Gonadal Maturation and Embryonic Development in the Deep-Sea Sponge-Associated Shrimp, Spongicola japonica Kubo (Crustacea: Decapoda: Spongicolidae)

Authors: Saito, Tomomi, and Koya, Yasunori

Source: Zoological Science, 18(4) : 567-576

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.567

Tomomi Saito and Yasunori Koya

1 Port of Nagoya Public Aquarium, Minato-ku, Nagoya 455-0033, Japan
2 Department of Biology, Faculty of Education, Gifu University, Gifu 501-1193, Japan

**ABSTRACT**—Development of the gonads and embryo in the deep-sea sponge-associated shrimp, *Spongicola japonica* Kubo, was observed based on field samples collected from July 1993 to June 2000. Histological observation of gonads revealed that the gonadal maturities were divided into 4 and 5 stages in males and females, respectively. In females the smallest mature size was 5.5–6.0 mm in carapace length (CL), and the beginning of oocyte development was related to body size. The beginning of spermatogenesis was not related to body size. Grouped males started spermatogenesis at more than 3.0 mm CL, but the immature testis was recognized at 4.0–4.5 mm CL in solitary males. Embryonic development was classified into 12 stages based on the morphology of embryonic appendages. Ovarian maturity was almost always synchronized with embryonic stages in ovigerous females, and the females with fully developed embryos had fully ripened ovaries. The ovigerous females molted after hatching juveniles, and laid new eggs. Reproductive seasonality was not recognized in *S. japonica*. The induction of reproduction in this species may be controlled not by any physical environmental factors, but by other factors such as body size.

**INTRODUCTION**

Japanese fauna of the family Spongicolidae include the following 5 species belonging to 2 genera: *Microprosthema validum* Stimpson, *M. scabricaudatum* (Richters), *Spongicola venusta* de Haan, *S. levigata* Hayashi and Ogawa, and *S. japonica* Kubo (Miyake, 1982; Hayashi and Ogawa, 1987; Hayashi, 1995). In particular, the deep-sea members of the family are known to live entrapped by a pair of shrimp in the atrium of hexactinellid sponges, and are attracting much attention from biologists. However, the life histories of the group members have long been poorly documented due to the difficulty of collecting a large series of samples from the deep-sea.

Recently, Saito (in press) described the development of external sexual characters and sexual pair sizes in *S. japonica*. However, this is insufficient to determine the minimum size at which they first participate in reproduction. This paper represents the first attempt to classify the developmental stages of gonads of both sexes of *S. japonica* to further our knowledge of its reproductive biology and the factors inducing reproduction.

According to previous studies of embryonic development in deep-sea shrimp, the incubation period is a long one; e.g., the eggs of *Oplophorus spinosus* are estimated to take about 145 days to develop at 12°C, and those of *Systellaspis debilis* a similar period (Herring, 1974). *S. japonica* is described as having a direct larval development (Saito and Konishi, 1999), and the incubation period would take as much time as those abbreviated larval development species. Therefore, it can be estimated from the developmental stages of embryos whether the reproductive seasonality of *S. japonica* is present or not. In this paper, that embryonic development is described and compared on each sampling day to clarify reproductive seasonality.

**MATERIALS AND METHODS**

*Spongicola japonica* Kubo, associated with the deep-sea hexactinellid sponge *Euplectella oweni* Herklots and Marshall, were collected at a depth of 300 m off Makurazaki (130°E, 31°N), East China Sea, as a by-product of commercial shrimp trawling from July 1993 to June 2000 except during the closed fishing season from January to March. Gathered sponges were placed separately into 1-liter vials with cooled seawater at 10.0–12.0°C on the deck of a trawler and transported within 10 hr to the Port of Nagoya Public Aquarium (PNPA). In the laboratory the sponges were dissected, and the shrimp in the atrium were counted, their dorsal postorbital carapace length (CL) measured, and then sexed by the presence of gonophores. The composition pattern of shrimp in a flawless sponge host is divided into 3 types by sex and numbers, i.e., solitary, sexually paired, and grouped. Solitary denotes a shrimp found alone in a perfectly flaw-
Fig. 1. Gonads of *Spongicola japonica* Kubo. A: Immature testis composed of epithelial cells and a few spermatogonia (arrowhead). B: Spermatogonia observed in the testis of early spermatogenesis stage. C: Primary spermatocytes. D: Bent testis at the late spermatogenesis stage mainly composed of spermatocytes (c) and spermatids (t). E: Secondary spermatocytes. F: Spermatids. G: Spermatophores (s) formed in the vas deferens. Note thick epithelia (arrowhead) lining the vas deferens. H: Young-stage oocytes. I: Pre-vitellogenic oocyte with small vacuoles in the cytoplasm. J: Small vitellogenic oocyte. K: Spermatophore observed in female genital tract. Scale bars: B, C, E and F=10 µm; A, H, I and K=50 µm; D, G and J=100 µm.

less host sponge. Sexually paired indicates a male above 5.2 mm CL and a female above 5.8 mm CL, which is the minimum size of a pair to be found in an ovigerous female (Saito, in press). The grouped type covers cases of multiple individuals except for those sexually paired. Specimens used in the gonadal, embryonic and captive studies are shown in Appendix 1, Tables 3 and 4, respectively. For observation of the growth-reproductive pattern, 8 sexual pairs of shrimp were kept separately in cubic cages with 10-cm sides, mesh size of 0.5 mm and suspended in a dimly lit tank holding 500 liters of aerated sea water with a filtration system using 1.2 m³ of crushed coral and silica sand under the following conditions: 0–10 lux in illumination intensity, 20.0±1.0°C water temperature, pH 7.8–8.1 and 34.0–36.0
ppt in salinity. Minced fish was given as food every day using a fine-tipped pipette. Dissection and measurement of shrimp and eggs were conducted using a stereomicroscope, and drawings were made with the aid of an attached drawing tube after fixation in 5% buffered formalin. In particular, ovary and embryo photographs were taken just before fixation. For histological observation, the whole animal was fixed in Bouin’s solution for at least 48 hr. After fixation, samples were dehydrated in a graded ethanol series, cleared with xylene, and embedded in paraffin. Sections were cut at 6 µm, and stained with Delafield’s hematoxylin and eosin.

RESULTS

Testis

Testicular maturity was divided into the following 4 stages based on histological observations.

Stage I (immature): the gonadal anlage of the male is still small and composed of epithelial cells and a few spermatogonia (arrowhead) (Fig. 1A).

Stage II (early spermatogenesis): spermatogenesis begins in the lobules where spermatogonia (Fig. 1B) and primary spermatocytes (Fig. 1C) are located. Spent testis corresponds to this stage.

Stage III (late spermatogenesis): testis has complex bends (Fig. 1D). Various stages of germ cells including primary spermatocytes (c), secondary spermatocytes (Fig. 1E) and spermatids (t) (Fig. 1F) are observed throughout the testis (Fig. 1D).

Stage IV (mature): spermatids are released into the vas deferens, where epithelia (arrowhead) become thick and spermatophores (s) are formed (Fig. 1G).

Table 1 shows the maturity stages of testes in relation to CL of shrimp. All the males below the 2.5-mm CL class had stage I testes. Most shrimp above the 3.0-mm CL class started spermatogenesis, though stage I testis was recognized in a 4.5-mm CL male which was collected as a solitary (see Appendix 1).

Ovary

Ovarian maturity was divided into the following 5 stages based on the condition of oogenesis and the diameter of the most abundant ova in the ovary.

Table 1. Components of gonadal maturity in each class of CL in Spongicola japonica Kubo

<table>
<thead>
<tr>
<th>CL class (mm)</th>
<th>–2.0</th>
<th>–2.5</th>
<th>–3.0</th>
<th>–3.5</th>
<th>–4.0</th>
<th>–4.5</th>
<th>–5.0</th>
<th>–5.5</th>
<th>–6.0</th>
<th>–6.5</th>
<th>–7.0</th>
<th>–7.5</th>
<th>–8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of examined males</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicular stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of examined females</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Ovarian stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Stage I (immature): the most abundant oocytes in the ovary are at an early stage (~50 µm; Fig. 1H).

Stage II (pre-vitellogenic): oocytes (50–200 µm; Fig. 1I) with centrally located large nuclei and small vacuoles are found in the cytoplasm.

Stage III (early vitellogenesis): small vitellogenic oocytes (200–700 µm; Fig. 1J, 4A’, B’) with yolk globules are observed. Spent ovaries, which are flaccid and transparent (Fig. 4A’), correspond to this stage. They are largely empty and contain only a few remnant ova.

Stage IV (mid vitellogenesis): medium-size vitellogenic oocytes (700–1200 µm; Fig. 4C’, D’) with large vacuoles and yolk globules are observed in the cytoplasm.

Stage V (late vitellogenesis): large vitellogenic oocytes (1200 µm–; Fig. 4E’) are observed.

Table 1 shows the maturity stage of the ovary in relation to CL of shrimp. All females below the 3.5-mm CL class had stage I ovaries, and females in 3.5–5.5 mm CL range had stage II ovaries. Females in the 5.5–6.0 mm CL range had stage II or V ovaries. Females above the 6.0 mm CL class had stage III–V ovaries. Spermatophores were found in the genital tract of 4 of 10 ovigerous females having stage IV or V ovaries (Fig. 1K) among which the stages of embryos varied (Table 5).

Embryonic development

The eggs were globular in shape during the early stages, becoming gradually oval as their size increased. The yolk was cobalt blue in color in contrast to the translucent embryo. The diameter of S. japonica eggs increased from 1.78±0.16 mm (mean±SD) to 2.32±0.12 mm in the course of embryonic development, which was divided into 12 stages based on morphological characters, appendages and ratio of yolk area to total egg area in lateral view (Fig. 2, 3, 4; Table 2). All egg measurements indicate the long axis of mean±SD.

Stage I (Fig. 2A, 4A): Fully grown oocytes. Beginning of embryonic development. Yolk area about 100% in lateral view. The eggs are globular and have a diameter of 1.78±0.16 mm (n=27).

Stage II (Fig. 2B, B’, 4B): Egg nauplius. Optic lobes distinct. Antennules, antennae and mandibular buds rudimentary.
Fig. 2. Embryonic developmental stages I–VI in Spongicola japonica Kubo. A–F: stage I–VI; A, B, E' and F': stage I, II, V and VI egg in lateral view; B, C and D: embryo in ventral view; E, F: telson of stage V, VI embryo; E', F': antenna of stage V, VI embryo. lab: labrum; mnd: mandible; opt: optic lobe; tel: telson; thr. abd.: thoracic abdominal plate; 1–2a: antennule, antenna; 1 br: forebrain; 2–3gan: 2nd–3rd ganglia, 1–2 mxl: maxillule, maxilla; 1–3 mxp: 1st–3rd maxillipeds; 1–2 per: 1st–2nd pereopods. Scale bars = 1.0 mm.
Fig. 3. Embryonic developmental stages VII–XII in *Spongicola japonica* Kubo. B', D', F': stage VIII, X and XII egg in lateral view; A–F: telson of stage VII-XII embryo. pro: uropodal protopod; uro: uropod. Scale bars = 1.0 mm.
Fig. 4. Embryonic developmental stage and the next clutch of oocytes in ovary of female Spongicola japonica Kubo. A–E: stage I, II, IV, IX and XII embryo; A'–E': ovaries of female corresponding to each embryonic stage; A': stage III ovary; B': stage III ovary; C', D': stage IV ovary; E': stage V ovary. Scale bars=1.0 mm.

Rod-shaped antennules and bifurcated antennae bear no terminal process. Thoracic-abdominal plate visible. Embryo thin, and rate of yolk area 78% in lateral view. Egg diameter=1.69±0.12 mm (n=10).

Stage III (Fig. 2C): Embryo thicker than in previous stage. Telson slightly bifid. Maxillules, maxillae, maxillipeds 1–3 and pereopod 1 appeared. Yolk area about 70% in lateral view. Egg diameter=1.73±0.11 mm (n=21).

Stage IV (Fig. 2D, 4C): Maxillules, maxillae, maxillipeds 1–3 and pereopods 1–2 developed. Forebrain, ganglia 2–3 are observed. Terminal process on each lobe of antenna and antennules. Telson bilobed. Yolk area about 65% in lateral view. Egg diameter=1.85±0.08 mm (n=24).

Stage V (Fig. 2E, E', E''): Telsonal segmentation. Yolk area about 60% in lateral view. Egg diameter=1.86±0.14 mm (n=20).

Stage VI (Fig. 2F, F', F''): Telson deeply cleft with six spines on each lobe. Two terminal and 8 plumose setae are observed on exopod and endopod of antenna, respectively. Yolk area about 57% in lateral view. Egg diameter=1.98±0.11 mm (n=63).

Stage VII (Fig. 3A): Eye pigmented. Telsonal lobes united featuring 5–6 spines on each lobe. Uropodal buds rudimentary and slightly notched from 6th abdominal somite. Yolk area about 47% in lateral view. Egg diameter=2.03±0.15 mm (n=12).

Stage VIII (Fig. 3B, B'): Telson enlarged with 5–6 spines on each lobe. Uropodal buds more developed than at previous stage. Yolk area about 42% in lateral view. Egg diameter=1.86±0.11 mm (n=6).

Stage IX (Fig. 3C, 4D): Each telsonal lobe bears 8 spines. Heartbeat, yolk and limb pigment recognized. Yolk area about 35% in lateral view. Egg diameter=2.19±0.06 mm (n=20).

Stage X (Fig. 3D, D'): Each telsonal lobe bears 7–9 plumose setae. Bifid uropodal buds rudimentary. Yolk area about 30% in lateral view. Egg diameter=1.97±0.07 mm (n=45).

Stage XI (Fig. 3E): Eye stalked but not separated from carapace. Uropod developed to 3/4 of telson length. Distal shaft of telsional setae, which are not covered with thin membrane, are plumose. Yolk area about 20% in lateral view. Egg diameter=2.21±0.13 mm (n=94).

Stage XII (Fig. 3F, F', 4E): Just before hatching. Eye stalked and separated from carapace. Uropodal protopod recognized. Yolk area about 17% in lateral view. Egg diameter=2.32±0.12 mm (n=12).
Table 2. Egg diameter and embryonic development of *Spongicola japonica* Kubo

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of samples</th>
<th>Egg diameter</th>
<th>Main characteristics</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovigerous females</td>
<td>Eggs</td>
<td>Long axis mean ±SD (mm)</td>
<td>Rate (%) of yolk area</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>27</td>
<td>1.78±0.16</td>
<td>100</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>10</td>
<td>1.69±0.12</td>
<td>78</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>21</td>
<td>1.73±0.11</td>
<td>70</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>24</td>
<td>1.85±0.08</td>
<td>65</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>20</td>
<td>1.86±0.14</td>
<td>60</td>
</tr>
<tr>
<td>VI</td>
<td>6</td>
<td>63</td>
<td>1.98±0.11</td>
<td>57</td>
</tr>
<tr>
<td>VII</td>
<td>2</td>
<td>12</td>
<td>2.03±0.15</td>
<td>47</td>
</tr>
<tr>
<td>VIII</td>
<td>2</td>
<td>6</td>
<td>1.86±0.11</td>
<td>42</td>
</tr>
<tr>
<td>IX</td>
<td>2</td>
<td>20</td>
<td>2.19±0.06</td>
<td>35</td>
</tr>
<tr>
<td>X</td>
<td>4</td>
<td>45</td>
<td>1.97±0.07</td>
<td>30</td>
</tr>
<tr>
<td>XI</td>
<td>8</td>
<td>94</td>
<td>2.21±0.13</td>
<td>20</td>
</tr>
<tr>
<td>XII</td>
<td>4</td>
<td>12</td>
<td>2.32±0.12</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 3. Number of ovigerous females of *Spongicola japonica* Kubo categorized by embryonic developmental stages at each sampling day

<table>
<thead>
<tr>
<th>Date</th>
<th>Females above 5.8 mm CL</th>
<th>Ovigerous females examined</th>
<th>Embryonic stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>11-Jul-93</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12-Apr-94</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5-Aug-94</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>15-Jun-96</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>25-Nov-96</td>
<td>8</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>15-Dec-96</td>
<td>9</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>12-Jun-97</td>
<td>12</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>4-Dec-97</td>
<td>14</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>4-Dec-99</td>
<td>17</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>2-Jun-00</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 4. Records of reproductive process in captive ovigerous females of *Spongicola japonica* Kubo

<table>
<thead>
<tr>
<th>Collection date</th>
<th>CL(mm)</th>
<th>Days after collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hatching</td>
</tr>
<tr>
<td>4-Dec-97</td>
<td>8.7</td>
<td>105</td>
</tr>
<tr>
<td>4-Dec-97</td>
<td>8.0</td>
<td>366</td>
</tr>
<tr>
<td>21-May-98</td>
<td>6.7</td>
<td>44</td>
</tr>
<tr>
<td>1-Dec-98</td>
<td>6.8</td>
<td>35</td>
</tr>
<tr>
<td>1-Dec-98</td>
<td>6.6</td>
<td>9</td>
</tr>
<tr>
<td>1-Dec-98</td>
<td>7.5</td>
<td>126</td>
</tr>
<tr>
<td>1-Dec-98</td>
<td>8.5</td>
<td>15</td>
</tr>
<tr>
<td>4-Dec-99</td>
<td>7.9</td>
<td>40</td>
</tr>
</tbody>
</table>
Reproductive seasonality and reproductive process

The number of ovigerous females categorized by embryonic developmental stage in each sampling day is given in Table 3. Most females above 5.8 mm CL were ovigerous, and the embryonic stages were synchronized within each egg mass, but varied among each female of the same sampling date. Reproductive seasonality was not recognized in S. japonica.

Table 4 gives records of the reproductive process in captive ovigerous females of S. japonica. Hatchings were seen in all 8 ovigerous females. Overall the time intervals between the start of the culture and hatching varied from 9 to 366 days. Most ovigerous females with fully developed embryos had fully ripe ovaries of stage V. Molting was observed from 3 to 41 days after hatching juveniles, and successive spawning 2 to 4 days after molting. No copulation was recognized. However, the females fell out the newly laid eggs from the brooding chamber within about a week, and their ovaries were not developed after spawning under laboratory conditions. Spent ovaries were found on the ovigerous females with newly spawned eggs. The developmental stages of the embryo and the maturity of the ovary of each ovigerous female were almost synchronized (Fig. 4, Table 5, Appendix 1).

**DISCUSSION**

Ovarian stages III–V were observed over the 5.5–6.0 mm CL range, which corresponded to the formative period of the brooding chamber and the minimum size of ovigerous females (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press). The present study demonstrated that the beginning of gonadal maturation was related to body growth (Saito, in press).

Farmer (1974) showed that spermatogenesis occurred throughout the year, and spermatophores were found in the vas deferens at all times in male N. norvegicus. In S. japonica, the testicular maturity of sexually paired males seems not always to be synchronous with the ovarian maturity of their paired females, but spermatophores were stored in the vas deferens of 8 of 9 sexually paired males. This fact indicates that paired males of S. japonica are capable of copulating with paired females at any time. The orders of the reproductive process of the coral banded shrimp Stenopus hispidus, a species related to S. japonica, and N. norvegicus were typically reported as: hatching larvae, followed by molting, copulation and spawning (Farmer, 1974; Zhang et al., 1998). However, it remains unclear when they became able to copulate in the reproductive process.

Spermatophores were seen to be stored in the genital tract of 4 of 10 ovigerous females. Waddy and Aiken (1991) showed that a single insemination was enough to fertilize 2 successive clutches of eggs in the over 120 mm CL of the American lobster Homarus americanus. They also observed inter-molt insemination in 20% of the individuals of that size that stored an insufficient amount of sperm. In S. japonica, the presence of spermatophores in the female genital tract suggests that females may be capable of holding sperm from a single copulation over successive spawnings, or that hatching juveniles or molting may not influence copulation.

The developmental stages of the embryo and maturity of the ovaries of each ovigerous female were almost synchronized. The synchronous type of ovary containing mostly single-stage oocytes enabled a single clutch during a spawning season to be continuous. Although the number of pre-puberty instars was not identifiable, the puberty molt was obvious from the full development of the brooding chamber. Only a single brood can be carried in a mature instar like the pelagic mysid shrimp Metamysis elongata, which carries up to 14 series of broods in successive instars (Clutter and Theilacker, 1971).

It is well known that large volumes of yolk are associated with longer durations of embryonic development (Rabalais and Gore, 1985). Herring (1974) studied embryonic development in the deep-sea carideans, Acanthephyra purpurea and Systellaspis debilis, that lay large lecithotropic eggs that hatch as post-larva (Gurney and Lebour, 1941). The estimated incubation periods at 12°C were 62 days in A. purpurea and 145 days in S. debilis. In both species, the nauplius eye pigment stage occurs midway into the estimated incubation period and the first chromatophore stage one-third of the way. The incub-
bation period of *S. japonica*, whose eggs contain much yolk and show direct larval development (Saito and Konishi, 1999), is not known precisely, but we can estimate from the captivity data that it took more than a year.

Orton (1920) predicted that deep-sea organisms would reproduce throughout the year, because the physical conditions at that depth had long been considered more static than that of shallow water. Rokop (1974, 1977) periodically monitored the reproductive condition of a variety of benthic invertebrates such as the brittle star *Ophiotholus normani*, the Scaphopoda, *Cadulus californicus*, and the paracarid crustaceans *Eurycope californiensis*, etc., and proved that year-round reproduction was common in deep-sea benthic animals. Since those studies, the reproductive seasonality of deep-sea decapod crustaceans has only been documented in few species. Wenner (1978) found year-round reproduction in the deep-sea polychelid lobsters *Stereomastis nana* and *S. sulTpa*. Sadakata (2000) confirmed reproductive seasonality in the northern shrimp *Pandalus eous* from the Japan Sea. Reproductive seasonality is recognized in the golden crab *Chaceon fenneri* (as *Geryon fenneri*), but not in *C. maritae* (as *G. maritae*) (Melville-Smith, 1987; Hinsch, 1988). Ohtomi and Matsuoka (1998) observed reproductive seasonality in the Jack-knife shrimp *Haliporoides sibogae*, the target species of the trawlers, not far from our study station where the bottom water temperature tended to be constant throughout the year at 9–10°C. Ohtomi (1997) and Ohtomi et al. (1998) reported that reproduction was induced by photoperiod in the deep-water shrimp *Solenocera melanther* and *Plesionika semilaeva* from Kagoshima Bay, where the bottom water temperature tended to be constant throughout the year at 15.8±0.5°C (Noro et al., 1991). In *S. japonica*, however, no reproductive seasonality is noted. Reproduction in this species may be controlled not by any environmental factors, but by other factors such as body size.

**ACKNOWLEDGEMENTS**

We are greatly indebted to Drs. M. Takeda of the National Science Museum and K. Konishi of the National Research Institute of Aquaculture for valuable advice and encouragement during this study. Cordial thanks are also extended to K. Ikeue for collecting materials, to Dr. J. Ohtomi of the Kagoshima University for useful information, to Dr. I. Uchida, the director, and to the PNPA staff for providing the opportunity and financial support to make this study possible.

**REFERENCES**


(Received October 24, 2000 / Accepted February 21, 2001)
## Appendix 1. Reproductive parameters of each specimen of *Spongicola japonica* Kubo used in the gonadal study

<table>
<thead>
<tr>
<th>Shrimp ID</th>
<th>Sampling date</th>
<th>CL (mm)</th>
<th>Host sponge ID</th>
<th>Composition pattern</th>
<th>ID of partner</th>
<th>Embryonic stage</th>
<th>Gonadal stage</th>
<th>Gonadal stage of partner</th>
<th>Spermatophore</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>611</td>
<td>17–Jun–96</td>
<td>1.9</td>
<td>632</td>
<td>S</td>
<td>616</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>853</td>
<td>15–Dec–96</td>
<td>2.1</td>
<td>827</td>
<td>G</td>
<td>710</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>851</td>
<td>15–Dec–96</td>
<td>2.2</td>
<td>827</td>
<td>G</td>
<td>614</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>621</td>
<td>17–Jun–96</td>
<td>3.1</td>
<td>647</td>
<td>G</td>
<td>1011</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>618</td>
<td>17–Jun–96</td>
<td>3.8</td>
<td>647</td>
<td>G</td>
<td>1022</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>620</td>
<td>17–Jun–96</td>
<td>4.1</td>
<td>647</td>
<td>G</td>
<td>103</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>815</td>
<td>15–Dec–96</td>
<td>4.5</td>
<td>815</td>
<td>S</td>
<td>1011</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1006</td>
<td>4–Dec–97</td>
<td>4.5</td>
<td>1004</td>
<td>S</td>
<td>1011</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1039</td>
<td>4–Dec–97</td>
<td>4.5</td>
<td>1020</td>
<td>S</td>
<td>1011</td>
<td>III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1133</td>
<td>21–May–98</td>
<td>4.8</td>
<td>1118</td>
<td>G</td>
<td>1118</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>619</td>
<td>17–Jun–96</td>
<td>5.2</td>
<td>647</td>
<td>G</td>
<td>616</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>709</td>
<td>25–Nov–96</td>
<td>5.5</td>
<td>703</td>
<td>P</td>
<td>710</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>613</td>
<td>17–Jun–96</td>
<td>5.5</td>
<td>644</td>
<td>P</td>
<td>614</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1027</td>
<td>4–Dec–97</td>
<td>5.6</td>
<td>1011</td>
<td>S</td>
<td>1011</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1049</td>
<td>4–Dec–97</td>
<td>6.1</td>
<td>1022</td>
<td>P</td>
<td>1050</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>11–Jul–93</td>
<td>6.7</td>
<td>102</td>
<td>P</td>
<td>103</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>847</td>
<td>15–Dec–96</td>
<td>7.0</td>
<td>824</td>
<td>P</td>
<td>846</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>202</td>
<td>12–Apr–94</td>
<td>7.0</td>
<td>201</td>
<td>P</td>
<td>201</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>11–Jul–93</td>
<td>7.0</td>
<td>101</td>
<td>P</td>
<td>101</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEMALE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>852</td>
<td>15–Dec–96</td>
<td>2.0</td>
<td>827</td>
<td>G</td>
<td>–</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>854</td>
<td>15–Dec–96</td>
<td>2.2</td>
<td>827</td>
<td>G</td>
<td>–</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>850</td>
<td>15–Dec–96</td>
<td>2.4</td>
<td>827</td>
<td>G</td>
<td>–</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>617</td>
<td>17–Jun–96</td>
<td>3.1</td>
<td>647</td>
<td>G</td>
<td>–</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>607</td>
<td>17–Jun–96</td>
<td>3.3</td>
<td>624</td>
<td>S</td>
<td>–</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>612</td>
<td>17–Jun–96</td>
<td>3.9</td>
<td>643</td>
<td>S</td>
<td>–</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>855</td>
<td>15–Dec–96</td>
<td>4.1</td>
<td>827</td>
<td>G</td>
<td>–</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>849</td>
<td>15–Dec–96</td>
<td>4.4</td>
<td>827</td>
<td>G</td>
<td>–</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>608</td>
<td>17–Jun–96</td>
<td>4.8</td>
<td>628</td>
<td>S</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>811</td>
<td>15–Dec–96</td>
<td>5.1</td>
<td>814</td>
<td>S</td>
<td>–</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>928</td>
<td>12–Jun–97</td>
<td>5.6</td>
<td>918</td>
<td>?</td>
<td>–</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1204</td>
<td>1–Dec–98</td>
<td>5.6</td>
<td>1207</td>
<td>S</td>
<td>–</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>937</td>
<td>5–Dec–95</td>
<td>5.7</td>
<td>505</td>
<td>?</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1303</td>
<td>4–Jun–99</td>
<td>5.8</td>
<td>1302</td>
<td>G</td>
<td>–</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>11–Jun–93</td>
<td>7.6</td>
<td>101</td>
<td>P</td>
<td>102</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>614</td>
<td>17–Jun–96</td>
<td>6.5</td>
<td>644</td>
<td>P</td>
<td>613</td>
<td>III</td>
<td>III</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>616</td>
<td>17–Jun–97</td>
<td>6.9</td>
<td>647</td>
<td>G</td>
<td>619</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
<td>+</td>
</tr>
<tr>
<td>710</td>
<td>25–Nov–96</td>
<td>6.9</td>
<td>703</td>
<td>P</td>
<td>709</td>
<td>VI</td>
<td>IV</td>
<td>III</td>
<td>+</td>
</tr>
<tr>
<td>103</td>
<td>11–Jun–93</td>
<td>7.2</td>
<td>102</td>
<td>P</td>
<td>104</td>
<td>VII</td>
<td>IV</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>201</td>
<td>12–Apr–94</td>
<td>7.3</td>
<td>201</td>
<td>P</td>
<td>202</td>
<td>V</td>
<td>X</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>1050</td>
<td>4–Dec–97</td>
<td>7.3</td>
<td>1022</td>
<td>P</td>
<td>1049</td>
<td>XI</td>
<td>V</td>
<td>IV</td>
<td>+</td>
</tr>
<tr>
<td>846</td>
<td>15–Dec–96</td>
<td>7.9</td>
<td>824</td>
<td>P</td>
<td>847</td>
<td>XII</td>
<td>V</td>
<td>IV</td>
<td></td>
</tr>
</tbody>
</table>

*FEMALE

| 1471      | 4–Dec–99     | 7.4     | 1429           | P                   | I            | III           |              |                          |               |
| 1419      | 4–Dec–99     | 7.1     | 1409           | P                   | II           | III           |              |                          |               |
| 1501      | 2–Jun–00     | 7.3     | 1501           | P                   | IV           | IV           |              |                          |               |
| 1467      | 4–Dec–99     | 6.8     | 1426           | P                   | IV           | IV           |              |                          |               |
| 1524      | 2–Jun–00     | 7.7     | ?              | ?                   | IV           | IV           |              |                          |               |
| 1448      | 4–Dec–99     | 7.3     | ?              | ?                   | VI           | IV           |              |                          |               |
| 1417      | 4–Dec–99     | 7.5     | 1407           | P                   | VII          | IV           |              |                          |               |
| 1503      | 2–Jun–00     | 7.6     | 1502           | P                   | IX           | IV           |              |                          |               |
| 1436      | 4–Dec–99     | 7.9     | ?              | ?                   | X            | V            |              |                          |               |
| 1470      | 4–Dec–99     | 6.7     | 1428           | P                   | X            | V            |              |                          |               |
| 1507      | 2–Jun–00     | 7.6     | 1504           | P                   | XI           | V            |              |                          |               |
| 1464      | 4–Dec–99     | 7.9     | 1425           | P                   | XII          | V            |              |                          |               |

S: solitary, P: sexually paired, G: grouped, -: ova not observed, +: spermatophore observed in the female genital tract, ?: host sponges were broken and composition pattern couldn’t be decided, *: ovaries were staged through the carapace under stereoscopic microscope. Broken line indicate the minimum size of sexually paired.