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Developmental Duration and Morphology of the Sea Star *Asterias amurensis*, in Tongyeong, Korea

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Abstract – The process of embryogenesis and larval development of the asteroid sea star *Asterias amurensis* (Lütken) was observed, with special attention paid to morphological change and larval duration. In reproductive season, mature sea stars were collected under floating net cages, located in Tongyeong, southern Korea. The mature eggs are 138 μm in average diameter, semi-translucent and orange in color, sperms in good condition appear light cream to white-gray in color. Embryos develop through the holoblastic equal cleavage stage and a wrinkled blastula stage that lasts about 9 hours after fertilization. Gastrulae bearing an expanded archenteron hatch from the fertilization envelope 22 hours after fertilization. At the end of gastrulation, rudiments of the left and right coelom are formed. By day 2, larvae possess complete alimentary canal and begin to feed. At this stage, the larva is called early bipinnaria. In 6-day-old larvae, the pre- and post- oral ciliated bands form complete circuits and the bipinnarial processes start to develop. By day 12, the lateral and anterior projection of the larval wall processes along the ciliated bands begins to thicken and curl, and the ciliated bands become more prominent. By day 32, early brachiolaria are presented with three pairs of brachiolar arms. Advanced brachiolaria with a well-developed brachiolar complex (three pairs of brachia and central adhesive disc) occur 6 weeks after fertilization. In the field, spawning of the sea star was observed in April to May, settlement form larvae and just settlements seem to occur from June to July, and early juveniles occur from August to September. Although we had not described the end of brachiolaria stage, it can be tentatively estimated that the duration of the pelagic stage of *A. amurensis* is 40 to 50 days.

Key words – *Asterias amurensis*, sea star, embryogenesis, larval development

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1. Introduction

The sea star of the genus *Asterias* is one of the most abundant and widely distributed echinoderms of the Northern Hemisphere. Among them, *Asterias amurensis*, commonly referred to as the northern Pacific sea star, is native to the coasts of Korea, southeastern Russia, Japan, and the extended Bering Sea coasts of Alaska (Fisher 1930; Onguru and Okutani 1991). However, the distribution of the sea star has now been extended, following its infiltration into a number of other countries, including Australia (Ward and Andrew 1995). *A. amurensis* is a notoriously efficient predator of bivalves such as mussels, scallops and clams, and well known to aggregate in large numbers on shellfish grounds (Hatanaka and Kosaka 1958). Additionally, it is an opportunistic feeder on a variety of epi- and in-faunal species. The outbreak of this species can badly damage natural and cultured shellfish beds (Nojima *et al.* 1986). Due to the important role that *A. amurensis* plays as a "keystone" benthic predator, considerable research effort has been undertaken to document its reproductive cycle with seasonally predictable gametogenesis and gonad weight (Kim 1968; Pearse and Walker 1986; Byrne *et al.* 1997), feeding behavior (Kim 1969; Lockhart and Ritz 2001), impact on native species (Ross *et al.* 2003), and spatial and size distribution of local population (Morrice 1995). Larvae of *A. amurensis* have been reared (Bruce *et al.* 1995; Lee *et al.* 2004), but information on the duration of larval development of *A. amurensis* is lacking (Kasyanov *et al.* 1980). Although *A. amurensis* is native to Korea, not only larval development, but also general ecological data (reproductive cycle, distribution, etc.) have not been reported with its Korean population.

The present study describes the sequences and morphological changes during the development of *A. amurensis* and provides an estimate of larval duration and reference material for identification of field-collected larvae.

2. Materials and Methods

Mature *Asterias amurensis* were collected under floating net cages, located in Jangdudo, Tongyeong southern Korea (34°45'N, 128°23'E) during the spawning season (April to May), from 2001 to 2002. Samples of 26 - 40 sea stars (arm radius, R = 140 - 155 mm) were collected from depths of 5 - 10 m by scuba. In the laboratory, the sea stars were maintained in flowing seawater aquaria. The ripest males and females were selected for spawning using the method described by Keesing *et al.* (1997). The female gonad is orange in color, and testes in good condition are a very light cream, almost white-gray (Fig. 1). A number of fertilized eggs were obtained as a result of natural spawning that occurred while specimens were being transported in an ice chest with seawater. In addition to the natural fertilization, successful artificial fertilization was realized as follows; spawning was induced with injection of 1 ml of 1-methy ladenine (10.3M solution) into each arm of the sea star above the gonad according to the methods described by Kanatani (1969). Once spawning had begun, a dilute sperm suspension was added to the vessels (20 l) containing mature ova to effect insemination. Flow water was used before the blastula stage, and vigorous swimming larvae were transported to the 200 l larval rearing tanks, while unhatched eggs and nonvigorous swimmers were removed. The larvae were reared in the tanks containing filtered seawater (31‰), at about natural surface seawater temperature (17 to 21°C). After the second day, the larvae were fed phytoplankton species *Chaetoceros calcitrans*, *Isochrysis galbana*, *Chlorella ellipsoidea* and *Dunaliella tertiolecta*, each grown in monoculture. Feeding was carried out daily, until the end of this study, with a mixture of 5000 cells/ml. The times reaching every developmental stage were determined when 70% embryos were at a given stage. Larval staging criteria followed Byrne and Barker (1991), and the external morphological features of sperm and each stage were observed and photographed with a scanning electron microscope (SEM), a microscope, and a stereomicroscope.

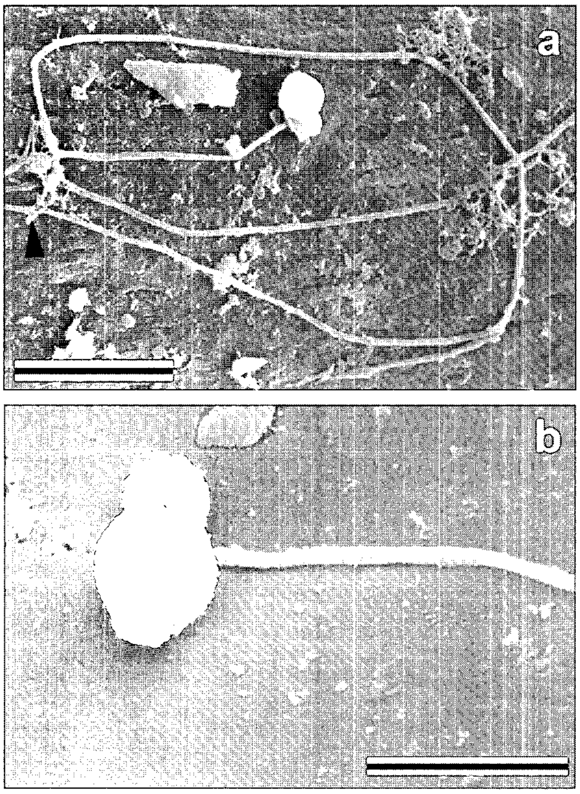

Fig. 1. Sperm of *Asterias amurensis*: a. scale=5 μm; b. scale=2.5 μm.

Table 1. Developmental events schedule of *Asterias amuresis* (17-20°C)

Time after fertilization	Larval characteristics
0 min	fertilization
107 min	2-cell
187 min	4-cell
232 min	8-cell
363 min	16, 32-cell
553 min (9 hrs)	early coeloblastula
18 hrs	blastrula
22 hrs	rotating embryo
25 hrs	early gastrula
38 hrs	gastrula
40-46 hrs	dipleurula
52-59 hrs	early bipinnaria
144 hrs (6 d)	bipinnaria
480 hrs (20 d)	advanced bipinnaria
768 hrs (32 d)	early brachiolaria
984 hrs (41 d)	mid brachiolaria

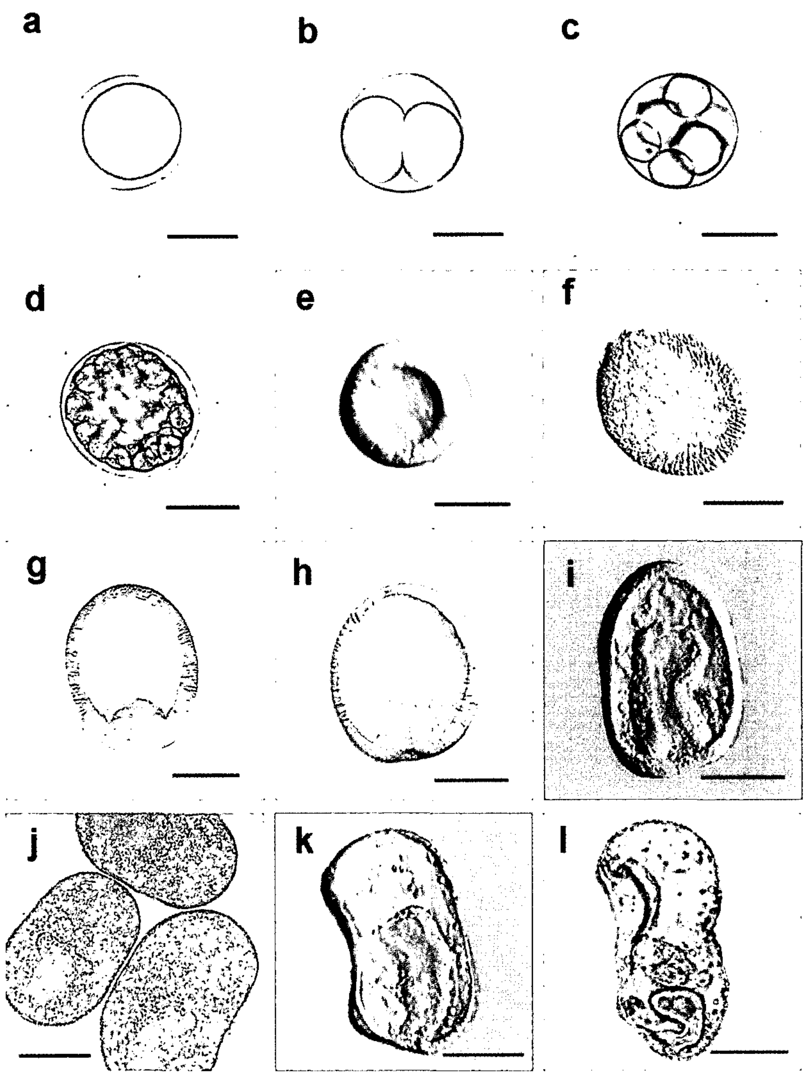

Fig. 2. Development of *Asterias amurensis*: a. fertilized egg; b. two-cell stage; c. eight-cell; d. early coeloblastula; e. late coeloblastula, rotating in fertilization envelope; f. hatched coeloblastula; g, h. early gastrula; i. advanced gastrula; j. late gastrula; k. late gastrula, lateral view (stomodaeum becomes the mouth); l. dipleurula, lateral view. Scale bar indicates 100 μm.

3. Results

The length of sperm is about 55 μm including pointed head and tail (Fig. 1a, b). Ova are negatively buoyant but were easily suspended by slight water movement. Mature ova were spherical, orange in color, semitranslucent, 137.6 μm (±10.8 μm, n=20) in diameter, and exist a granular texture in plasm.

The developmental schedule of *A. amurensis* was in Table 1. The fertilization envelope is apparent about 5 minutes after insemination (Fig. 2a). The first and second polar bodies are visible in the perivitelline space within 40 minutes. Cleavage is the typical echinoderm pattern of holoblastic and radial cleavage. The first cleavage occurs in a plane through the animal and vegetal poles about 107 minutes after fertilization, and subsequent cleavages take place at intervals of about 80 minutes (Fig. 2b), and the 8-cell, 16-cell and 32-cell stage 3.8 to 6.5 hours after fertilization (Fig. 2c). Early coeloblastulae are reached at about 9 hours after fertilization. As a result of cleavages, a blastula with rather loosely bound blastomeres forms (Fig. 2d). The wrinkled blastula stage began with the appearance of an ingression tract on the surface 18 hours after fertilization. Subsequently, the ingression tracts gradually increased in number and size. The embryos hatch the fertilization envelope, begin to rotate by means of cilia that uniformly cover the body surface about 22 hours after fertilization (Fig. 2e). Gastrulation takes place at the vegetal pole, before the complete disappearance of the egression tracts. Larvae hatch as coeloblastulae, and the gastrulae become free-swimming larvae 25 hours after fertilization (Fig. 2f). Early gastrulae were nearly spherical and were 160 μm in diameter (Fig. 2g, h). At hatching, larvae begin to elongate and form a shallow blastopore. They continue to elongate with growth of the archenteron into the blastocoel. The mesenchyme cells that form the surface of the hollow ball all seem to adhere strongly to each other and enter the blastocoel at the vegetal pole (Fig. 2i). 38 hours after fertilization, late gastrulae develop when right and left enterocoels form as pouches off the expanded tip of the archenteron (Fig. 2j). A shallow stomodeum is present, and the blind end of the archenteron bends towards the oral surface. During this stage, the posterior bends ventrally, and the blastopore is regarded as the larval anus, and the archenteron develops into the larval gut. By 40 to 46 hours, the blind end of archenteron fuses with the stomodeal invagination. Now the complex gut is formed (Fig. 2k), and the larvae become early bipinnaria. By this stage, the larvae are 250 μm in length and 190 μm in width, and most of them swim near the surface of the rearing tank and begin to feed. The cardiac sphincter is present 52 to 59 hours after fertilization and separates the developing esophagus from the stomach. The hydropore is present and extends dorsally out of the left enterocoel (Fig. 2l). The mouth widens and takes on a gaping appearance. Pre- and post-oral ciliated bands begin to develop and the enterocoels are present in the form of small pouches on either side of the anchenteron at the level of the cardiac spincter. In 6-day-old larvae, the pre- and post- oral ciliary bands form complete circuits and the bipinnarial processes start to develop (Fig. 3a). The oral hood curves over the mouth and both anterior and lateral bipinnaria processes become more pronounced. Larvae exhibit posterior flexure and contraction of the cardiac sphincter. By day 8, the oral hood becomes pointed. The enterocoels extend both anteriorly to the mouth and commence extension in a posterior fashion (Fig. 3b). By day 12, the lateral and anterior projection of the larval wall processes, along the ciliated bands, begins to thicken and curl, and the ciliated

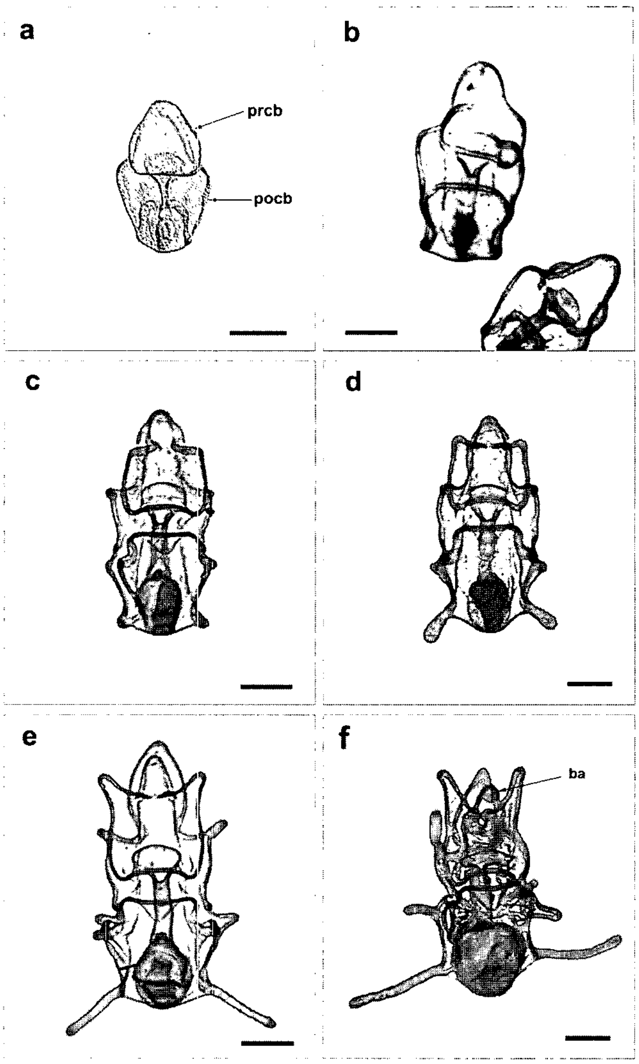

Fig. 3. Pelagic larvae of *Asterias amurensis*: a. early bipinnaria; b. bipinnaria; c. advanced bipinnaria; d, late bipinnaria; e. brachiolaria; f. late brachiolaria; prcb. postoral ciliated band, prob. preoral ciliated band, ba. brachiolaria arms. Scale bar indicates 200 μm.

bands become more prominent. 20-day-old larvae are now late bipinnaria (Fig. 3c). By this stage, the ventral horn completes its growth fusing with the right enterocoel. In the preoral hood, the coelom extends in an anterior fashion beyond the median dorsal process, forming the lumen of the future median brachiolar arm. At the base of the median dorsal process, the anterior coelom gives rise to two lateral extensions destined to be the coelomic lumina of the posterior brachiolar arms. 32 days after fertilization, early brachiolaria are present with three pairs of brachiolar arms (Fig. 3d). As the body and processes grow, the size of the band increases and the processes become long, flexible arms (Fig. 3e, f). Four-week-old larvae are 1,160 in length (±70 μm, n=25). Advanced brachiolaria were present 6 weeks after fertilization (Fig. 3f). These larvae have a well-developed brachiolar complex, comprised of the three pairs of brachia and a central adhesive disc.

4. Discussion

Development of *Asterias amurensis* is similar to other asteroids that develop indirectly through a pelagic planktotrophic bipinnaria and brachiolaria larva (Strathmann 1978), but the duration of the larval development and rearing conditions differ from species. Generally, the embryos and larvae of asteroid species develop more rapidly in warmer waters (Strathmann 1978; Barker and Nichols 1983). Table 2 shows that the developmental differences between previous reports on *A. amurensis*. The duration of the larval stage closely followed the description given by Bruce *et al.* (1995), although larvae took about 12 days to reach fusion of the axohydrocoel compared to 30 to 36 days and 78 days reported by Bruce *et al.* and Komatsu (in Bruce *et al.* 1995), respectively. The shorter development time obtained in the present study may have resulted from the larvae being reared at higher temperatures than those in Bruce et al. In similar temperature conditions, in the present study and Kasyanov, it is reported that the fusion time of the axohydrocoel were 10 to 12 days after fertilization. After this stage, the development in this study was faster than in previous reports. Larval duration of 115 to 120 days (by Komatsu in Bruce *et al.* 1995), 66 to 91 days (Bruce *et al.* 1995), and 50 to 60 days (by Kasyanov in Bruce *et al.* 1995) are predicted for *Asterias amurensis* larvae developing at 10 to 12°C, 11 to 14°C, and 19°C respectively. Estimates of larval duration in sea stars are based largely on laboratory-reared organisms. In most cases, some degree of abnormality and considerable variation in both development rate and time to settlement has been recorded (Barker 1979; Byrne and Barker 1991). Several factors can influence developmental rate and larval duration in sea stars, including temperature, feeding regime, size of rearing containers, and provision of appropriate settlement substrate (Baker 1979; Barker and Nichols 1983). Very few estimates are available for sea star larval duration in the field and thus it is difficult to assess whether the observed variability in development reflects natural variability or laboratory artifact. Barker and Nichols (1983) estimated the minimum larval duration of *Asterias rubens* in the field to be 77 to 84 days based on monitoring of spawning, larval distribution and settlement. They also referred that larval duration of the Genus *Asterias* species appears to be both protracted and variable.

In conclusion, the brachiolaria stage and duration of larval development of *A. amurensis* has not been satisfactorily described. However, comparing present photographs and data with those of Kume and Dan (1968) and previous reports, we adopted larval staging criteria, which we have not described at the end of brachiolaria stage. However, we reveal the mid- brachiolar stage (three brachiolar arms, long pigmented larval processes) and settlement form larvae and just settlements seem to occur from June to July, and early juvenile occur from August to September. We can tentatively estimate the duration of the pelagic stage of *A. amurensis* at 40 to 50 days.

Table 2. Developmental times for laboratory rearing experiments of *Asterias amurensis*

Author & water temperature	Komatsu[+] 10°C	Bruce *et al.*[+] 15°C	Kasynov[+] 19°C	Present study 17-20°C
Stage				
Blastula	15-16 hr	12-14 hr	14-16 hr	18 hr
Gastrula		40 hr		38 hr
Early bipinnaria	3 d	3 d	3 d	52-59 hr
Bipinnaria (pre and post ciliated band)		5 d	5 d	6 d
enterocoels fused axohydrocoel	78 d	30-36 d	10-11 d	12 d
Branchial development	102 d	-	40-45 d	32 d
Brachiolaria-adhesive disk	115-120 d	-	50-60 d	41-50 d

+; refer to Bruce *et al.* (1995)

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