OBSERVATIONS OF THE OVARIAN EGGS AND SPAWNING HABITS IN *Euphausia superba* DANA

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Abstract: The development of ovarian eggs of a total of 197 individuals of *Euphausia superba* DANA was observed. One of three material sources was from the stomachs of minke whales caught by the CHIYO MARU fleet during the 1973/74 season, while others were collected by net during two cruises of the R.V. KAIYO MARU in 1979/80 and 1980/81. Histologically, the oogenetic development of ovarian eggs in female *E. superba* (23–57 mm) was classified into the following nine stages: I. oogonia stage, II. early chromatin nucleolus stage, III. late chromatin nucleolus stage, IV. early peripheral nucleolus stage, V. middle peripheral nucleolus stage, VI. late peripheral nucleolus stage, VII. early vitellogenic stage, VIII. late vitellogenic stage, and IX. final maturity stage. In females smaller than 30 mm (T.L.) from November to February, ovarian eggs were composed largely of the stages I to III, whereas those in animals larger than 31 mm collected in early to mid January showed a more developed stage composition. Near the end of January a few post-spawning krill larger than 35 mm began to occur and their relative abundance in the population increased toward early February. Changes in the composition of ovarian eggs by the developmental stages during late January to February suggested that some females of 35–40 mm may spawn within the current breeding season whereas the remaining non-spawning females of similar body size may spawn first in the subsequent breeding season. In the ovary of post-spawning females some oocytes of stages II to III were observed, which were considered to become mature by the beginning of the subsequent breeding season. These results strongly suggest that *E. superba* is likely to spawn separately, twice or more in different breeding seasons throughout its life-span. The number of eggs released by 26 individuals of 44–54 mm in the shipboard rearing experiments ranged from 627 to 3115 ($\bar{x}$=1417, s=117) per female. This figure was considerably smaller than the known apparent fecundity of this animal.

1. Introduction

Reproduction biology such as the growth rates and longevity of the Antarctic krill, *Euphausia superba* DANA, has been studied largely on the composition of body size (*e.g.* RUUD, 1932; BARGMANN, 1937; MARR, 1962; IVANOV, 1970; MACKINTOSH, 1972, 1973, 1974; NEMOTO, 1975; KOCK and STEIN, 1978; FEVOLDEN, 1979), but some related knowledge also came from rearing experiments (MCWHINNIE and DENYS, 1978a; MURANO *et al.*, 1979). On the other hand, subjects relating to the production of krill have been studied on the basis of morphological characteristics of maturation and spawning ecology, such as fecundity, period, place, and spawning times (SHEVTSOV and MAKAROV,

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1969; MAKAROV, 1971, 1975, 1976; MARR, 1962; VORONINA, 1974; EVERSON, 1977).

Recent study of the secondary sexual characteristics using samples collected during the winter season indicated that *E. superba* possibly spawns more than twice in different breeding seasons covering two years (*e.g.* MAKAROV, 1976; KOCK and STEIN, 1978). DENYS *et al.* (1981) found that female krill reared under laboratory conditions did not die after spawning but regressed to a more juvenile appearance, and suggested that it may spawn for two successive years. Their studies were largely based on morphological characteristics as an indication of maturation, and particularly the size frequency distribution was analyzed in order to determine the extent of physiological maturation in smaller sized krill. However, throughout such studies on the maturation and the breeding of this species the characteristics of the ovary in post-spawning females have been less studied up to date; and studies on the reproductive ecology such as the brood size, fecundity, and process of sexual maturation are needed. The present study aims to provide more detailed evidence of the maturation process of the ovary and spawning habits, along with possible spawning frequencies and periodicity.

2. Material and Methods

2.1. Samplings

1) *E. superba* was collected from the first stomach of minke whale (*Balaenoptera acutorostrata bonaerensis*) caught by the whaling factory ship, CHIYO MARU in the area of 63°37′S, 85°08′E to 69°59′S, 01°13′W of the Antarctic Ocean during the 1973/74 season (Fig. 1). All materials collected were fixed with 10% formalin. Among many samples collected, a total of 65 krill ranging 23–57 mm in body length were randomly

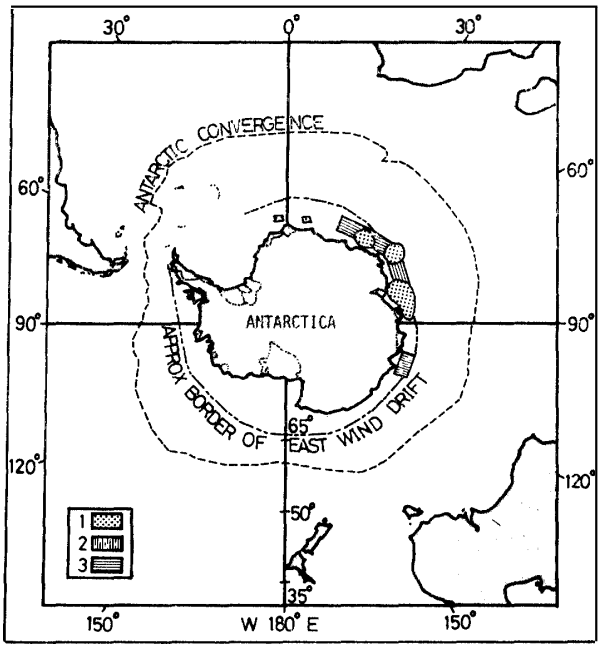

Fig. 1. Sampling areas of Euphausia superba *in the Antarctic Ocean. 1: CHIYO MARU (1973/74), 2: KAIYO MARU (1979/80), 3: KAIYO MARU (1980/81).*

selected for the histological observations of the ovary.

2) During the pre-FIBEX cruise of the R.V. KAIYO MARU of the Fisheries Agency in 1979/80, krill were collected with a KOC-A net in the area of 64°41.5′S, 101°49.3′E to 65°00.1′S, 119°59.8′E in the Antarctic Ocean. All materials collected were firstly fixed with Bouin's fluid, and a week later were transferred to 80% ethyl alcohol for longer preservation. A total of 85 females of 29–55 mm collected in January were selected for the histological observations. During this cruise 20 matured females, collected by an oblique haul between 0–36 m depth using the KOC-A net at 64°45.5′S, 114°04.0′E on January 24, were used for the tentative shipboard rearing experiment.

3) During the FIBEX cruise of the R.V. KAIYO MARU in 1980/81, oblique tows from 0 to less than 100 m were made with a KOC-A net at several stations located between 66°48.4′S, 69°49.7′E and 66°50.0′S, 71°06.2′E on February 3, and a total of 39 female krill were reared on the ship. After checking that the spawning took place during late January to early February under laboratory conditions, 27 spawned individuals of 44–53 mm were selected and fixed in the formalin solution for the histological observations.

2.2. Shipboard rearing experiments

The shipboard rearing experiments using a fish hold were conducted twice during the KAIYO MARU cruise for periods between January 24 and February 25 in 1980 and between February 3 and 8 in 1981. Immediately after sampling, the mature females of healthy appearance were sorted out from the samples and kept alive in a plastic container ($40 \times 50 \times 30$ cm) filled with 30 liters of surface seawater.

In order to acclimate the krill to artificial conditions on the ship, the container was placed in a chilled fish hold of total darkness. The water temperature during this treatment was controlled to be at about $+0.4$°C in the case of 1980, and at 0°C~ -1°C in 1981. After 24-hour acclimation, the animals were transferred to separate vessels with the capacity of 0.2–3.0 liters, each of which contained a single animal. The fish hold was lighted for 2–3 hours by a 60 watt electric lamp once or twice a day for the observations. Throughout experiments the water of rearing vessels was exchanged with fresh surface seawater at intervals of one or two days. The net phytoplankton, mostly diatoms, was given to them as food, but sometimes cultured phytoplankton was also used as food.

The total number of eggs released per female was counted in every vessel after removing the spawned animal from the vessel.

2.3. Histological preparations

Length from the anterior tip of the rostrum to the posterior end of the uropod was measured as the total length of krill (MAUCHLINE, 1981) throughout the present study. For the histological examination of the ovary, the cephalothorax was separated from the abdominal segments and embedded in paraffin. Histological sections, 10 μm thick, of the whole cephalothorax stained by the hematoxylin-eosin double staining method were prepared. In order to determine the most representative sectioning planes of the cephalothorax for the observation of the ovary, many materials sectioned on different planes were observed. It was revealed from these observations along

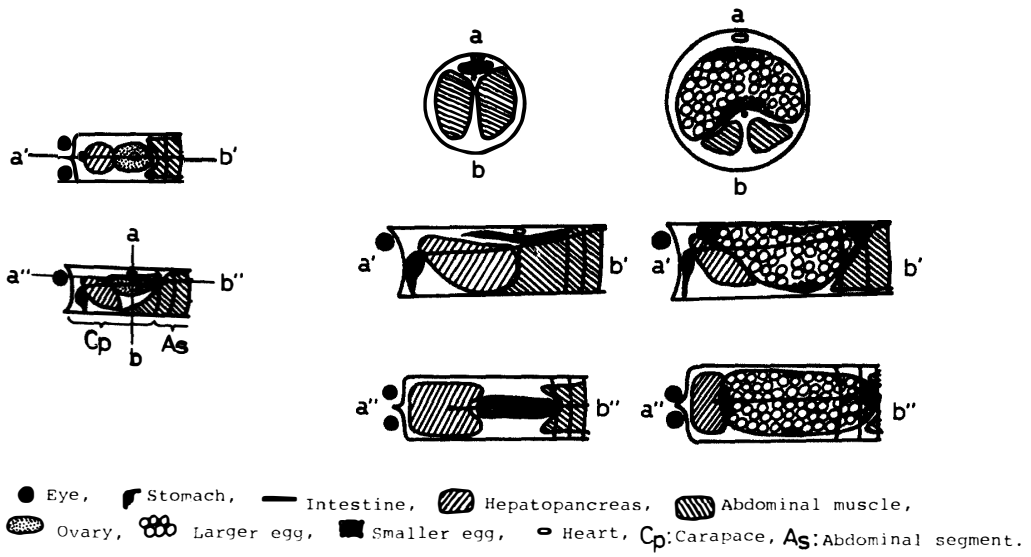

Fig. 2. Schematic planes of histological sections of the cephalothorax in E. superba. *The marks a–b, a′–b′ and a″–b″ indicate the transversal plane, sagittal plane and dorsoventral plane, respectively (left row). Figures corresponding to each sectional plane show both for a smaller immature female (middle row) and a larger mature female (right row).*

with generally known anatomical characteristics of the krill reproductive system (*e.g.* ZIMMER, 1913; RAAB, 1915; BARGMANN, 1937) that the whole ovary of *E. superba* occupied almost symmetrically the cephalothorax against the sagittal plane at the median part of the cephalothorax (Fig. 2, a′–b′), and that the ovarian eggs show a similar composition in both sides of the body. On the other hand, when the cephalothorax was sectioned on the dorsoventral plane (Fig. 2, a″–b″), the sections showed quite biassed distribution of the ovarian eggs. On the basis of these observations, sections along the sagittal plane close to the central part of the cephalothorax were considered to be the most representative sections for the estimation of the average composition of the ovarian eggs. That is, the composition of ovarian eggs over the whole ovary was estimated from these sections. In all the animals examined the germ cells were classified histologically by the development of maturation, and the number of cells on a section was counted for each maturity stage in order to know the composition of ovarian eggs from their relative frequencies of occurrence. The average length of the long axis of the egg and the nucleus diameters were measured on 50 germ cells.

3. Results

3.1. Histological observations of ovary

3.1.1. Maturity stages of ovarian eggs

Sections of the cephalothorax with undeveloped and developed ovaries along the sagittal plane are shown in Figs. 3–6. It is noticed from the figures that the ovary extends to the first or second abdominal segment in accordance with the growth of ovary size by maturation. Referring to the developmental stages of ovarian eggs in fishes (*e.g.* YAMAMOTO *et al.*, 1965; YAMAMOTO, 1977), ovarian eggs in *E. superba* were divided

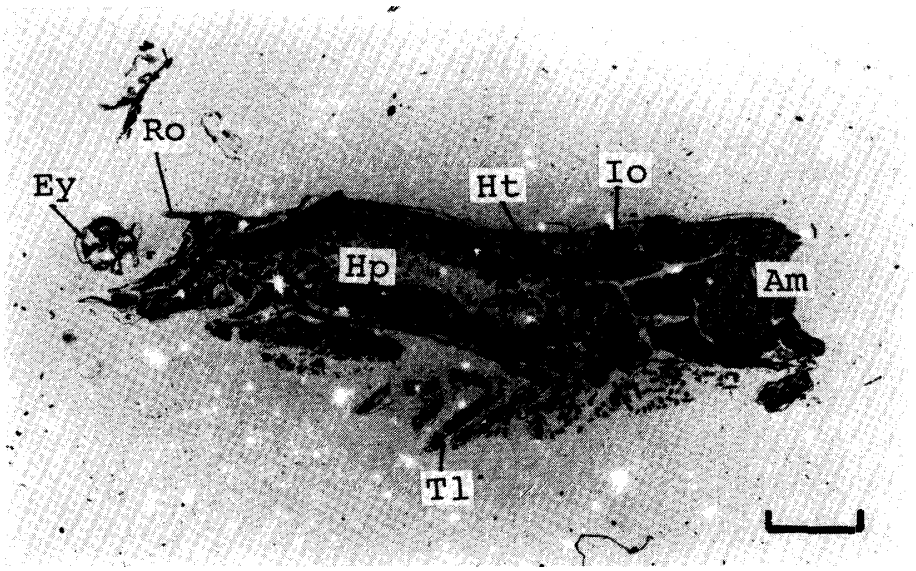

Fig. 3. Section of the cephalothorax along the sagittal plane in an immature female of E. superba. *Undeveloped ovary contains follicles. T.L. 30 mm, 22/XI/1973. Scale: 1 mm. Am: abdominal muscle, Ey: eye, Ht: heart, Hp: hepatopancreas, Io: immature ovary, Ro: rostrum, Tl: thoracic leg.*

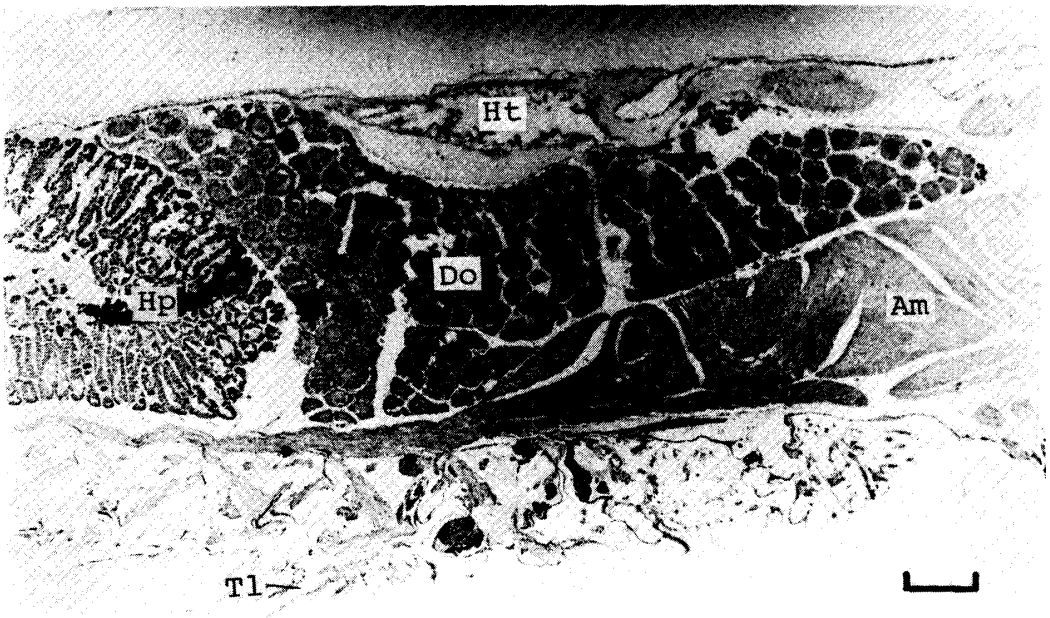

Fig. 4. Section of a developed ovary along the sagittal plane in matured E. superba. *T.L. 57 mm, 10/I/1974. Scale: 1 mm. Do: developed ovary (other legends are shown in Fig. 3).*

into nine developmental stages (see also Pls. 1–3) by the degree of maturity as follows:

I. Oogonia stage. The diameter of oogonium varies from 9.8 to 29.4 μm (av. 19.7 μm). The oogonia is slightly stained by hematoxylin (Pl. 1, Fig. 1).

II. Early chromatin nucleolus stage. The diameter of oocytes is 19.8–39.6 μm (av. 25.2 μm). The cytoplasm is stained heavily by hematoxylin (Pl. 1, Fig. 2) and a few cells with expanded cytoplasm are also included in this stage.

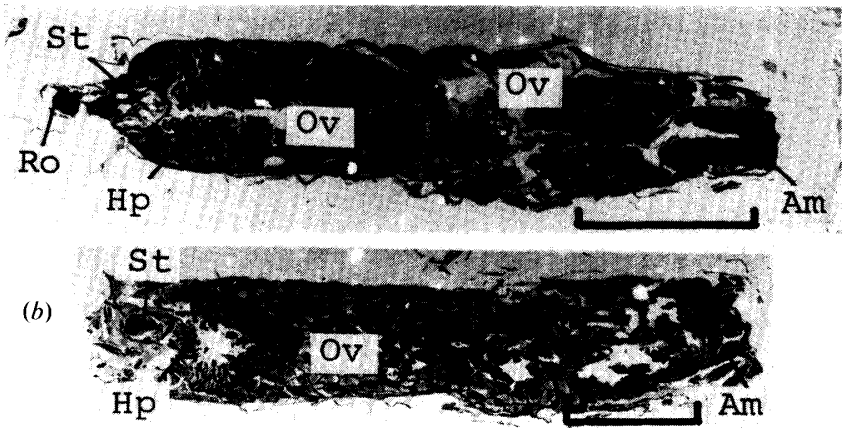

Fig. 5. Sections of the cephalothorax along the horizontal plane in female E. superba. *(a) Section at upper dorsad. T.L. 40 mm, 25/I/1980, (b) Section at the principal axis, T.L. 53 mm, 29/I/1980. Scale: 5 mm. Ov: ovary, St: stomach (other legends are shown in Fig. 3).*

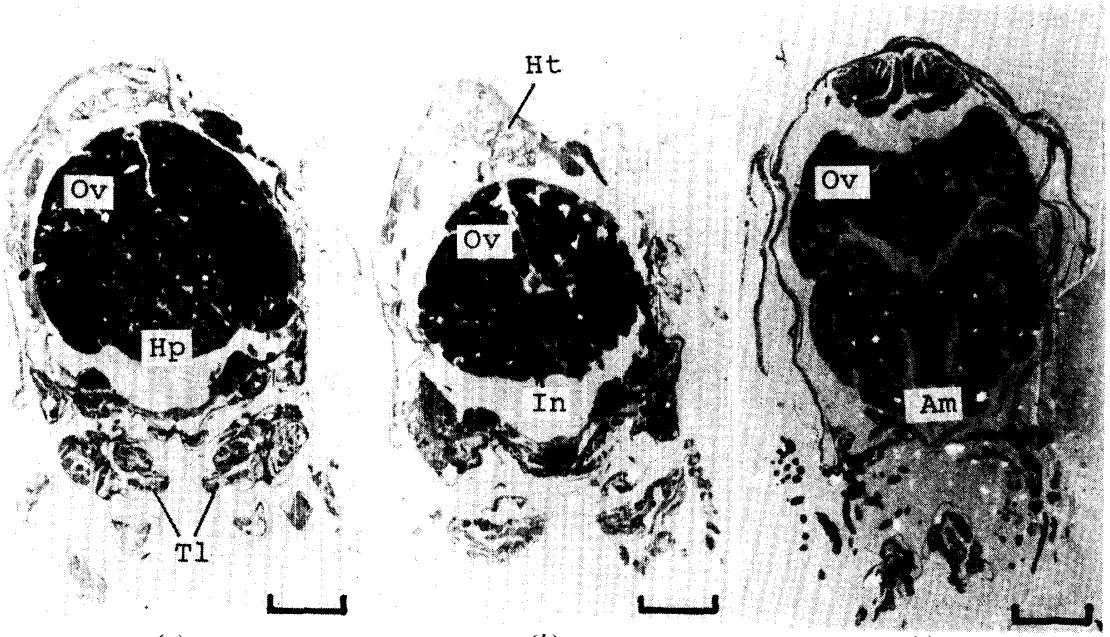

Fig. 6. Sections of the cephalothorax along the transversal plane in mature female of E. superba. *(a) Anterior part, (b) Central part, (c) Posterior part. T.L. 53 mm, 2/II/1973. Scale: 1 mm. In: intestine (other legends are shown in Figs. 3 and 5).*

III. Late chromatin nucleolus stage. The diameter is 19.8–69.3 μm (av. 44.9μm). Remarkably enlarged cytoplasm stained by hematoxylin is observed (Pl. 1, Fig. 3).

IV. Early peripheral nucleolus stage. The diameter of germ cell and the nucleus is 175–375 μm (av. 245μm) and 75–175μm (av. 131 μm) respectively. Diameter of egg is larger than that of the earlier stages. The germ cell has a nucleus heavily stained purple by hematoxylin, but the peripheral nucleolus is somewhat obscure. The cytoplasm is well stained red by eosin (Pl. 1, Fig. 4).

V. Middle peripheral nucleolus stage. The diameter of germ cell and the nucleus is 200–325 μm (av. 256μm) and 75–175 μm (av. 136μm) respectively. The nucleus

and the cytoplasm are of similar coloration to the early peripheral nucleolus stage. The peripheral nucleolus is distinct at the margin of the nucleus (Pl. 2, Fig. 1).

VI. Late peripheral nucleolus stage. The diameter of germ cell and the nucleus is 250–425 μm (av. 315 μm) and 100–200 μm (av. 145 μm) respectively. The morphology and the coloration are similar to stages IV and V, but a few small droplets are found in the cytoplasm (Pl. 2, Fig. 2).

VII. Early vitellogenic stage. The diameter of germ cell and the nucleus is 250–600 μm (av. 338 μm) and 75–200 μm (av. 154 μm) respectively. The nucleus and the cytoplasm are similarly colored to stages IV–VI. The characteristic features in this stage are the increase of small droplets in the cytoplasm, growth in cell diameter, and the clear boundary between adjacent germ cells (Pl. 2, Fig. 3).

VIII. Late vitellogenic stage. The diameter of germ cell and the nucleus is 350–550 μm (av. 408 μm) and 150–225 μm (av. 184 μm) respectively. Many larger droplets become clear in the cytoplasm, which indicates a considerable accumulation of vitellogenic substances (Pl. 3, Fig. 1).

IX. Final maturity stage. The cytoplasm in the germ cell is slightly stained red

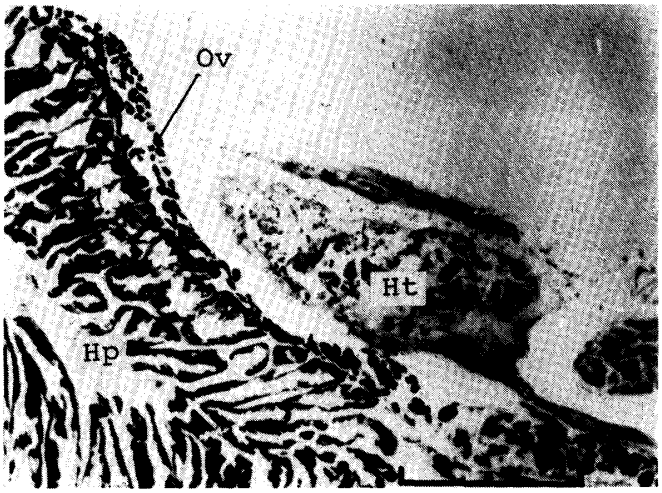

Fig. 7. Structure of undeveloped ovary in immature E. superba. *T.L. 30 mm. 22/XI/1973. Scale: 1 mm (other legends are shown in Figs. 3 and 5).*

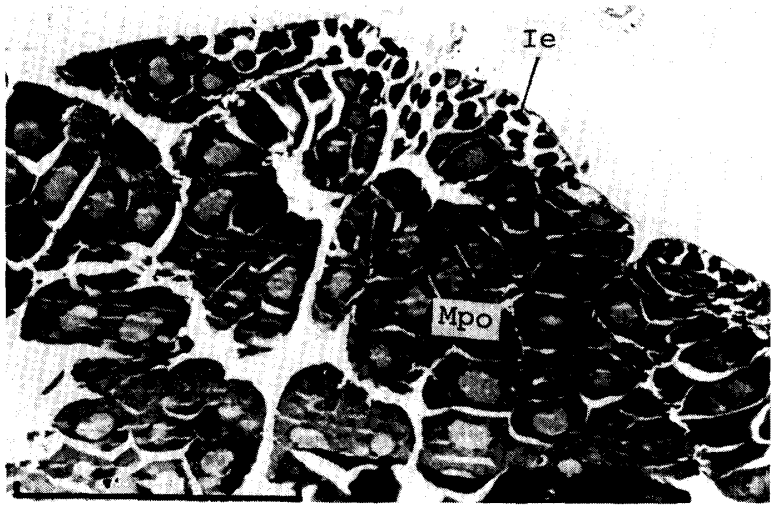

Fig. 8. Structure of developing ovary in maturing E. superba. *T.L. 39 mm. 19/XII/1973. Scale: 1 mm. Mpo: oocytes of the middle peripheral nucleolus stage, Ie: immature eggs.*

by eosin. The diameter of germ cell is 450–775 μm (av. 573 μm) and the contour of the nucleus becomes indistinct. This stage is considered to be the most matured stage before fertilization and ovulation take place (Pl. 3, Fig. 2).

3.1.2. Composition of ovarian eggs

From the composition of ovarian eggs by their developmental stages, the maturity of each individual became distinguishable. Sexually immature krill retained eggs from the oogonia to oocytes of the late chromatin nucleolus stage (Fig. 7), whereas half-mature krill retained oocytes from the early chromatin nucleolus stage or the early peripheral nucleolus stage to the early vitellogenic stage (Figs. 8–9). Mature krill, on the other hand, simply or dominantly retained oocytes from the late vitellogenic stage to the final maturity stage (Figs. 10–11). In immature krill the eggs from the oogonia

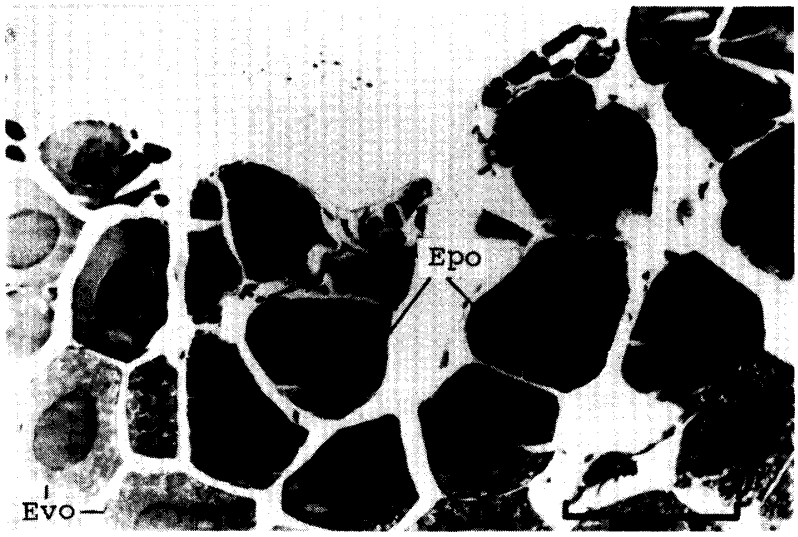

Fig. 9. Arrangements of eggs in various maturation stages in developing ovary of E. superba. *T.L. 37 mm. 29/I/1980. Scale: 400 μm. Epo: oocytes of the peripheral nucleolus stage, Evo: oocytes of the early vitellogenic stage.*

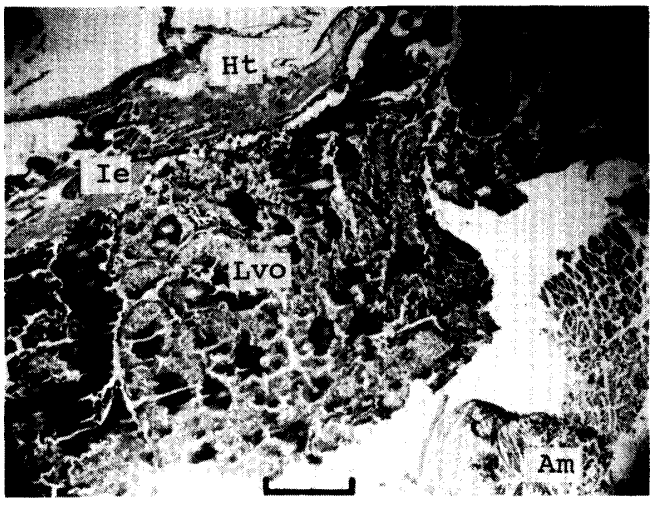

Fig. 10. Arrangements of eggs in mature ovary of E. superba. *T.L. 40 mm. 31/I/1980. Scale: 400 μm. Lvo: oocytes of the late vitellogenic stage (other legends are shown in Figs. 3 and 8).*

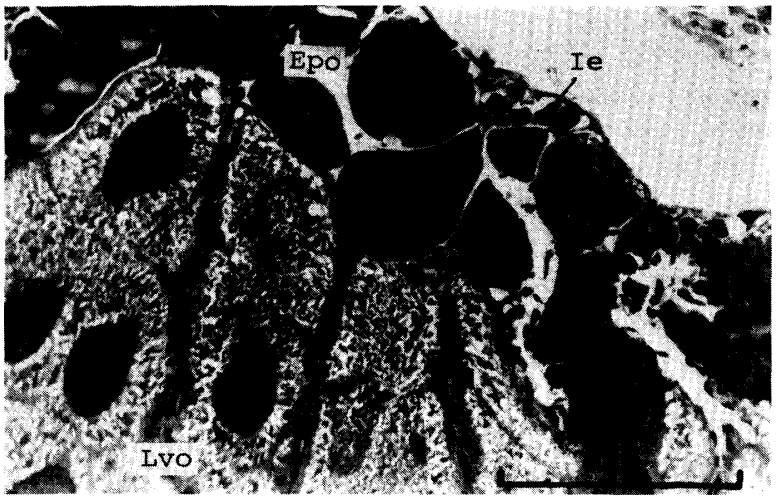

Fig. 11. Arrangements of eggs in mature ovary of E. superba. *The boundary of eggs of each maturity stage is clearly visible. T.L. 54 mm. 21/I/1980. Scale: 400 μm (legends are shown in Figs. 8, 9 and 10).*

to oocytes of the late chromatin nucleolus stage are generally distributed in the marginal parts of the ovary closer to anterior, posterior, and beneath the heart; while the oocytes of the early peripheral nucleolus stage to the early vitellogenic stage occupied more the inside of the ovary (see Figs. 8–9). In mature krill the oocytes of the late vitellogenic stage and the final maturity stage are distributed largely more inside of the ovary (see Figs. 10–11). However, younger eggs of various maturity stages were always present to some extent even in the ovary of mature krill.

3.1.3. Ovary after ovulation

In the ovary of post-spawning females there was observed a small amount of oocytes of the early to late chromatin nucleolus stages (Fig. 12). In several post-spawning females there were also found some mature eggs, though small in number (Fig. 13). The number of immature oocytes existing in the ovary ranged from 63 to 480 cells per

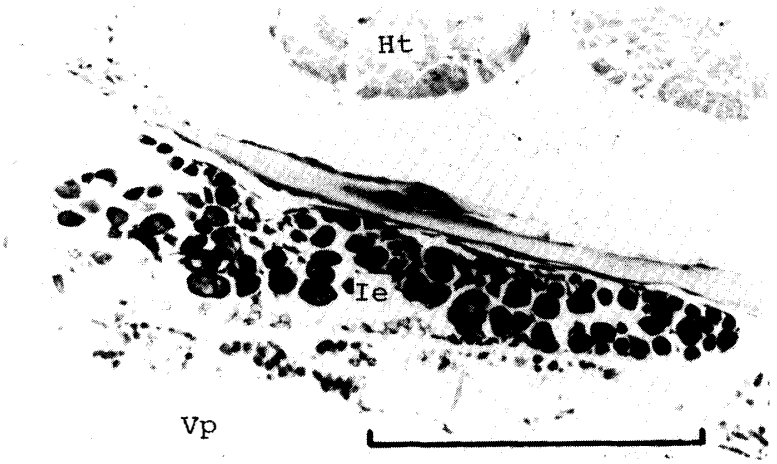

Fig. 12. Immature eggs remaining in the ovary after spawning. T.L. 35 mm. 18/I/1974. Scale: 400 μm. Vp: vacancy in the ovary (other legends are shown in Figs. 3 and 8).

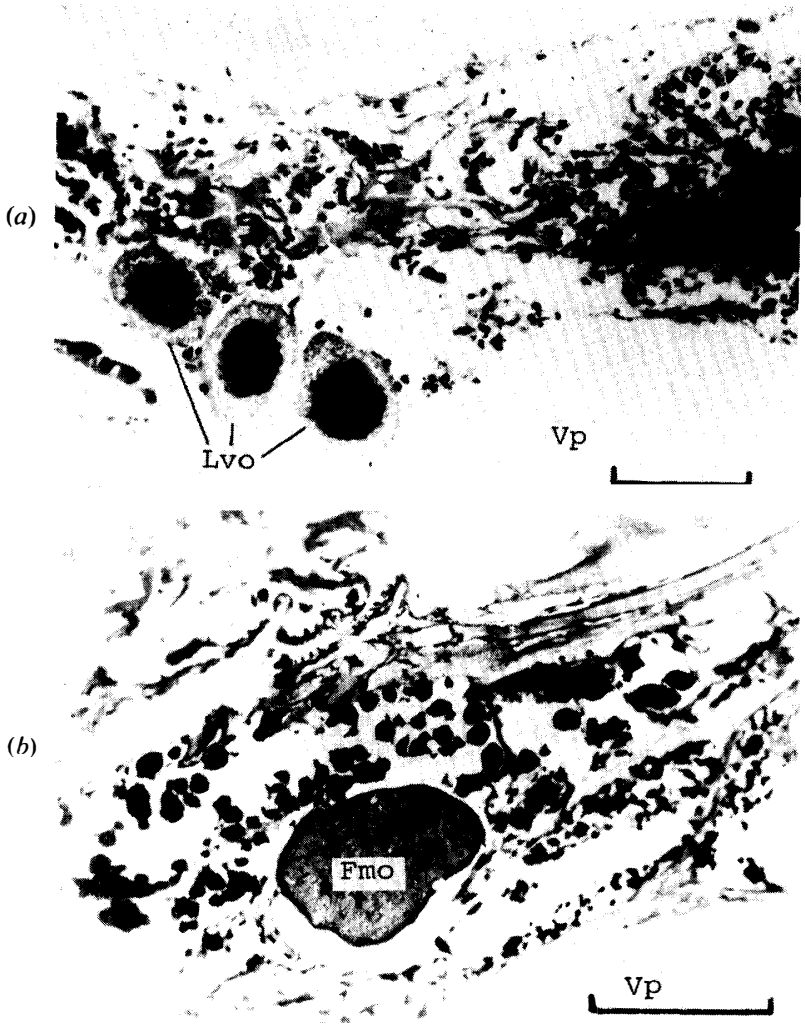

Fig. 13. Oocytes at the late vitellogenic stage (a) and at the final maturity stage (b) left in the ovary after spawning. (a) T.L. 48 mm. 27/I/1974. Scale: 500 μm. (b) T.L. 46 mm. 4/II/1974. Scale: 500 μm. Fmo: oocytes of the final maturity stage (other legends are shown in Figs. 10 and 12).

individual in 17 post-spawning females. In 24 out of 27 post-spawning krill reared in the laboratory, maturity stages of ovarian eggs showed a similar result to the case of net-sampled krill (Table 1). The remaining three individuals carried many mature eggs, and were considered unusual.

3.2. Number of eggs spawned

The number of eggs actually released was 1140–1688 eggs ($\bar{x}=1309$, SE=79) in a total of six individuals of 49–54 mm total length in the 1979/80 rearing experiment (see KIKUNO, 1981) and 627–3115 eggs ($\bar{x}=1526$, SE=145) in 20 individuals of 44–53 mm in the 1980/81 experiment (Table 2). Due to the small number of observations, the relationship between the number of eggs spawned and the krill size was not clear. The fecundity in *E. superba*, however, seems to vary considerably within the size classes because the histological observations of ovary sectioned at a median part along the sagit-

Table 1. Maturity stages of ovarian eggs in laboratory reared, post-spawning E. superba.

Individual No.	Total length (mm)	Number of eggs spawned	Maturity stages of ovarian eggs after spawning: Oogonia	Oocytes of the early chromatin nucleolus stage: Number**	%	Oocytes of the late chromatin nucleolus stage: Number**	%	Oocytes of the final maturity stage: Number**	%
1	51	2230	0	0	0	0	0	0	0
2	50	1140	0	230	32.9	468	67.0	1	0.1
3	46	1142	unknown*	*		no count		no count	
4	48	1121	0	0	0	101	28.2	257	71.8
5	44	1373	0	0	0	0	0	0	0
6	49	no count	*	*		120	98.4	2	1.6
7	49	1774	0	19	12.0	83	52.5	56	35.4
8	53	2114	*	*		132	93.0	10	7.0
9	51	no count	0	72	17.6	37	9.0	301	73.4
10	47	1928	*	*		69	86.3	11	13.7
11	46	672	*	*		54	83.1	11	16.9
12	47	1901	*	*		59	66.3	30	33.7
13	49	3115	*	*		85	100.0	0	0
14	51	1798	*	*		188	81.4	43	18.6
15	50	674	*	*		174	80.9	41	19.1
16	49	no count	0	0	0	4	19.0	17	81.0
17	46	1310	*	*		115	98.3	2	1.7
18	49	857	*	*		126	94.7	7	5.3
19	45	1070	*	*		53	76.8	16	23.2
20	53	2515	*	*		67	27.0	181	73.0
21	48	no count	*	*		>55	—	0	0
22	50	no count	*	*		>286	—	8	—
23	51	1928	*	*		102	91.9	9	8.1
24	52	1898	0	0	0	45	58.4	32	41.6
25	47	no count	0	0	0	0	0	0	0
26	48	no count	0	57	16.9	274	81.1	7	2.1
27	—	no count	*	*		116	94.3	7	5.7

* Due to difficulty to distinguish the difference of oogonia and oocytes of the early chromatin nucleolus stage from other internal organs, their numbers were not counted separately.

** Number of egg cells found on a histological section.

tal plane showed that the number of mature eggs was about ten in 35 mm krill, whereas it was about 200 in a 53 mm krill (Fig. 14).

3.3. Monthly changes in the composition of ovarian eggs

Composition of ovarian eggs by developmental stages in *E. superba* is shown in Fig. 15. Ovarian eggs in a size class smaller than 30 mm collected from November to February were composed largely of oogonia to oocytes of the late chromatin nucleolus stage, while those in animals larger than 31 mm were composed very little of oogonia to oocytes of the late peripheral nucleolus stage from November to December with highest frequency in oocytes of the middle peripheral nucleolus stage, and those oocytes were remarkably developed to attain the vitellogenic stage towards early to mid Janu-

Table 2. Number of eggs laid by E. superba *reared in laboratory.*

Sample	Year	Number of eggs spawned	Total length (mm)	Wet body weight (g)	Sample	Year	Number of eggs spawned	Total length (mm)	Wet body weight (g)
1	1979/80	1218*	49	—	14	1980/81	1901	47	1.22
2	〃	1236*	49	—	15	〃	627	46	1.28
3	〃	1320*	50	—	16	〃	1928	47	0.98
4	〃	1140*	51	—	17	〃	3115	49	1.22
5	〃	1251*	54	—	18	〃	1798	51	1.34
6	〃	1688*	50	—	19	〃	674	50	1.47
7	1980/81	2230	51	1.22	20	〃	1310	46	1.08
8	〃	1140	50	1.19	21	〃	857	49	1.61
9	〃	1142	46	0.94	22	〃	1073	45	1.08
10	〃	1121	48	1.27	23	〃	2515	53	1.71
11	〃	1373	44	1.01	24	〃	1928	51	1.32
12	〃	1774	49	1.19	25	〃	1898	52	1.31
13	〃	2114	53	1.60	26	〃	1989	—	—

* After the results by KIKUNO (1981).

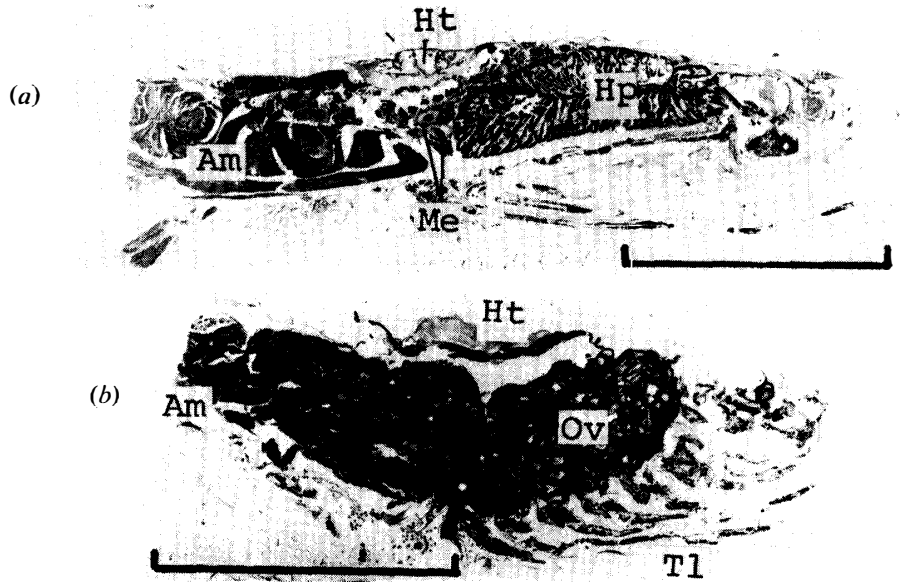

Fig. 14. Difference in the amount of ovarian eggs between (a) smaller and (b) larger sized E. superba. *(a) T.L. 36 mm. 30/I/1980. Scale: 10 mm. (b) T.L. 51 mm. 31/I/1980. Scale: 10 mm. Me: mature egg (other legends are shown in Figs. 3 and 5).*

ary. In late January a few post-spawning krill larger than the 31–35 mm size class began to occur, and the number of these size classes increased toward February. This indicates that the spawning had been carried out sometime around mid January. In the ovary of spawned females no well developed oocytes were left, but some amount of oocytes of the early and late chromatin nucleolus stages remained. The composition of ovarian eggs of *E. superba* collected by the net sampling in January of the 1979/80 season is shown in Fig. 16. It can be seen in this figure that the ovarian eggs are likely to develop considerably towards late January as shown in Fig. 15, but there were no post-spawn-

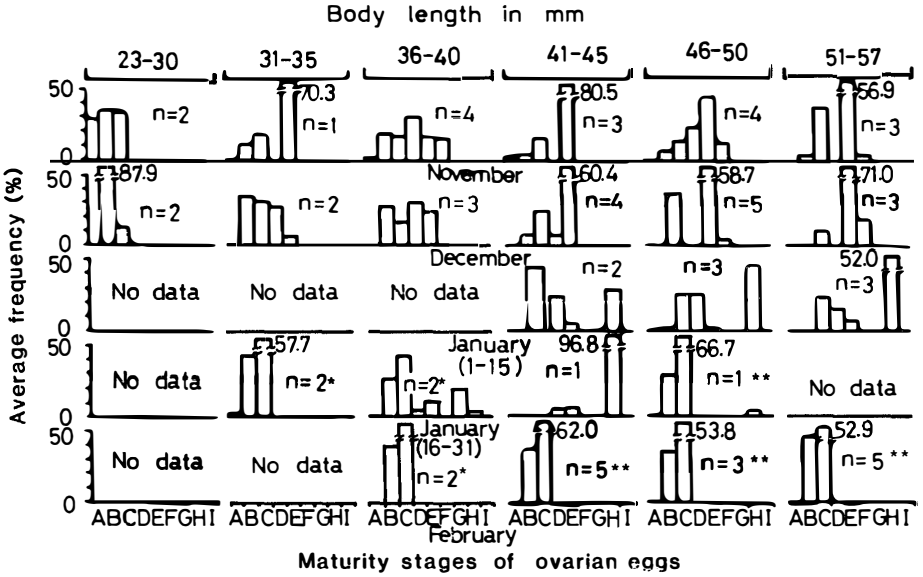

Fig. 15. Composition of ovarian eggs of E. superba *collected from the stomachs of minke whales in 1973/74. n: number of krill examined. A: oogonia stage, B: early chromatin nucleolus stage, C: late chromatin nucleolus stage, D: early peripheral nucleolus stage, E: middle peripheral nucleolus stage, F: late peripheral nucleolus stage, G: early vitellogenic stage, H: late vitellogenic stage, I: final maturity stage. *One of two animals spawned. **All spawned.*

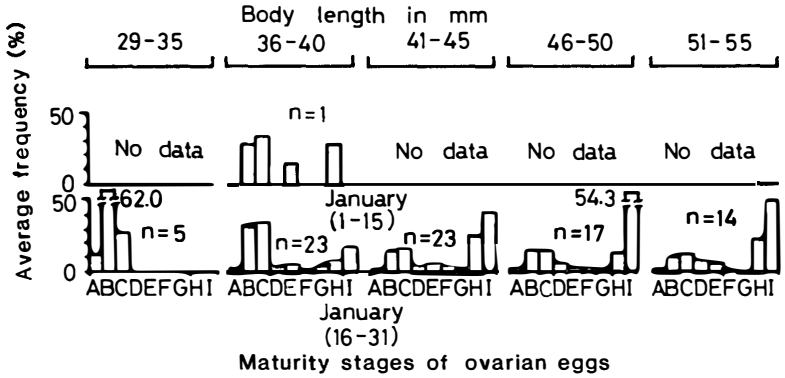

Fig. 16. Composition of ovarian eggs of E. superba *collected with the KOC-A net during the* KAIYO MARU *cruise in 1979/80 (legends are shown in Fig. 15).*

ing females during January in the 1979/80 season. The ovarian egg composition in krill larger than 41 mm was observed to be homogeneous, whereas it varied considerably from individual to individual in the 31–35 mm and 36–40 mm size classes which occurred in late January to February (see Fig. 15). Therefore, attention was directed to the composition of ovarian eggs in the 31–35 mm and 36–40 mm size classes to clarify the characteristics of maturation and spawning habits in these size classes (Fig. 17). The maturity composition of ovarian eggs showed various features differing within both size classes, but on the whole, the composition can be classified into four representative types, *i.e.*, 1) from oogonia to oocytes of the late chromatin nucleolus stage, 2) from

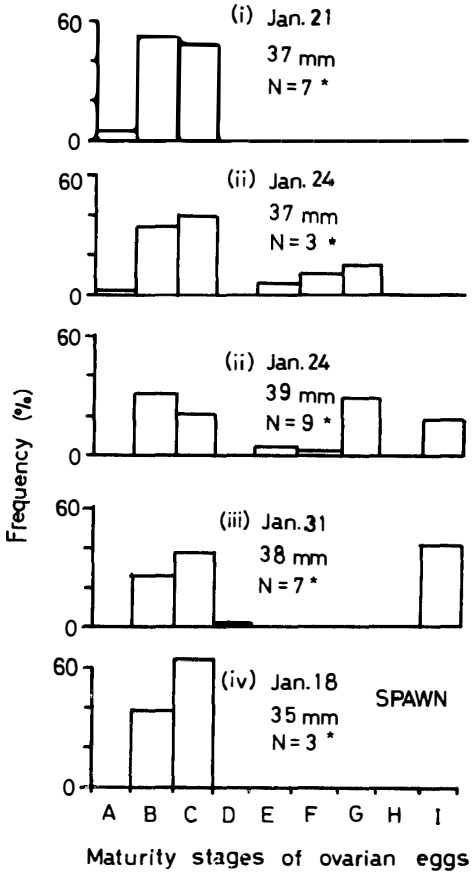

Fig. 17. Difference in the maturity composition of ovarian eggs among E. superba *of similar size classes. N: number of animals examined (legends are shown in Fig. 15).*

oogonia to oocytes of the early vitellogenic stage or oogonia to oocytes of the final maturity stage, 3) from oogonia to oocytes of the late chromatin nucleolus stage and the final maturity stage, and 4) from oogonia to oocytes of the early and late chromatin nucleolus stages existing in the ovary of post-spawning krill.

4. Discussion

Through the histological observations of ovarian eggs in female krill, one fact which became clear was a remarkably rapid progress in maturing of the gonad during early to mid January when the vitellogenesis of the ovarian egg begins its progress. The occurrence of post-spawning krill recognized histologically in late January to February indicates that *E. superba* occurring in the Indian Ocean Sector perform their spawning during these months. In the shipboard rearing experiments conducted in both the 1979/80 and 1980/81 seasons, the krill spawned at the end of January to early February. This also suggests the beginning of the spawning season during the abovementioned periods. Assuming the oocytes of early developmental stages such as peripheral nucleolus stages take more than a month to become well matured eggs ready to be spawned, the spawning period may be considered to go on until March. The mini-

mum size of post-spawning krill confirmed by the histological sections of its ovary was 35 mm in total length, and this size agrees with the minimum maturation size, 36 mm, obtained from the morphological observations for maturation of krill (NEMOTO and MURANO, 1979).

In the ovary of post-spawning females there was observed some amount of oocytes of the early and the late chromatin nucleolus stages. In some fish reproduction such younger ovarian eggs are known to begin to develop again towards subsequent spawning (*e.g.* HICKLING and RUTENBERG, 1936; YAMAMOTO, 1955, 1977). Assuming those oocytes in *E. superba* will begin to develop in February with the estimated rate for attaining appreciable maturation as shown in Fig. 15, they must become mature eggs in the early austral winter and then be spawned. This conception, however, does not agree with reported spawning periods, *i.e.* end of January to March (*e.g.* EVERSON, 1977), and seems to be difficult to accept because winter spawning of this species has not been previously reported. It is unfavorable to release eggs in winter hampered by many factors such as limited food and severe environmental conditions. In some shrimps it has been known that the immature oocytes left in the ovaries after spawning will develop so as to be spawned during another occasion (*e.g.* KING, 1948; IKEMATSU, 1955; YATSUYANAGI and MAEKAWA, 1956a, b, 1957; CUMMINGS, 1961; WILLIAMS, 1965). These evidences lead to a consideration that remaining eggs after spawning in *E. superba* would pass the winter and be released in the subsequent summer. The suggested fate and developmental process of these immature oocytes found in the ovary of post-spawning female krill may indicate similar conditions of ovarian eggs, known as the group synchronous type of maturation in fish (*e.g.* HICKLING and RUTENBERG, 1936; YAMAMOTO, 1955, 1977). This may lead to the consideration that *E. superba* spawns twice or more throughout its life span. As known by a very simple composition of ovarian eggs during the peak of the spawning period, *E. superba* perhaps spawns once within a current breeding season.

Analyzing the size-frequency distribution of *E. superba*, several workers considered that *E. superba* will become mature at the age of 2+ (RUUD, 1932; BARGMANN, 1945; NEMOTO, 1959), whereas some others considered that this animal will mature at the age of 3+ as the result of rearing experiments (MCWHINNIE and DENYS, 1978a, b; MURANO *et al.*, 1979). IVANOV (1970) and NEMOTO and MURANO (1979) estimated that the growth rate of *E. superba* would be 15–18 mm per year. If *E. superba* grows at the rate of 15 mm per year, and the well-known growth curve of this animal is taken into consideration, the post-spawning krill of 35–57 mm observed in this study must be composed of two groups of different year classes, *i.e.*, 2 and 3 years old.

From changes in the maturity composition of ovarian eggs during late January to early February, it was suggested that part of the female krill of 35–40 mm would perhaps lay eggs within the breeding season at the age of 2+, while another non-spawning krill of the same or similar body size may spawn first a year later at the age of 3+. MAKAROV (1976) reported that some females of 30–51 mm collected in September retained underdeveloped oocytes in the ovary. This fact again supports that some krill will spawn for the first time at the age of 3+ old. These considerations suggest that the oocytes of the early and late chromatin nucleolus stages occurring during the period from the end of January to early February will develop up to oocytes of the early peripheral

nucleolus stage in the following early summer. In the ovary of females possibly spawned at the age of 2+ old with the size of 35–40 mm, there were observed some immature oocytes, which may become mature by the following season. This result suggests the possibility of a second spawning, that is, *E. superba* conducts its first spawning during the current breeding season at 2+ years old, and probably a second one occurs during a different breeding season at 3+ years old.

In the present study the number of eggs spawned per female varied from 627 to 3115 ($\bar{x}=1417$, SE=117) through the experiments of both the 1979/80 and 1980/81 cruises. This figure is close to the 1000–4000 eggs reported by MCWHINNIE and DENYS (1978a). In the post-spawning animals, through these experiments, there was found histologically a very small number of unspawned mature eggs in the ovary. On the other hand, the number of eggs in the ovary of *E. superba* in the previous reports shows considerable variation. MAUCHLINE and FISHER (1969) reported 310–800 eggs, while NEMOTO *et al.* (1976) reported 2000–14000 eggs. Other estimations fall within these values (*e.g.* BARGMANN, 1945; JAZDZEWSKI *et al.*, 1978; MCWHINNIE and DENYS, 1978a; MAKAROV, 1975; NAUMOV, 1962; NEMOTO, 1974, 1975). To compare the fecundities with the number of spawned eggs in the present study, there is a considerable difference between them. The known fecundity of *E. superba* is generally based on counting the whole or a partial number of visually mature eggs in the ovary, or is indirectly estimated from the volume. Although the reason of such a difference was not clear, one of possible causes would depend on the method of the fecundity estimation such as counting mature eggs that are actually spawned within the current breeding period as in the present study.

Acknowledgments

We are greatly indebted to Drs. K. NASU and Y. KOMAKI of the Far Seas Fisheries Research Laboratory, Fisheries Agency, for their warmful encouragement and help during the pre-FIBEX and FIBEX cruises of the R.V. KAIYO MARU. We thank Prof. H. TAKAHASHI of Faculty of Fisheries, Hokkaido University for giving many advices on histological techniques. Captain T. TAKAHASHI and his crew kindly provided the ship's fish hold for the rearing experiments throughout both cruises, and their well-timed cooperation is greatly appreciated.

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(*Received November 6, 1982; Revised manuscript received February 21, 1983*)

Plate 1. Ovarian eggs of *E. superba*.

Fig. 1. Oogonia (Og). T.L. 35 mm, 16/I/1980. Scale: 100 μm.

Fig. 2. Oocytes of the early chromatin nucleolus stage (Eco). T.L. 37 mm, 25/I/1980. Scale: 100 μm.

Fig. 3. Oocytes of the late chromatin nucleolus stage (Lco). T.L. 34 mm, 21/I/1980. Scale: 100 μm.

Fig. 4. Oocytes of the early peripheral nucleolus stage (Epo). T.L. 47 mm, 22/I/1980. Scale: 100 μm. Cy: cytoplasm, N: nucleus.

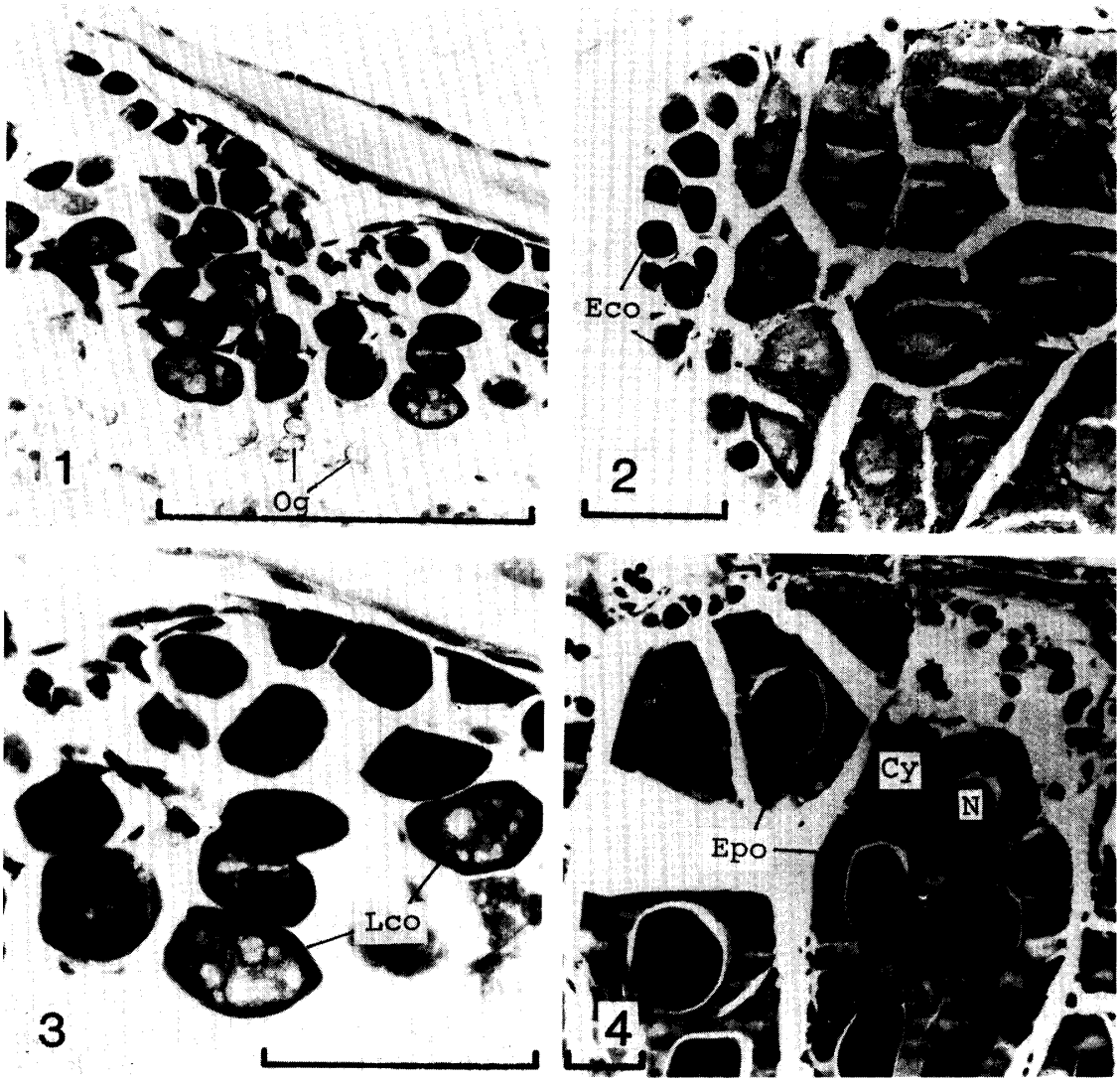
1
Og
2
Eco
3
Lco
4
Epo
Cy
N

Plate 2. Ovarian eggs of *E. superba.*

Fig. 1. Oocytes of the middle peripheral nucleolus stage (Mpo). T.L. 53 mm, 20/I/1980. (Legends are as shown in Pl. 1, Fig. 4.). Nl: nucleolus. Scale: 100 μm.

Fig. 2. Oocytes of the late peripheral nucleolus stage (Lpo). T.L. 50 mm, 21/I/1980. (Legends are as shown in Pl. 1, Fig. 4 and Pl. 2, Fig. 1). Scale: 100 μm.

Fig. 3. Oocytes of the early vitellogenic stage (Evo). T.L. 37 mm, 24/I/1980. (Legends are as shown in Pl. 1, Fig. 4). Scale: 100 μm.

Plate 2

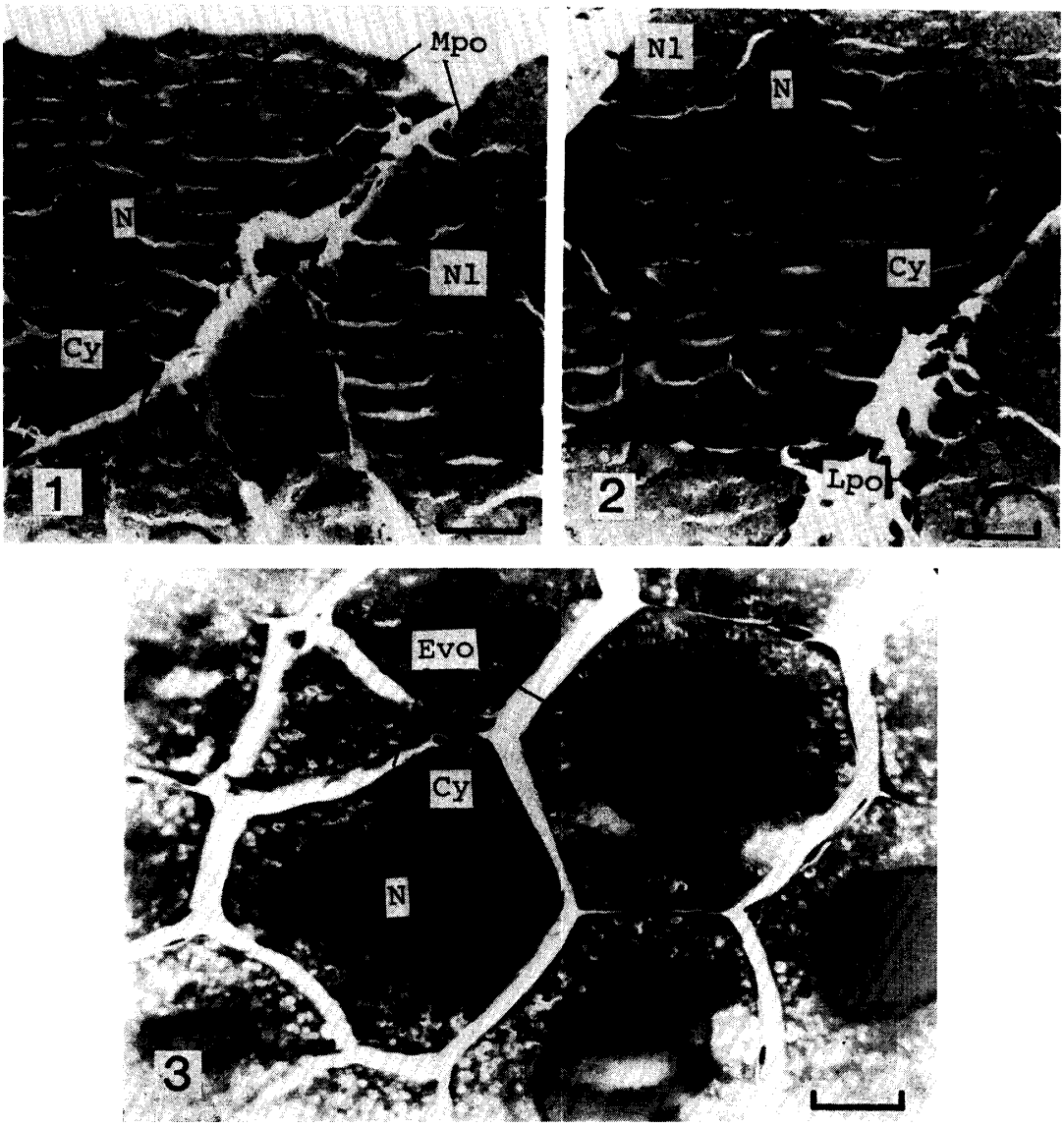

Plate 3. Ovarian eggs of *E. superba*.

Fig. 1. Oocytes of the late vitellogenic stage (Lvo). T. L. 48 mm, 22/I/1980. Scale: 100 μm. (Legends are as shown in Pl. 1, Fig. 4).

Fig. 2. Oocytes of the final maturity stage (Fmo). T. L. 47 mm, 22/I/1980. Scale: 100 μm. (Legends are as shown in Pl. 1, Fig. 4).

Plate 3