**Studies on Gonad Maturity in Some Marine Invertebrates—VIII.
Structure of Reproductive and Related Organs of a Foreign Barnacle,
Balanus eburneus, that appears in Lake Kamo-ko, Sado Island***

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Histological observations were made to elucidate the structure of the reproductive and other internal organs of the foreign barnacle, *Balanus eburneus*, which inhabits the bamboo-made rafts used for oyster cultivation in Lake Kamo-ko, a brackish lake located in Sado Island of the Japan Sea. The materials used were collected at monthly intervals during May to December, 1971.

The male genital organ consisting of the testis, seminal tubules, seminal vesicles and vas deferens, and its seasonal changes with special regard to the spermatogenesis are described. The ovary and its ovarioles that appear in an arborescent manner are mentioned, with a description of the developing ova and the larvae contained in the mantle cavity. It is believed that the breeding season might be in summer. Findings on the internal anatomy of the visceral organs are also stated.

In order to expand our knowledges relating to the maturity and structural pattern of the reproductive organ system of marine invertebrate animals, HONMA and his coworkers have tried to investigate successively the aging or seasonal changes in the prosobranch mollusk¹⁾, deep sea and littoral crabs²⁻⁶⁾ and moon-jelly⁷⁾.

The foreign barnacle, *Balanus eburneus*, propagated rapidly in the brackish lake, Kamo-ko, in Sado Island was first discovered by Mr. T. KITAMI, one of the members of the Sado Marine Biological Station, in September, 1965, and was reported by UENOMI^{8,9)} as an immigrant from the American Atlantic coast. Although this species spreads widely along the coasts of Sado Island and coasts of Niigata, in particular the jetties of Port Niigata, now, together with another foreign barnacle, *Balanus improvisus*^{10,11)}, there is no report dealing with the life history including breeding season and anatomy of the soft parts of this barnacle. Similarly, little is known comparatively on the histologic detail of the soft parts of sessile barnacles occurring in the Japanese Islands. Therefore, using the materials from Kamo-ko, a histologic examination was attempted to elucidate the structure of the reproductive organs. To make some comparison with other species, *Tetraclina squamosa* and *Chelonibia testudinaria*, both obtained from the vicinity of the Sado Marine Biological Station were also employed.

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Material and Methods

The material (*Balanus eburneus*) used in this study was collected from the bamboo-made rafts for oysters cultivation in Lake Kamo-ko, the largest and single brackish lake in Sado Island, the mouth of which communicates with Ryotsu Bay by a narrow channel (Figs. 1 and 2). At monthly intervals ten to twenty specimens of the entire animals were immersed in Bouin's or Bouin-Hollande's fixatives immediately after removed from the raft, and the period of collection extended from May to December, 1971.

The soft body without shell was then dehydrated, embedded in wax, cut serially at $8\ \mu$ thick, and stained with Delafield's hematoxylin-eosin, Heidenhain's iron hematoxylin-light green, azan triple stain, and periodic acid Schiff's method for demonstration of polysaccharides.

Fig. 1. Map of Lake Kamo-ko and its neighbourhood. Circle indicates the main collecting place. Inset map of Sado Island shows the locations of the lake and the Sado Marine Biological Station, Niigata University.

Fig. 2. A number of bamboo-made rafts for oyster cultivation floating on Lake Kamo-ko. Photo on early March, 1972.

The specimens of *Tetraclita squamosa* were collected from the rocky beaches just in front of the Sado Marine Biological Station of Niigata University, and the specimens of *Chelonibia testudinaria* were obtained from the carapace of the green turtle, *Chelonia mydas*, that was captured by the set net prepared off the coast of the Station.

Results

General morphology. Although the distributional pattern along the coasts of the Japanese Islands and the shape and mode of the thinner shell of *Balanus eburneus* have already been described by UTINOMI⁹⁾, no mention was made about the internal anatomy by him. In spite of 20 to 23 mm in diameter and about 15 mm in height of the calcareous plate, the soft body hanging by muscle strands to the shell is nearly as large as 12 mm. The soft body in flattened, long ovoid shape enveloped with the internal and external mantles has six pairs of the thoracic appendages, the feet, each of which is adorned with cirri. Near these feet, the penis in needle-like appearance projects. The mouth consisting of 4 pairs of appendages, such as maxilla, maxillule, palp of mandible and labrum, is open on the top of the body, and the palp of mandible is equipped with a pair of segments with bristles (Fig. 3).

Between the connective tissues that occupy and fill up the space of internal and external mantles, there are found a lot of ovarioles, muscle fibers and cement glands. A number of larvae including nauplii contained in the mantle cavity are frequently encountered. The general organization of *Tetraclita squamosa* and *Chelonibia testudinaria* is essentially similar to that of *Balanus eburneus* (Fig. 3).

The alimentary canal consists of 6 parts. They are: the mouth that is lined with cuticle layer, esophagus surrounded by the columnar epithelium, a spacious thin-walled stomach consisting of cubic cells, midgut with a number of diverticula opened into the

Fig. 3. Diagrammatic illustration of a longitudinal section in *Balanus eburneus* to show the internal anatomy.

M. mouth; Sh. shell; Mu. muscle; Mc. mantle cavity; Im. internal mantle; Em. external mantle; O. ovary; Ct. connective tissue; Cg. cement gland; P. penis; Ml. mass of larvae; A. anus; C. cirri; T. tergum; Sc. scutum; Lp. leaflike projection.

Fig. 4. Schematic drawing of a longitudinal section of the digestive and male reproductive organs in *Balanus eburneus*.

M. mouth; G. ganglion; E. esophagus; S. stomach; Mg. midgut gland; St. seminal tubule; I. intestine; Sv. seminal vesicle; Sd. sperm duct; P. penis; A. anus; Ed. ejaculatory duct; C. cirri.

upper part of intestine, intestine tapering toward the posterior region, and anus lined with cuticle (Fig. 4).

One of the largest ganglion is the so-called cerebral ganglia or the circum-esophageal commissure, lies in front of the rather vertical esophagus (Fig. 4).

Reproductive organs. Like the other thoracican barnacles, the present species is a hermaphroditic crustacean. The male genital organ system consists of four parts. They are: the testis with a number of seminal tubules, a pair of seminal vesicles, vas deferens (=sperm duct) and penis. Seminal tubules are distributed near and periphery of the intestine in an arborescent manner. Cross section of each tubule is almost round, measuring about 80 to 120 μ in diameter in the material in May.

Various stages of spermatogenic cells are found in this tubule, although development of these cells arising from the epithelium near the basement membrane to the center of the tubule. The primary spermatogonia located in the basal region of the tubule are larger in size with ovoid nuclei and lucid cytoplasm, while the secondary ones are somewhat smaller and darker, located above the former (Fig. 5). After mitosis the secondary spermatogonia develop into the primary spermatocytes which pass through the maturation divisions. Following the secondary maturation division, i.e., the reduction division, the spermatocytes are transformed into the spermatids (Fig. 5). It is easy to recognize the nursing cell, that is characteristic of larger size and lucent nucleus, scattered in these spermatogenic cells. The process of spermiogenesis is as follows. The chromatin substance first situated in the periphery of the nucleus appears to form a ring-like body. Following this, the shape of the spermatid becomes spherical with a vacuole-like body in its central region. Next, the spermatid projects and extends toward the direction of its longer axis forming bipolar long, slender cilia, and the spermatozoon is elaborated completely. By means of phase contrast microscopy, the sperm appears to be in an active state. The highest peak of the spermiogenesis takes place in May to June (Figs. 6 and 7). Toward August, the amount of the sperm contained in the seminal tubules and vesicles reaches the greatest level, but in some of the individuals, ejection of the sperm was gradually becoming increased from July. Simultaneously, a relative decrease in the number of spermatogenic cells occurs in the tubule. Toward October, it is difficult to find the gonadal material in the tubule and vesicle which have become atrophied and reduced (Fig. 8).

The vesicles located near both sides of the posterior part of the intestine are considerably large pouch with three or more constrictions. The vesicular wall consisting of a flat epithelium is coated more with thin muscular layer (Fig. 8). A pair of the vas deferens derived from these vesicles reduce their size gradually toward the penis. The epithelium consisting of the cubic to depressed cells with occasional cilia is covered with a thin muscular layer with the exception of the neighbouring region of the penis. Near the basal part of the penis, these two ducts join with each other to form a single canal,

and are directed toward the ejaculatory duct that centers in the penis. In other words, the penis coated with a chitinous layer contains a lot of longitudinal muscle (Fig. 9). In parallel with this muscle bands, a number of nerve fibers exist, enveloping the ejaculatory duct. This duct consists of a columnar epithelium with occasional occurrence of the cilia in the apical part and considerably thick basement membrane in the opposite side. The nuclei, spherical to ovoid in shape, are located near the basal margin. There are found numerous nerve fibers around the duct (Fig. 9). Although the thickness of the penis varies according to seasons, the diameter of it in a specimen of June 24, 1971 is about 600 μ , and an ejaculatory duct is 100 μ .

The most voluminous female genital organ is the ovary as an arborescent feature lying chiefly in the connective tissue spaces near the base, which are enveloped in the walls of the mantles. The ovarioles containing the developing eggs in various stages are surrounded by a thin stromatous membrane (Fig. 10). The ripe ovum in oval shape reaches to the diameter of 130 μ in longer axis and 80 μ in shorter with a lot of yolk deposition (Fig. 11). It is difficult to recognize the nucleus in a ripe ovum, while a round nucleus with a single prominent nucleolus is still seen in the center of the egg in process of maturation. In these younger ova, vacuoles that might be derived from oil droplets are found in the yolk globules. The spawning takes place in summer season.

A pair of slender oviducts open to the outside of the body at or near the bases of the first pair of cirri. These ducts contain the degenerative eggs in various stages of the development. In the connective tissue near the ovarioles, masses of the secretory cells in larger sizes are found. Some of them are intermingled with the ovarioles. The perikaryon of the cell is considerably large, stained homogeneously with hematoxylin or azocarmine. The nucleus, irregular or polymorphic in shape, has many nucleoli and peripheral shifted chromatin. In an older cell the size and the rate of irregularity of the nucleus are more pronounced, and the nucleoli also increase their number. These features have been diagnosed as the cement gland cell which elaborates the cement (Fig. 11). In addition to these findings, the nauplius larvae are encountered in the mantle cavity during August–September (Fig. 12).

Discussion

With regard to the reproduction of barnacles, comparatively many papers appeared on the breeding cycles^{12–17)}, and self-fertilization or copulation^{18,19)}, and so on. Reports dealing with histologic detail of reproductive organs and gametes are few, while the spermatozoa as revealed by electron microscopy has been described by MUNN and BARNES^{20,21)}. Therefore, the present study would be serviceable to this field of sessile barnacles. As gross morphology of reproductive organs and nearby structures has been made by HILGARD¹⁸⁾ and LACOMBE²²⁾, the authors have described the histological findings

with emphasis on the spermatogenesis of the present foreign barnacle, *Balanus eburneus*. With the exception of the larger size of the cells in *Tetraclina squamosa*, the general pattern of the organs and tissues among three species of barnacles examined in the present time are similar with one another. In other words, not a peculiar feature in the histologic pictures of the reproductive organs and nearby structures including changes in the gonadal materials was detected in the case of *Balanus eburneus*.

Although the detail of actual state of the copulation of *B. eburneus* could not be observed, INABA¹⁹⁾ described this manner in the case of *B. amphitrite hawaiiensis*. Based on the present histologic examination, it is likely that the copulation and further fertilization of *B. eburneus* will take place repeatedly during a single breeding season, i.e., summer season. Toward autumnal season, the seminal vesicle has atrophied and the spermatogenesis ceased, while a considerable number of the relict sperm were still retained. Accordingly, these relict sperm might be ingested and disposed by the phagocytes during winter season. As the life cycle of *B. eburneus* could not be determined, the real origin (presumably Saseho Naval Port) and course of the widespread propagation in Lake Kamo-ko and the coast of Niigata could also not be elucidated.

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Explanation of the plate

- Fig. 5.** Cross section of a seminal tubule showing three kinds of early spermatogenetic cells. They are: peripherally shifted, large spermatogonia (Sg), spermatocytes (Sc) with prominent chromatin clumps, and centrally shifted spermatids (St). This figure is based on a material collected on May 18, 1971. $\times 1,400$
- Fig. 6.** Cross section of a seminal tubule showing the various stages of spermatogenetic cells. Abbreviations are the same as in Fig. 5 with a bundle of sperms (Sp) that have long, bipolar projections. $\times 1,400$
- Fig. 7.** A mass of seminal tubules (St) filled with a lot of mature sperm. A material in summer season. $\times 80$
- Fig. 8.** Seminal vesicle (Sv) and several seminal tubules (St) of a specimen after the breeding season (October). Diminution of the size of tubule and decrease in the number of sperm in the vesicle are evident. $\times 80$
- Fig. 9.** Cross section of the penis. Near the central region, an ejaculatory duct (Ed) consisting of the high columnar cells is found. Well-developed longitudinal muscle (Mu) and a chitinous layer in the margin are also noticed. $\times 80$
- Fig. 10.** The maturing eggs in the ovarioles. A lot of yolk globules, a round, light nucleus with a prominent nucleolus, and several number of vacuoles derived from the oil droplets are seen in an ovum. $\times 110$
- Fig. 11.** The ovarioles (Ol) containing the ripe eggs near the cement gland (Cg) buried in the connective tissue. $\times 110$
- Fig. 12.** The developing larvae including nauplius (N) in the mantle cavity. A specimen on late August. $\times 110$