Oogonia, oocytes in protoplasmatic and trophoplasmatic growth (phase of vacuolization, intensive trophoplasmatic growth, ripening)	Functionally mature Hydrated eggs rest freely in ovaries
VI-VIII	400-1 000	2.5-9.5	Oogonia, oocytes in protoplasmatic and trophoplasmatic growth (phase of vacuolization, initial vitellogenesis, intensive trophoplasmatic growth). Empty follicular membranes.	Ripening of the next back of eggs after extrusion of previous batch
VI	No data available			Extrusion Ovaries after extrusion of last batch of ovulated eggs

Table 2: Number of batches of ripening egg cells for *E. carlsbergi* at maturity stage IV-V.

Body Weight (mg)	Ovaries (mg)	Total Number of Oocytes Over 100 µm in Diameter ($x10^3$)	Weight of 50 Hydrated Oocytes (mg)	Weight of Ovaries Filled with Hydrated Oocytes (mg)	Maturity Coefficient (C_1) (%)	Theoretical Maturity Coefficient* (C_2) (%)	Naumber of Batches (C_2/C_1)
8 100	1 191	44.2	10.8	9 550	14.7	118.0	8.0
16 150	2 050	40.3	17.5	14 100	12.7	87.3	6.9
9 350	994	23.7	14.2	6 730	10.6	72.0	6.8

* Theoretical maturity coefficient is calculated from weight of ovaries filled with transparent eggs.

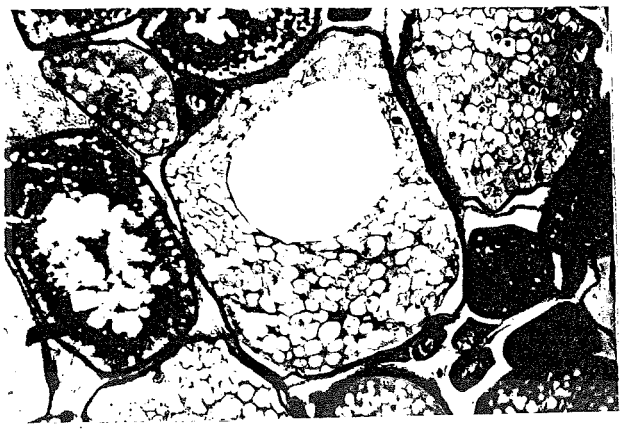

Figure 1: *E. carlsbergi* ovary at maturity stage IV-V. A hydrated oocyte in the centre. Magnification - 20 x 10.

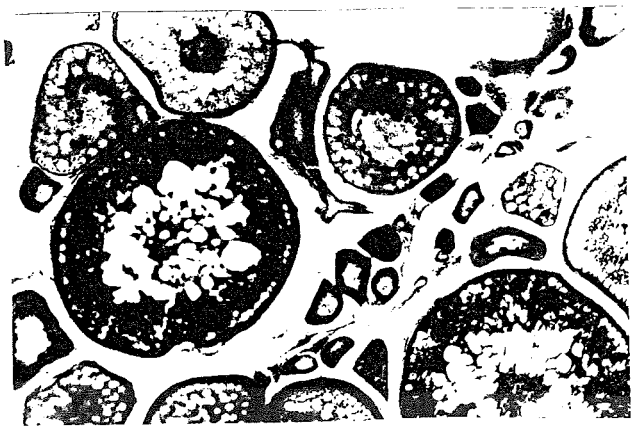

Figure 2: *E. carlsbergi* ovary at maturity stage VI-VIII. Residual follicle in the centre. Older oocytes in phase of intensive trophoplasmatic growth. Magnification - 20 x 10.

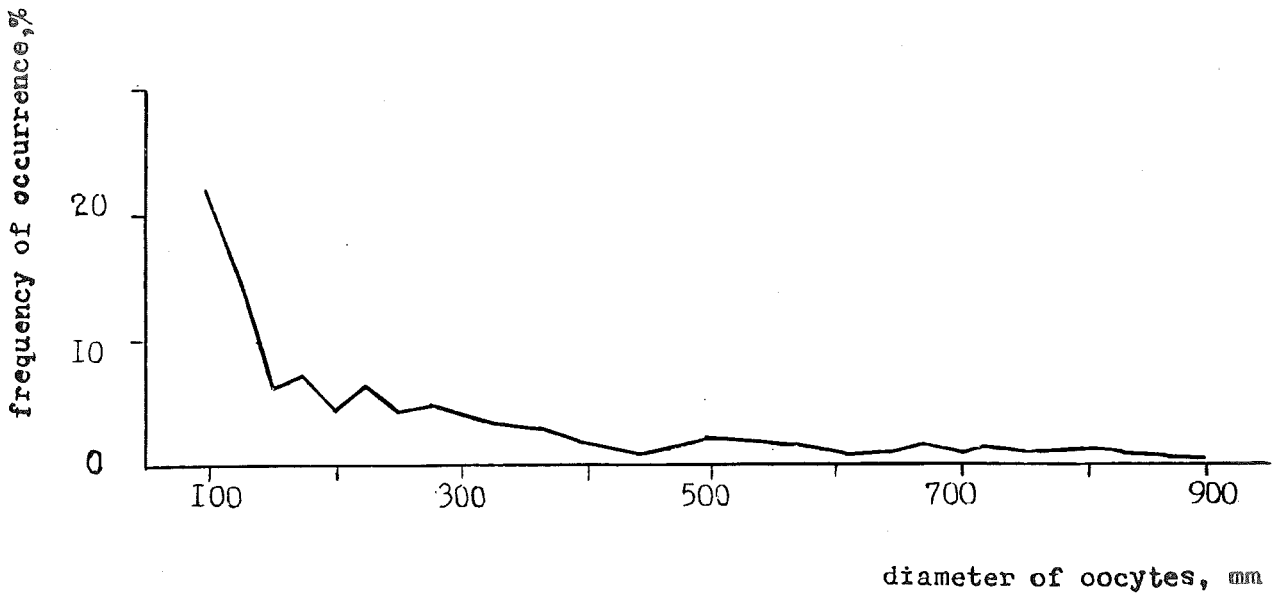

Figure 3: Size distribution of vitelline eggs in *E. carlsbergi* at maturity stage IV-V.

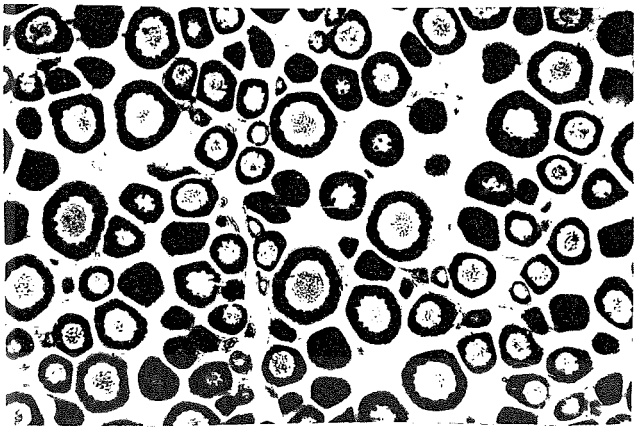

Figure 4: *E. carlsbergi* ovary at maturity stage II. Older oocytes in monolayer follicle phase. Magnification - 20 x 10.

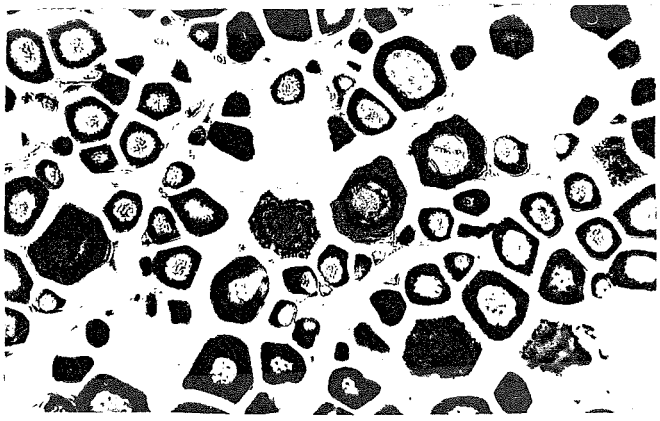

Figure 5: *E. carlsbergi* ovary at maturity stage II. Resorption of fast-growing oocytes. Magnification - 20 x 10.

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