Embryos and Rhynchoteuthion Paralarvae of the Jumbo Flying Squid * Dosidicus gigas (Cephalopoda) Obtained through Artificial Fertilization from Peruvian Waters

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Shipboard artificial fertilization experiments were carried out during November 1997 using four mature and mated females of *Dosidicus gigas* (320-407 mm in mantle length) from Peruvian waters. A total of 167 hatchlings were obtained from about 3600 eggs which were kept at 18°C. Oviducal gland powder from the closely related species, *Ommastrephes bartramii*, was effective in expansion of chorion, which is essential for normal embryonic development. Hatching occurred 6-9 days after fertilization. Paralarvae survived up to 10 days after hatching without feeding. Mantle length was 0.9-1.3 mm (mean 1.1 mm) at hatching and increased to 1.1-1.5 mm (mean 1.4 mm) on the 7th day after hatching. Proboscis suckers were equal in size. Length of long axis of the statolith increased from ca. 40 μm at hatching to ca. 60 μm on the 4th day after hatching and remained around 63-67 μm until the 10th day. Daily increments were indistinct in the statoliths.

Key words: Embryonic development, morphology, paralarvae, jumbo flying squid, statolith

The jumbo flying squid *Dosidicus gigas* (Orbigny, 1835) is the largest species of the family Ommastrephidae, attaining 120 cm in mantle length. It is distributed in the eastern Pacific Ocean east of 120°W and between 35°N and 50°S.1) Although this species has been commercially harvested, with the maximum annual catch of 165,000 metric tons in 1994, its biology remains largely unknown.2,3) In particular, information about their early life stages is limited. Nesis1) and Yamaguchi and Okutani4) summarized morphology and distribution of rhynchoteuthion paralarvae of *D. gigas*. Although an extraordinary abundance of rhynchoteuthions in the eastern tropical Pacific was reported,5) these paralarvae could not be identified to species—they were either *Sthenoteuthis oualaniensis* or *Dosidicus gigas* due to the current lack of sufficient published morphological description of paralarval *D. gigas*. Nesis6) reported that *D. gigas* spawn in the austral spring and summer somewhere in the area over the continental slope of Peru. However, Masuda et al.7) concluded that the spawning season is year-round as evidenced from hatching date distribution, which was estimated from statolith microstructure and from occurrences of mature females.

Commercial catch rates of the *D. gigas* fishery in Peruvian waters fluctuated drastically between El Nino years and normal years.8) Since the life span of *D. gigas* is assumed to be one or two years,9) fluctuation of stock abundance could be directly affected by survival rates of paralarvae and juveniles which are thought to be quite low in ommastrephid squids.1,8)

Artificial fertilization techniques have been established for routinely obtaining the ommastrephid embryos on board research vessels for *Ommastrephes bartramii*,10) *Sthenoteuthis oualaniensis*,10) *Todarodes pacificus*,10) and *Illex argentinus*.11) The effect of temperature on survival rates of paralarvae of *T. pacificus* has been examined on the basis of rearing experiments.12) Such a study would be useful for understanding fluctuations of stock abundance in *D. gigas*. This report describes results on the artificial fertilization of *D. gigas* in Peruvian waters during a cooperative survey between Japan and Peru in 1997.

Material and Methods

Shipboard Experiments and Observation

We used four mature and mated females of *Dosidicus gigas* obtained from Peruvian waters with jigs on board the Japanese research vessel Kaiyo Maru (Table 1). The procedures of artificial insemination followed those of Sakurai and Ikeda13) and Sakurai et al.9)

For each experiment, we prepared 0.2 μm filtered seawater in order to avoid bacterial infection. Immediately before insemination, we also prepared ‘jelly water’ by adding 30 ml of filtered seawater to 20-30 mg of lyophilized oviducal gland powder, which was processed from *Ommastrephes bartramii* by Y. Sakurai of the Hokkaido University.

Within 30 min after capture, we dissected the specimens and extracted ripe eggs from their oviducts and sperm masses from seminal receptacles on the buccal membranes; both were separately placed on sterilized petri dishes (10 mm high and 52 mm in diameter). Sperm was activated by chopping sperm masses with fine forceps and by adding a drop of filtered seawater to 20-30 mg of lyophilized oviducal gland powder, which was processed from *Ommastrephes bartramii* by Y. Sakurai of the Hokkaido University.
Table 1. Outline of the artificial fertilization experiments of *Dosidicus gigas*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Station</th>
<th>Date of artificial insemination</th>
<th>Time of artificial insemination</th>
<th>Latitude (S)</th>
<th>Longitude (W)</th>
<th>Sea surface temperature (°C)</th>
<th>Mantle length (mm) of female used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>313</td>
<td>1997/10/24</td>
<td>19:25</td>
<td>5°30'</td>
<td>85°40'</td>
<td>25.5</td>
<td>407</td>
</tr>
<tr>
<td>2</td>
<td>331</td>
<td>1997/11/2</td>
<td>19:25</td>
<td>9°30'</td>
<td>81°00'</td>
<td>24.1</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>340</td>
<td>1997/11/8</td>
<td>19:50</td>
<td>11°29'</td>
<td>80°00'</td>
<td>23.5</td>
<td>337</td>
</tr>
<tr>
<td>4</td>
<td>344</td>
<td>1997/11/10</td>
<td>19:20</td>
<td>12°30'</td>
<td>80°00'</td>
<td>23.8</td>
<td>378</td>
</tr>
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Shipboard rearing

<table>
<thead>
<tr>
<th>No. of frozen specimens from shipboard rearing</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Just before hatching</td>
<td>3</td>
</tr>
<tr>
<td>Hatching paralarvae</td>
<td>6</td>
</tr>
<tr>
<td>1-day-old paralarvae</td>
<td>16</td>
</tr>
<tr>
<td>2-day-old paralarvae</td>
<td>15</td>
</tr>
<tr>
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<tr>
<td>4-day-old paralarvae</td>
<td>5</td>
</tr>
<tr>
<td>5-day-old paralarvae</td>
<td>4</td>
</tr>
<tr>
<td>6-day-old paralarvae</td>
<td>4</td>
</tr>
<tr>
<td>7-day-old paralarvae</td>
<td>4</td>
</tr>
<tr>
<td>8-day-old paralarvae</td>
<td>1</td>
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<tr>
<td>9-day-old paralarvae</td>
<td>1</td>
</tr>
<tr>
<td>10-day-old paralarvae</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
</tr>
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IMARPE laboratory rearing

<table>
<thead>
<tr>
<th>No. of eggs used (A)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>No. of hatchlings (B)</td>
<td>500</td>
</tr>
<tr>
<td>Hatching rate (B/A)</td>
<td>24</td>
</tr>
<tr>
<td>Hatching date</td>
<td>1997/11/17-18</td>
</tr>
</tbody>
</table>

30 min after the insemination, we divided the fertilized eggs to three dishes, each of which contained 200-300 eggs. We added filtered seawater to a depth of 5-7 mm and kept the dishes in an incubator at a temperature of 22°C with light for Experiment 1 (Station 313) or 18°C in darkness for Experiments 2-4 (Stations 331, 340, 344; Table 1). We changed seawater once a day except for Experiment 1 where we did not change water. Paralarvae were not fed.

We observed eggs and paralarvae twice a day (at approximately 7 and 20 hrs) with a Nikon dissecting microscope, photographed them with a 35 mm still camera, and recorded with a digital video camera. Embryos and paralarvae were selected and either fixed with ethanol or frozen at -30°C and kept for later laboratory observation (Table 1). Sizes of embryos and paralarvae were not measured on board because of lack of a micrometer.

Ontogenetic stages were adapted from those of Watanabe *et al.* who presented atlas of development of the Japanese common squid *Todarodes pacificus*.

Laboratory Experiments and Observation

Four paralarvae obtained from Experiment 2 and approximately 1300 eggs from Experiments 3 and 4 were transported to the Institute del Mar del Peru (IMARPE) in Callao, Peru on November 12, 1997. Each embryo had evident chorion and the vitellic sac. These paralarvae and embryos were placed in an incubator that was kept at 18°C in darkness. Filtered and sterilised seawater was changed daily. From the sample of Experiment 3, eight individuals were preserved for later cytogenetics and others studies.

Frozen samples from shipboard rearing experiments were thawed in the National Research Institute of Far Seas Fisheries (NRIFSF) in Japan and immediately observed with a Zeiss microscope (model Stemi 2000C) connected to a high resolution monitor through a 3CCD camera (Sony DXC970MD). Mantle length, head width (maximum width of head excluding eyes) and proboscis length (from the base to tip excluding its suckers) were measured and photographed with a Zeiss image analysing system (model KS-200). Statoliths were extracted from arbitrarily selected paralarvae and embryos. Long and short axes of each statolith were measured with a Zeiss microscope (model Axiovert 25) connected to the Zeiss image analysing system.

Results

Development of Embryos and Paralarvae

One to several hours after the fertilization, ripe eggs usually changed their shapes from oval (ca. 1.3 mm x 0.9 mm) to spherical with expanded chorion and perivitelline space. Some of the eggs did not expand their chorion. Embryonic
development was confirmed about 12 h after the fertilization. No embryos in Experiment 1 developed beyond Stage 15. In Experiments 2–4, the chorions further expanded on the fourth day after the insemination. We transferred these embryos to other dishes with filtered seawater. Eggs without chorion expansion did not develop further. Four days after fertilization, embryos expanded vertically and perimordia of eyes, funnel and arms were recognized (Stage 20, Fig. 1A). Between four and five days after the fertilization, a funnel, arms and reddish pigments on mantle and eyes became evident (Stage 21, Fig. 1B). On the sixth day, the embryos began to rotate within the further developed perivitelline space and reddish and yellowish pigments became more distinct (Stage 24–25, Fig. 1C). Egg diameters at these stages were almost twice those of undeveloped eggs.

Hatching occurred at Stages 26–27 from 6 to 8 days after fertilization with a peak at the seventh day. Embryos kept in the laboratory of IMARPE hatched from 7 to 9 days (Experiment 4) or from 9 to 10 days after fertilization (Experiment 3, Table 1), however, stages were not recorded. The earlier hatching on board ship might be due to the effect of the ship’s movement or other related stimuli. Embryos emerged from eggs with the posterior tip of the mantle first. Mantle lengths at hatching measured 0.9–1.3 mm based on frozen specimens.

Hatching rate of shipboard-reared embryos was about 1% in Experiments 2 and 3, and was 11% in Experiment 4, whereas that of embryos kept in IMARPE laboratory was 5% in Experiments 3 and 4 (Table 1). The number of embryos with abnormal shape was 12 out of 103 paralarvae in the present study.

**Morphology and Behavior of Paralarvae**

Incipient fins and proboscis were evident in the first day paralarvae and the ratio of proboscis length to mantle length gradually increased from ca. 20% at hatching to 40–60% on the seventh day from hatching (Fig. 1D–E and Fig. 3). Lateral and internal proboscis suckers were equal in size (Figs. 1 and 2). No photophores were observed on the eyes or intestine throughout the period of observation. The ink sac was observed before hatching (Fig. 1C) and hatchlings ejected their ink when frightened. Pigments were generally yellowish at the time of hatching but gradually became reddish and decreased in size with considerable variation (Fig. 1). Pigments on mantle were arranged in two transverse rows. Eye pigmentation was reddish just after hatching and became darker after the fourth day. Internal yolk was almost exhausted on the seventh day after hatching.

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**Fig. 1.** *Dosidicus gigas* shipboard reared embryos (A-C) and paralarvae (D-E) obtained from artificially fertilized eggs (all photographs are from live animals).

Time elapsed after the insemination for embryos are: 4 days and 11 hours (A), 4 days and 23 hours (B), and 6 days (C); days elapsed after hatching are: 1 day (E) and 4 days (F).

**Fig. 2.** Two *Dosidicus gigas* 6-day-old paralarvae (ethanol-fixed samples) showing different posture and chromatophore patterns. Bar = 1 mm.

**Fig. 3.** Relationship between age and proportion of proboscis length to mantle length (ML) of shipboard reared *Dosidicus gigas* paralarvae.
Fig. 4. Relationship between age and mantle length (ML) of shipboard reared *Dosidicus gigas* paralarvae. Solid dots indicate mean ML with a fitted line.

The maximum observed life was 10 days in Experiment 2 (shipboard experiments) without feeding or 9 days in the IMARPE laboratory. The feeding experiments were carried out in IMARPE with cultured rotifers or with amino acids obtained from collagen digestion. No feeding behaviour was observed. Death might have derived partly from the protozoa contamination of the culturing media of the rotifers. After four days from hatching the paralarvae became weak.

Paralarvae actively swam in the posterior direction, usually with the abdomen up. Reacting to manipulation, paralarvae withdrew their heads into their mantles. We often observed paralarvae extending their proboscis almost along longitudinal axis, but we did not observe movement of proboscis onto the surface of the mantle or to the mouth.

**Growth**

Mantle length of paralarvae examined ranged from 0.9 to 1.8 mm and mean mantle length gradually increased with age, with considerable variation within and among ages (Fig. 4). The regression line fitted to mean mantle lengths (ML) at ages of 0–7 days after hatching is expressed as $ML = 0.0314x + 1.1429$, where $x$ is age in day.

Statoliths were thin and easily broken while extracting from the statocysts with fine needles. Length of long axis of the statolith increased with age from ca. 40μm at hatching to ca. 60μm four days after hatching (Fig. 5). Statoliths from 6, 8, and 10-day-old paralarvae were similar in length (63–67μm), suggesting an effect of starvation on growth. Although growth of statoliths was thus evident until four days after hatching, daily increments were indistinct in the observed statoliths.

**Discussion**

The oviducal gland powder from *Ommastrephes bartramii* was effective for expansion of the chorion of *Dosidicus gigas*. This extends the previous results, which showed its effectiveness among three ommastrephids, *Todarodes pacificus*, *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*.

The causes of the death of embryos in Experiment 1 could be attributed to either higher temperature (22°C), water change, or light conditions. Although Sakurai and Ikeda suggested water changes, Sakurai et al. reported no necessity of it since oxygen was not a limiting factor for embryonic development.

Among many possible causes of the low hatching rate in the present study, the high ratio of eggs without expanded chorion, which may have resulted from use of oviducal gland jelly from *O. bartramii*, is probably important. Sakai and Brunetti suggested that anoxia and high pressure caused by the unexpanded chorion could have been derived mortality of *Illex argentinus* embryos. Occurrences of abnormal embryos were sometimes frequent in the shipboard experiments for *O. bartramii* and *T. pacificus*. The lower ratio of abnormal embryos reported here, compared to the previous studies, may be attributed to calm sea conditions including a port call.

Morphology of these paralarvae (Figs. 1 and 2) was similar to that reported by Nesis and also similar to *O. bartramii* and *S. oualaniensis*. Proboscis suckers were equal in size, although Nesis reported that lateral suckers are slightly larger than the others. Among ommastrephid paralarvae in the eastern Pacific Ocean, only *D. gigas* and *S. oualaniensis* share a condition of equal-sized proboscis suckers. However, morphological data so far obtained from artificial rearing of these two sympatric species (Sakurai et al. and the present study) indicate no specific difference.

From statolith microstructure, Balch et al. and Bigelow and Landgraph estimated ages of paralarvae of *Illex* sp. and *Ommastrephes bartramii*, respectively. Bigelow and Landgraph also noted that increments within 15-μm area immediately distal to the nucleus were weak and assumed this condition corresponding to the yolk stage. In the present study, no distinct increments were observed. Statoliths of artificially fertilized paralarvae of *Illex argentinus* have similarly indistinct increments (M. Sakai, Fisheries and Aquaculture International, Tokyo, pers. comm.), suggesting that this is an effect of artificial rearing, e.g., no diel changes in light condition. However, it may be possible to use statolith size to estimate ages of wild paralarvae as evidenced from age-statolith length relationship at least until the sixth day after hatching, thereafter growth of statoliths was not observed (Fig. 5).

Starvation is the most plausible explanation of the death
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References