Mem. Fac. Fish. Kagoshima Univ.,
Vol. 39, pp. 129~135 (1990)

Maturation of the Spiny Lobster *Panulirus japonicus*

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Keywords: Spiny lobster, *Panulirus japonicus*, reproductive system, ovary maturation.

Abstract

Male and female reproductive systems of the spiny lobster *Panulirus japonicus* were studied anatomically. Development of the ovary was observed histologically to classify its maturation process of the oocyte. The reproductive system of the males consisted of the testis, vas deferens and spiral-shaped genital apparatus which was provided externally. For the females, the ovary and oviduct were its components. The ovary showed a H-shape, and reddish orange at the matured condition. The vas deferens and ovary changed their size on each reproductive cycle. Oocytes in the ovary were classified into three phases, based on the condition of vitellogenesis. They were further divided into six stages from differences of the diameter and cytoplasmic characters. Furthermore, the eyestalk ablation was conducted. Based on the results of the operation, physiological interference of the eyestalk in the maturation was discussed.

Reproductive systems of the spiny lobster *Panulirus japonicus* are basically similar to those of other Nephropidae[1] and Palinuridae[2] species. The external and internal reproductive organs as well as arrangement of the genital apertures were described in *P.japonicus* by Okamura[3]. However, little has been known on their changes due to the reproductive cycle, although there have been some studies related to the embryology, breeding season, spawning times, fecundity or resources.

The female minimum size at maturity was reported as the carapace length of 38 mm, whereas it corresponded to the carapace length of 42 mm and body weight of 80 g, assumed to be 1.5-2 years after puerulus larvae[4]. Regarding the time of spawning, it took place twice annually[4-6]. It seems valuable and important from a standpoint of fishery resources to clarify the genital development accompanied by the maturation. This study was thus conducted to confirm the structures of the male and female reproductive systems, and to investigate histological changes of the oocytes related to the maturation.

The removal of the eyestalk in decapods was known to yield a signifinant acceleration of the ovarian development and/or molting cycle[7], even though such experiment has not yet been conducted in this *Panulirus japonicus*. A surgical experiment with the eyestalk ablation

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was carried out to investigate the physiological role of the eyestalk in the reproductive and molting mechanisms.

Materials and Methods

Adults of the Japanese spiny lobster *Panulirus japonicus* were obtained from the markets[4]. They were kept alive in 500*l* tanks in the laboratory. Males and females were distinguishable from each other by their shapes of the dactylopodite of the fifth pereiopod[3]. The body weight (BW) and carapace length (CL) of each lobster were recorded. For the females, the ovary was weighed, and the gonadosomatic index (GSI) was calculated as the ratio of ovary weightx100/BW. Respective genital organs were described anatomically after weighing. The histological investigation was conducted on the ovary development with the treatments of Bouin's fixative, EtOH dehydration, paraffin embedding, and staining of the sliced specimens with hematoxylin-eosin or PAS-hematoxylin.

The eyestalk ablation was applied to the females. The operation of both sides was done unilaterally with ten-day intervals. The operation technique was to press the stalk portion with hot pincettes, stopping the blood flow. They were reared within two weeks, while feeding clams. Then, they were sacrificed to the histological observation of the ovary.

All the above-mentioned experiments were conducted during May 12 to July 4, 1990.

Results and Discussion

Reproductive System

Males: The internal reproductive system of males is shown in Fig.1. The matured testis

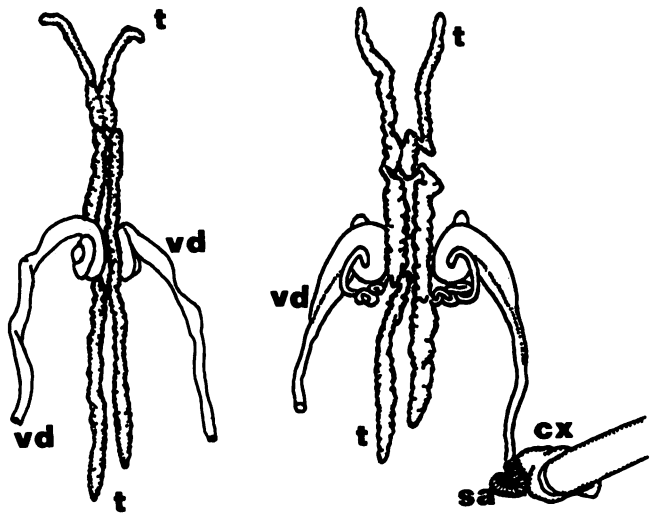

Fig. 1. Male reproductive system. left, ventral view (BW 186 g, CL 57 mm). right, dorsal view (BW 132 g, CL 52 mm). cx, coxopodite of the fifth pereiopod; sa, spiral apparatus; t, testis; vd, vas deferens.

showed a semi-transparent or slightly yellowish organ, smaller than the ovary of females[3]. It was situated on the dorsal of the midgut gland, beneath the heart. It was H-shaped, like other lobsters[1,2,8], with two anterior lobes extending to the dorsolateral stomach, and two posterior lobes extending backwards to the 1st abdominal segment. The posterior lobes lied between the dorsolateral abdominal muscles alongside the midgut. The paired vasa deferentia arose on the outside of the posterior lobes and opened on the coxopodites of the fifth pereiopods. The opening site at each coxopodite was provided with a chitinous and spiral-shaped apparatus. The vas deferens appeared to consist of three parts: a proximal region of the highly coiled tube, an intermediate region of the thick tube of which initial part showed a helix condition, and a distal ejaculatory region of the slender tube. The spermatophores seemed to be produced in the secretory area of the proximal and intermediate vasa deferentia and were stored there until copulation occurred. The testis seemed to produce not only spermatids but also nutrient cells observable in the spermatophores. The enlarged tube of the intermediate vas deferens changed its size according to the maturation cycle, and contained a mucous or hyaline-like substance. The matrix surrounding the sperms was formed from this substance.

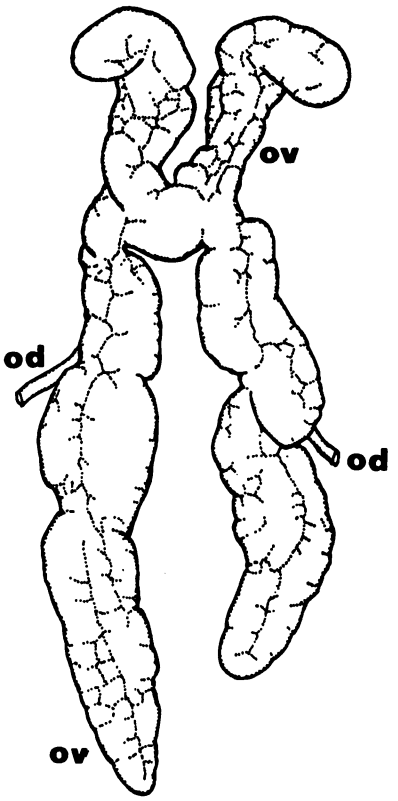

Fig. 2. Reproductive system of the matured female (BW 200 g, ovary wt. 19.7g, CL 61mm), dorsal view. The distal part of the oviduct is not presented in this figure. ov, ovary; od,oviduct.

Females: The internal reproductive system of females is shown in Fig.2. The ovary was situated on the dorsal of the midgut gland, beneath the heart. It showed a H-shape like other lobsters[1,8,9]. Its anterior lobes extended to the cephalic region with their tips turning upwardly. Its posterior lobes in mature condition extended to the fourth abdominal segment, alongside the midgut. The posterior lobes differed in length from each other. The left of them was longer in general[3]. An oviduct arose around the mid-point on each side of the ovary posterior to the connection between the two halves. It was a semi-transparent and slender tube, and opened on the coxopodite of the third pereiopod. There was no apparatus of the genital organ at that opening site. The thelycum was not prepared, differing from the cases in Nephropidae species[1]. The appearance of the ovary during development has scarcely been reported in this species. The ovary showed externally white or weakly yellowish in immatured condition. It changed to reddish orange at the completely matured condition. This color was almost similar to the case in the spiny lobster *Panulirus homarus*[10] of which ripe ovary showed

coral red, but differed from those of other spiny and clawed lobsters like *Jasus*[8], *Homarus*[11] and *Nephrops* species[12]. The latter was rich brick red, dark green or royal blue. Such different coloration seemed to occur due to qualitatively differing lipid deposition during vitellogenesis. Its shape and size would also change during the development, although detailed studies concerning them are deficient. The author ever made an effort in the breeding season to clarify the relationship between the ovary weight and its maturation condition. A calculated value of the gonadosomatic index was 9.5% in a completely matured female(200 g BW, 61 mm CL).

Histology of Ovary and Developmental Oocytes

The ovarian wall consisted of outer connective tissue with blood vessels and inner germinal epithelium. Layers of outer epithelium and smooth muscle were not recognized in this species, differing from other spiny or clawed lobsters[11,13,14]. The connective tissue changed its thickness periodically according to the ovarian reproductive cycle. For example, its thickness decreased from 0.13 mm to 0.04 mm in individuals of 150-200 g BW. The germinal epithelium formed inward folds running the length of the ovary, and from that epithelium ova developed, being surrounded by a single layer of flat cells, *i.e.* follicular cells. Egg diameters reached around 0.5 mm for matured ova. Development of the oocytes was classified histologically into three phases and six stages (Fig.3).

a) Non-vitellogenesis phase : It was an early and immatured stage of development. The maximum diameter of oocytes was 120-130 μm. Each nucleus was around 30 μm, and contained commonly a nucleolus as well as dispersed chromatin. The cytoplasm was stained with hematoxylin.

b) Primary vitellogenesis phase : This was divided into three stages. At the first stage, the oocyte and nucleus diameters were 130-140 μm and 40-50 μm, respectively. The cytoplasm was stained with hematoxylin, possessing peripherally not so many vacuoles. At the second stage, the diameters of the oocyte and nucleus were around 150 μm and 40-50 μm, respectively. The cytoplasm reduced its hematoxylin-positive character and became eosinophilic. The peripheral vacuoles increased remarkably in number, and were distributed around the nucleus. The third stage showed the maximum diameter of the oocytes approximately 250 μm with the nucleus of 40-50 μm. Eosinophilic granules appeared peripherally among the distributed vacuoles. The egg membrane began to be formed between the oocyte and follicular cells.

c) Secondary vitellogenesis phase : It was an accumulation period of yolk. It was divided into two stages. The oocytes of the first stage showed the diameter range of 260-480 μm and their nuclei of about 20 μm. In the cytoplasm, eosinophilic yolk granules appeared abundantly. At the second stage, the maximum diameter of the oocytes reached about 500 μm. The nuclei showed a decrease in size and finally became difficult to be observed. The thickness of the egg membrane was measured 4-8 μm. This stage corresponded to the completely matured condition of the oocyte.

Compared to the ovarian maturation in other decapods like Natantia species[15], the maturation process and therefore its cycle in these lobsters were very slow. Especially, the

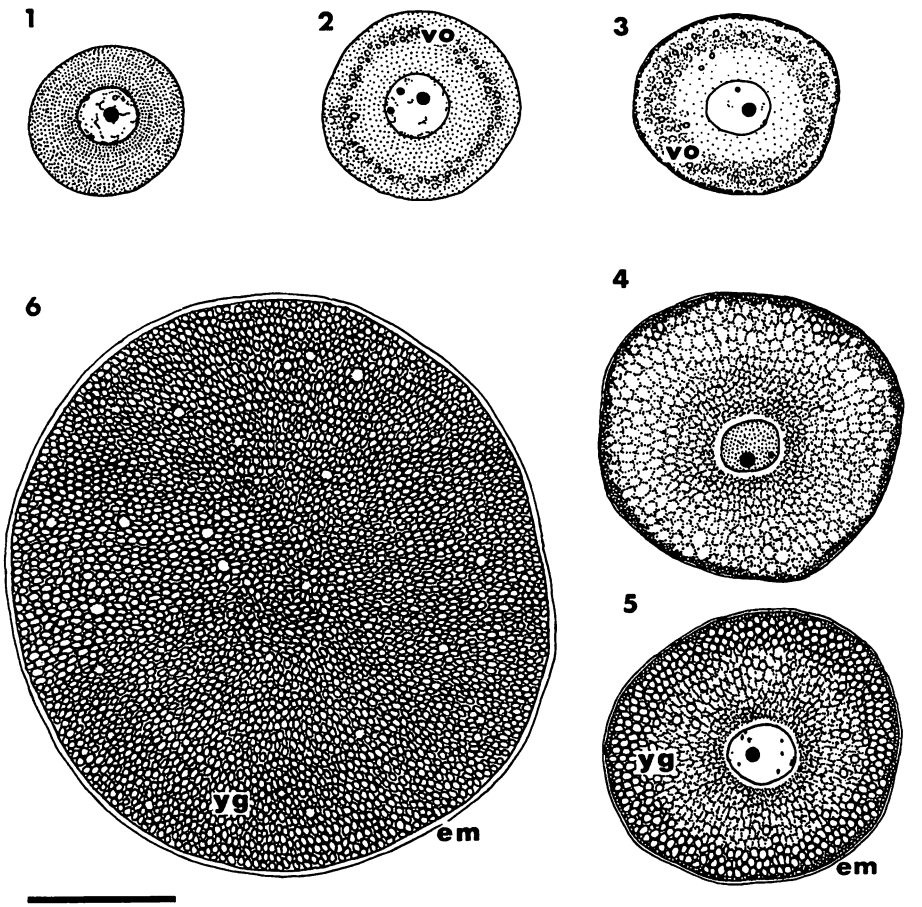

Fig. 3. Developmental stages of the oocytes observed histologically. Follicular cells are not shown in these figures. scale = 125 μ m. em, egg membrane; vo, vacuole; yg, yolk granule. 1, immature stage in non-vitellogenesis; 2, first stage in primary vitellogenesis; 3, second stage in primary vitellogenesis; 4, third stage in primary vitellogenesis; 5, first stage in secondary vitellogenesis; 6, second stage in secondary vitellogenesis.

secondary vitellogenesis phase seemed to require much longer time for its achievement. The long process of yolk accumulation has probably a reversional potential, and reabsorption from the oocyte yolk would occur according to physiologically unsuitable or critical conditions of nutrition and environment.

Eyestalk Ablation

Within two weeks after the eyestalk ablation (water temperature of 24-30 ℃), some of the females of 150-200 g BW spawned. However, the other did not spawn or in a case died.

The non-spawned individual of 244 g BW showed to be at the premolt stage. Its gonadosomatic index was 0.61 % and oocytes in the ovary were at the phase of primary vitellogene-

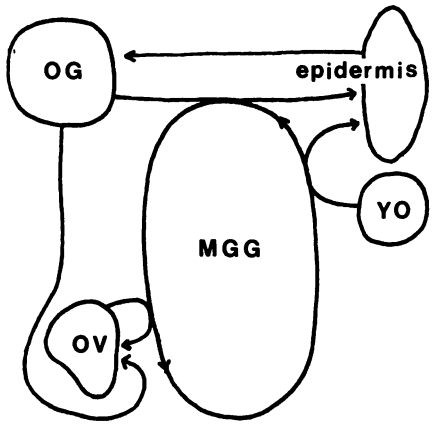

Fig. 4. Physiological interactions supposed in the spiny lobster *Panulirus japonicus*. MGG, midgut gland; OG, optic ganglion; OV, ovary; YO, y organ. At the intermolt stage, the optic ganglion inhibits the molting metabolism of the midgut gland (see antagonistic directions of both arrows). The molting metabolism of the midgut gland relates trophically to the ovary development also. The ovary receives another trophic regulation of the optic ganglion. The epidermis is controlled by the optic ganglion and y organ. The molting metabolism of the midgut gland accerelates the y organ's work to the epidermis.

sis. The ova, in some cases, showed reabsorption processes of yolk at the secondary vitellogenesis phase. The spawned females had nevertheless nearly riped oocytes in the ovary. This phenomenon seemed to be an incomplete exhaust and correspond to abnormal spawning caused by the eyestalk removal, or as in the case of the natural conditions[4,5], its ovary equaled the normal one which prepared the ova condition for a following spawning. In any case, these results seemed to indicate that the eyestalk not only produced the ovary-inhibiting hormone but also integrated the physiological metabolisms of the molting and ovarian development. The author thus supposes a schema of this lobster's physiological interaction of the molting and maturation in Fig.4. At the intermolt stage, the optic ganglion in the eyestalk inhibits the molting metabolism of the midgut gland (see antagonistic directions of both arrows). The molting metabolism of the midgut gland also relates trophically to the ovary deveolopment. The ovary receives another trophic regulaion of the optic ganglion. Chitin secretion of the epidermis is controlled by the optic ganglion and y organ. The molting metabolism of the midgut gland accerelates the y organ's work on the epidermis activity. Adiyodi and Adiyodi[7] deduced the existences of the molting and molt-inhibiting hormones as well as the gonad-stimulating and gonad-inhibiting hormones, although none of them have so far been proved. Thus, we need more substantial knowledge on the metabolic mechanisms of the molting and maturation.

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