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ORIGINAL ARTICLE

Biology

Histological studies on the spent ovaries of aged snow crabs *Chionoecetes opilio* caught in the Sea of Japan

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Abstract Aged female snow crabs *Chionoecetes opilio*, 9 or so years old after their final molt, were collected sporadically from artificial reefs established off the coast of Wakasa Bay, Sea of Japan. In order to clarify the degenerative changes that occur in spent ovaries, the ovaries of these crabs were examined histologically. The ovaries were found to be reduced in both size and volume, with most showing significant oosorption by follicular epithelial cells. Reserve fund eggs and unspawned eggs were rarely detected, with the exception of some in atretic follicles. One to dozens of orange to black nodules scattered over the ovary consisted of masses of degenerative ova in various stages of oolysis. Since nodular formation was recognized in 26% of these aged individuals, it seems very likely that such crabs are unable to spawn healthy eggs.

Keywords Aged snow crab · *Chionoecetes opilio* · Ovarian histology · Sea of Japan · Spent ovary

Introduction

The snow crab *Chionoecetes opilio* is an important fishery resource in the northwestern Atlantic and north Pacific, having recently been placed under strict fisheries management [1, 2]. Similarly, in the Sea of Japan, snow crab fisheries are conducted under strict regulations, with artificial reserve reefs having been established in selected areas for this purpose [3, 4]. General conservation measures, culturing techniques and research needs on the ecology and life history of snow crabs have also been addressed [5–7].

The breeding cycle and reproductive potential of female snow crabs in the Sea of Japan indicate that the last molt apparently occurs during July–October, thereby enabling the adult stage to be attained. Immediately after, these females mate with males in order to achieve the first oviposition, and they subsequently clutch the eggs to their abdomens [4, 8]. These females are referred to as primiparous females here. After one and half a years, the fertilized eggs are liberated as prezoea (during February–April). Subsequently, the females exhibit repeated annual hatching, copulation and spawning within a one-year cycle [9–11], and are considered multiparous females. Although the lifespan of female crabs following the last molt is still unknown, release experiments with tagged crabs conducted in the Sea of Japan resulted in subsequent recaptures 7 years after [4]. On the other hand, female snow crabs in the Bering Sea are known to survive 6–7 years after their final molt [12], compared with 4–5 years off the Canadian Atlantic coast [13, 14].

It has been suggested that the reproductive ability of females may decline after they reach a certain (advanced) age, since decreases in the number of eggs and sperm in the spermatophore have been noted [15, 16]. However, no histological studies have been carried out on the ovaries of aged crabs.

Many histological studies on various crab species have reported a reserve fund of young oocytes, ripe eggs, and atretic eggs [9, 17–20]. On the other hand, many reserve fund eggs contaminated with nodules were found in the

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aged snow crabs examined during the present study, thereby presenting an opportunity to describe such degenerative changes. The nomenclature adopted here for oocyte details follows that used in previous studies of the reproductive organs of marine animals [21] (Fig. 5).

Materials and methods

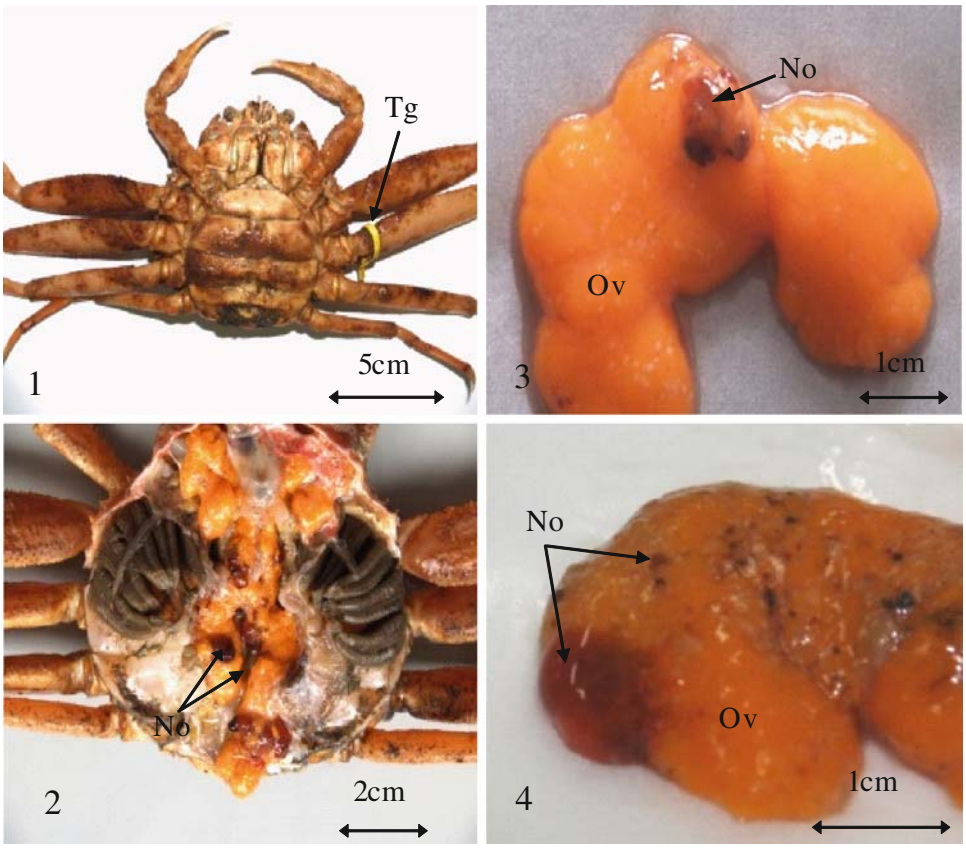
One hundred six aged snow crab individuals were selected from the snow crab catch by deep-sea motor trawlers of the Echizen-cho Fisheries Cooperative Association, Fukui Prefecture, operating in December 2007. The aged crabs were easily distinguished by their yellowish-brown exoskeletons with dark scars, parts of which were ulcerated and fouled with various attached organisms.

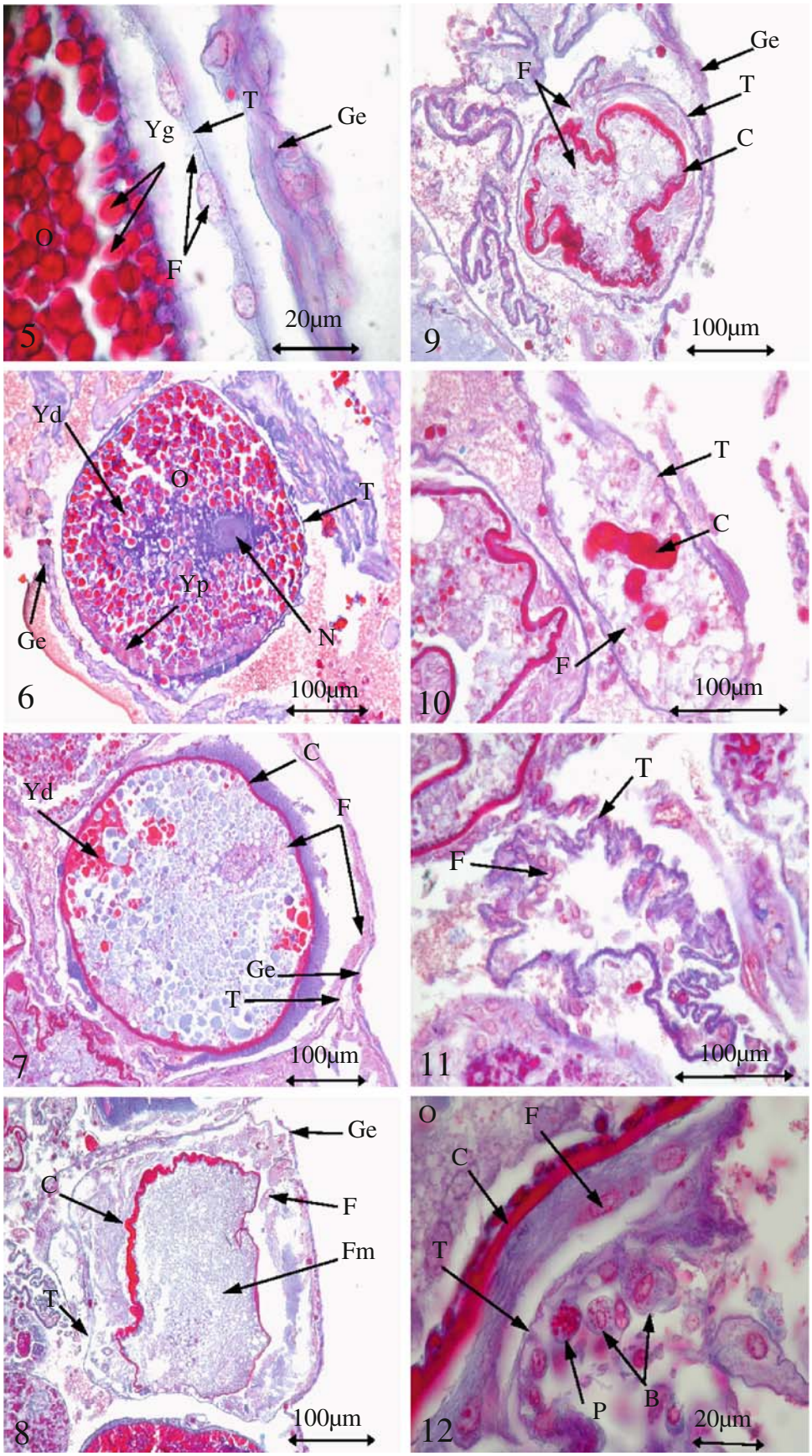
The crabs were measured (carapace width), and body weight, ovarian weight and color tone, numbers of ovarian and fertilized eggs, spermathecal load of the paired spermathecae [16, 22], and embryonic developmental stages were noted. Gonadosomatic index (GSI) was calculated, following Kon and Honma [9]. One individual had been tagged 8 years and 1 month previously, with no eggs being encountered. As this crab had been at least one and a half years old at its final molt, it was considered to be over 9 and a half years old (Fig. 1).

Figs. 5–12 **5** High-power view of the general surface structural pattern of part of the oocyte in a snow crab *Chionoecetes opilio* (azan stain). **6** A degenerative and/or atretic follicle showing collapse of the follicular epithelial membrane. Note the nucleus located near the center of the oocyte, and rich yolk droplets and yolk platelets (azan stain). **7** A degenerative oocyte surrounded by a distinct oolemma. Note the mass of nuclei invading the interior of the oocyte (azan stain). **8** Oocyte showing a significant degenerative change in the follicular epithelial cells, with, conversely, development of the oolemma (deep red azan stain). Note the network structure of the cytoplasm. **9** Oocyte showing significant invasion by follicular epithelial cells, accompanying a decrease in and degeneration of follicular cells between the oolemma and theca. Note two ovulation scars on the *left side* (azan stain). **10** Two degenerated oocytes with distinct condensed oolemma (azan stain). **11** Oocyte showing an ovulation scar containing a few follicular epithelial cells with expanded round nuclei. Note absence of oolemma (azan stain). **12** High-power view of part of a degenerative oocyte surrounded by a distinct oolemma, follicular epithelial layer and theca. Outside the oocyte, an aggregation of blood cells and phagocytes is evident (azan stain)

Ovaries with nodules, found in 27 individuals (Figs. 2, 3, 4), were immersed in Bouin's solution prepared by picric acid saturated with sea water. Ovarian blocks were dehydrated through an alcohol series, embedded in paraffin, cut to a thickness of 5 μm, and stained with hematoxylin–eosin (HE) double stain, azan trichrome and periodic acid Schiff reaction (PAS) to disclose polysaccharides. Sections were observed under a light microscope. In addition, the ovaries

Figs. 1–4 **1** Ventral view of an adult female snow crab, caught 8 years and 1 month after being tagged. **2** Ovary of an aged snow crab showing several pale crimson to red-tinted nodules. **3** Part of the ovary in an aged snow crab showing a red-tinted nodule with a partial brownish color. **4** Part of the ovary in an aged snow crab showing a large nodule tinted with dark red and numerous tiny dark-colored nodules

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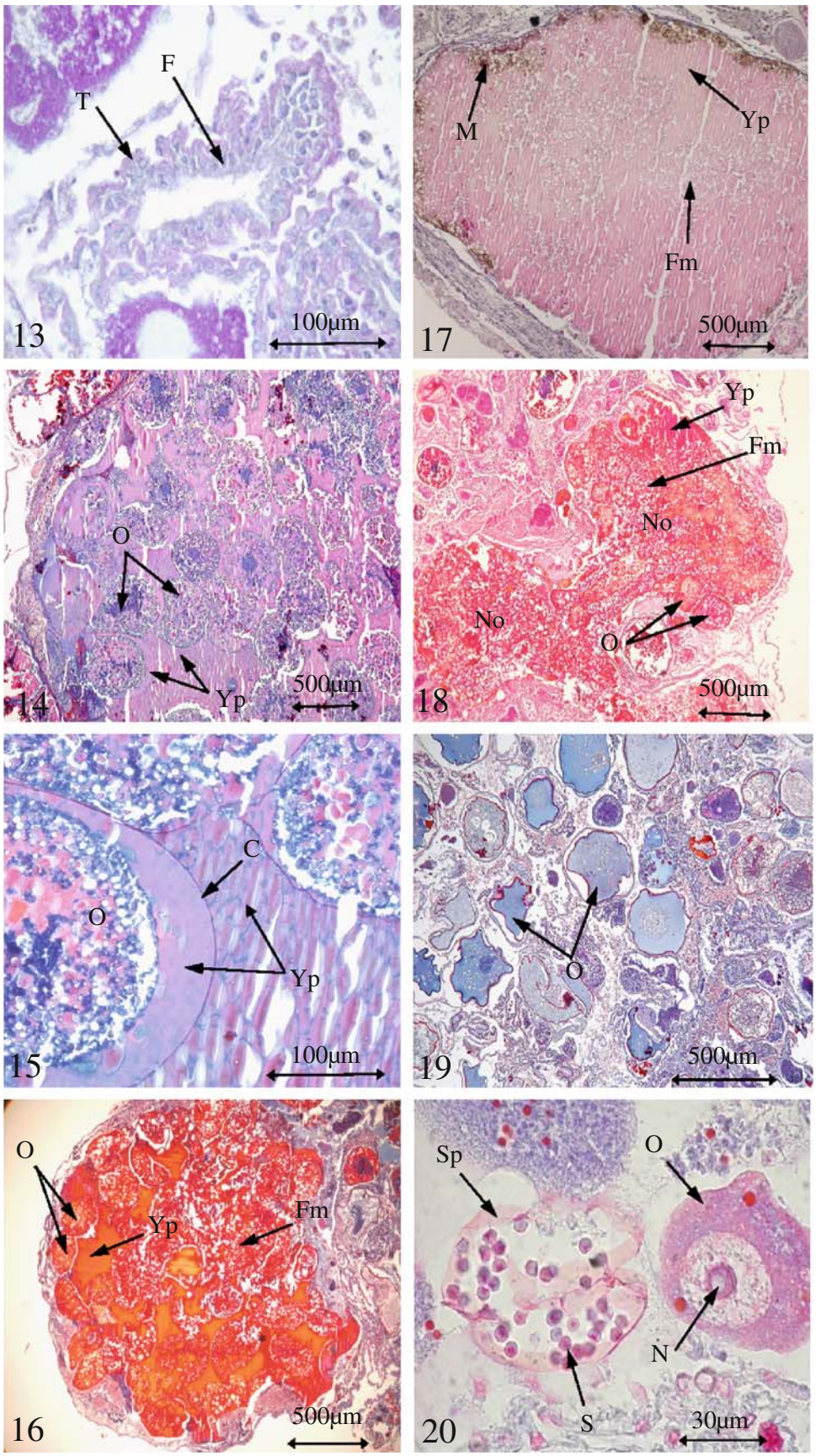
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◀ **Figs. 13–20** **13** An ovulation scar showing relict follicular cells and thecal remnant (azan stain). **14** Low-power view of a section through part of a nodule (*pale crimson tint*). Note the aggregation of degenerative oocytes (azan stain). **15** Enlarged view of part of a nodule indicated in **14** (azan stain). **16** Low-power view of a section through a nodule (*red tint*), showing a marked increase in the number of yolk plates (azan stain). **17** Low-power view of a cross-section of a nodule (*dark tint*). Note melanin aggregation in the marginal region (HE stain). **18** Low-power view of a section through a nodule (*red tint*) showing invasion of the anterior portion toward the interior of ovary (azan stain). **19** Low-power view of a section of ovary showing many oocytes in various stages of degeneration (azan stain). **20** High-power view of a spermatheca (containing many round sperms) in the ovary (*left side*). Note a residual young oocyte in the peripheral nucleolus stage (*right side*) (azan stain). *B*, blood cells (=hemocytes); *C*, oolemma (=chorion); *F*, follicular epithelial cells; *Fm*, foamy yolk; *Ge*, germinal epithelium; *M*, melanin granules; *N*, nucleus; *No*, nodule; *O*, oocyte; *Ov*, ovary; *P*, phagocyte; *S*, sperm; *Sp*, spermatheca; *T*, theca; *Tg*, tag; *Yd*, yolk droplet; *Yg*, yolk globule; *Yp*, yolk plate

of three pubescent females just prior to their final molt (tenth molting stage), two adult females immediately after their last molt that had spawned primiparous eggs, and three young females, the exoskeletons of which were injury-free and non-dusky, were also fixed and prepared for histological examination. These crabs were caught by a motor trawl net operated by the research vessel of Fukui Prefectural Fisheries Experimental Station.

Results

Macroscopy

The aged ovaries had up to dozens of soft nodules, variously tinted (for example, pale crimson, red, brown and black). Dark nodules were particularly numerous compared with the rest.

Specimens brooding fewer than five thousand eggs accounted for some 70 individuals (66%) of the 106 specimens, but there was little difference between those with (70%) or without (65%) nodules. Furthermore, the mean number of eggs brooding and the mean spermathecal load of individuals examined were also approximately same in both nodule-free (56,368 eggs, 74 mg) and noduled individuals (60,834, 79 mg).

Microscopy

Most of the ovarian eggs were in a strongly degenerative stage, associated with considerable oolysis in the process of atresia. Yolk globules, united with each other, gradually formed large spherules and platelets, finally developing into a single large plate which moved toward the egg margin (distal portion) (Fig. 6). After that, a marked decrease in the amount of yolk globules and platelets occurred (Fig. 7), the residual material staining pale red (PAS) or pale blue (azan) with an indistinct network structure (Fig. 8). At this stage, an invasion of follicular epithelial cells was noted: each cell contained a large round nucleus and fine granules in the cytoplasm, derived from oolysis of the egg body. The oocyte size was reduced and the ovum shape strongly distorted.

As oosorption proceeded, an increase in the thickness of the oolemma (=chorion) was noted, staining deeply with azan (Fig. 9). Similarly, the theca, tinted pale purplish-red, increased in thickness and gradually became distinct as distortion of the ovum increased. Inflated follicular cells with expanded round nuclei were scattered both inside and outside the oocytes, with the outer cells gathering in locally stratified layers outside the oolemma (Fig. 9).

With further oocyte degeneration, convolution of the oolemma became more advanced and showed several deep invaginations. The theca revealed a slight indentation, with the space between oolemma and thecal layer being occupied by follicular epithelial cells, each of which contained an expanded nucleus (Fig. 9).

With further degeneration and oosorption, the oolemma fragmented (Fig. 10). Accordingly, the amount of deep red material was very small at this stage. Subsequently, no oolemma components were seen (Fig. 11). The theca showed strong indentation, with only a few follicular epithelial cells being evident. Outside the thecal layer (but not inside), a small number of large phagocytes with red granules and blood cells (=hemocytes) were found (Fig. 12).

The oocytes were greatly reduced, revealing a similar picture for atretic follicles, although the latter were lined with a layer of cuboidal cells adjoining the inside of the thecal cells (Fig. 13).

The nodules, pale crimson or red-tinted, consisted of a large conglomerate mass of fused degenerative vitellogenic oocytes (Fig. 14) enveloped by a membrane of fibrous connective tissue in which some areas of melanin granule deposition were detected. The diameter of each oocyte, surrounded by a thin oolemma (0.5–2.0 μm in thickness) and stained red by azan, was ca. 500 μm. However, it was difficult to identify both the follicular and thecal cell layers. The ooplasm was occupied by various stages of yolk production, including yolk globules (5–20 μm), liquefactive yolk droplets, yolk plates and yolk granules (1–3 μm). In the central portion, a nucleus consisting of a mass of fine purplish granules was stained by azan. Some oocytes appeared to have an unusual peripheral structure surrounded by circular yolk plates (Fig. 15). As this feature progressed, an increase in the number of yolk plates was noted, in contrast to a distinct decrease in yolk drops and yolk granules (Fig. 16). Azan stain demonstrated the close

affinity of these purplish yolk plates, both inside and outside the oocytes.

No evidence of oocytes was found in completely darkened nodules. In addition, other cellular components, such as follicle cells, phagocytes and blood cells, such as those indicated in Fig. 12, were not detected. The envelope was lined with a thin layer of pigment, almost all of which was melanin granules (Fig. 17). Generally, the brownish and further darkened nodules were long ovoids or cylindrical in shape (Fig. 17), although the tip of the nodule occasionally penetrated deep into the ovary, as indicated in Fig. 18.

On the other hand, the nodule envelopes, which were brown in color, formed from a mass of more advanced degenerated eggs without yolk globules, although only the yolk plate remained, as some of the mass had been converted into a fine granular network or reticular structure (Fig. 18).

The ovarian tissue of the crabs with dusky exoskeletons showed denaturized secondary vitellogenic oocytes in addition to the strongly degenerated oocytes. No normal oocytes were recognized anywhere in the ovaries (Fig. 19).

In the ovary of one of the primiparous crabs, immediately after first spawning, a spermatheca containing hundreds of sperm in cross-section was encountered among the young oocytes (Fig. 20).

Discussion

The maximum estimated age recorded is something over 9 and a half years after the final molt for a female *C. opilio.*

The degeneration of aged crab oocytes can be seen from two perspectives: oosorption of follicular action, as well as oolysis and successive conglomeration. In addition, the occurrence of atretic eggs and ovulation scars provide evidence of successive changes prior to final ovulation.

Successive degenerative phases of oocytes and reserve fund eggs in several crab species have been reported on briefly by several authors [9, 18–20]. Moreover, the effects of starvation have also been documented [23–25]. However, the present study differs from the above in that it focuses on microscopic images of nodular formation and the follicular epithelial activities exhibited by spent aged crabs.

It seems likely that nodular formation was brought about by regressive changes in many unspawned eggs, in which the aggregation and deposition of melanin granules were noted.

Furthermore, it is highly doubtful that eggs held by aged snow crabs are likely to spawn after fertilization due to the very low number of normal matured eggs and also the small number and/or density of viable sperm. The relationship between spermathecal load (quantity) and fertilizable potency in the genus *Chionoecetes* has been documented by several researchers [16, 22, 26–30]. However, the present study calculated only the spermathecal load (rather than potential sperm activity), in addition to assessing the number of normal mature eggs that had the potential for ovulation. A histological examination showed that none of the present specimens contained such eggs.

Hinsch [19] reported sperm cells in the ovary of the golden crab, whereas the existence of spermatheca in the snow crab ovary was determined in the present study. In another investigation of *C. opilio*, Sainte-Marie et al. [31] recognized that sperms infiltrated the space between the chorionic envelop of the oocyte and its cell membrane, and raised the possibility of fertilization within the ovary. However, the present study is the first to demonstrate spermatheca in females of that species.

Since 1988, artificial reserves for the protection of female snow crabs have been established in western parts of the Sea of Japan. These have been relatively effective, as demonstrated by the aged females caught near and around the reserves. In fact, over the ca. 20 years since the reserves were created, a gradual increase in the proportion of aged crabs that do not contribute to reproduction has been noted. Accordingly, in order to preserve suitable habitats for young adult females, some efforts must be directed into reducing the numbers of these aged crabs.

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