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SOME ASPECTS ON REARING LARVAE AND LARVAL DEVELOPMENT OF *TRIPNEUSTES GRATILLA* (L.) (ECHINODERMATA: ECHINOIDEA)[1]

CHANG-PO CHEN[2] and JIN-QUAN RUN

Institute of Zoology, Academia Sinica,
Nankang, Taipei, Taiwan 11529,
Republic of China

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Chang-Po Chen and Jin-Quan Run (1988) Some aspects on rearing larvae and larval development of *Tripneustes gratilla* (L.) (Echinodermata: Echinoidea). *Bull. Inst. Zool., Academia Sinica* **27**(3): 151-157. Larvae of *T. gratilla* were raised to metamorphosis in beakers containing filtered seawater (35‰ S) under constant temperature (25°C) and dim light (ca. 500 lux). Seawater was neither aerated nor agitated and was changed every 4-5 days. The alga *Isochrysis* aff. *galbana* (10^4 to 10^5 cells/ml) was supplied as food. The embryo reached the gastrula stage at 22 h and the prism stage at 30 h after fertilization. The larvae reached the feeding pluteus stage at 4 days. The plutei increased in size and had 4 pairs of arms by 18 days. The vestibule appeared at about 23 days, the tube feet and pedicellariae appeared subsequently, and the plutei became competent for metamorphosis at 30 days.

Key words: Sea-urchin.

Tripneustes gratilla is a commom, shallow-water, regular sea urchin in the tropical seas of Taiwan and spawns during September to October as water-temperature starts to decrease (Chen and Chang, 1981). The gonads of *T. gratilla* are edible and have highly commercial value. Thus, the population size of T. *gratilla* has decreased due to overfishing. Artificial seeding of juvenile sea urchins to the coast zone may be able to replenish the population size. Mortensen (1937) has reared some larvae of *T. gratilla* to metamorphosis, but he fed the larvae by adding fresh natural seawater everyday or every few days, and the data on culture method and larval development are fragmentary. Therefore, in order to get a large quantity of juveniles, the present study intended to find a practical procedure of culturing the larvae to metamorphosis in laboratory, and examine the chronology of larval development in *T. gratilla*.

Based on the general culture methods (Hinegardner, 1969; Leahy, 1986), we focused on finding the proper algal species cultured in laboratory and its appropriate concentration as food for larvae of *Tripneustes gratilla*. Because algae are usually seperated from the culture medium before being used to feed larvae (Hinegardner, 1969), the effect of algal culture medium on this larval growing was examined.

MATERIALS AND METHODS

Sexually mature males and females of *Tripneustes gratilla* (L.) were collected from

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2. To whom reprint request should be sent.

northern Taiwan in September, 1986, and injected with 0.5 M KCl into the coelom through the peristomial membrane to induce spawning. Eggs were seminated artificially and then washed with filtered seawater. The larvae were raised in 500 ml beakers containing planktonic algae and filtered seawater (35‰ S) at 25°C and dim light (ca. 500 lux of fluoresent light). Seawater was changed every 4 to 5 days, but was neither aerated nor agitated. The development of the larva *T. gratilla* was recorded and photographed.

In order to find the appropriate algae species for feeding, 5-day old larvae were raised at a density of 4.2 larvae per ml of seawater and were fed with one of the following species of algae: *Chaetoceros gracilic*, *Isochrysis* aff. *galbana* and *Tetraselmis chuii*. Algal cells were added to the larvae culture each day to keep a concentration about 10^5 cells/ml. After 5 days, the body length (A) and width (B/C ratio) (Fig. 1) of the larvae were measured.

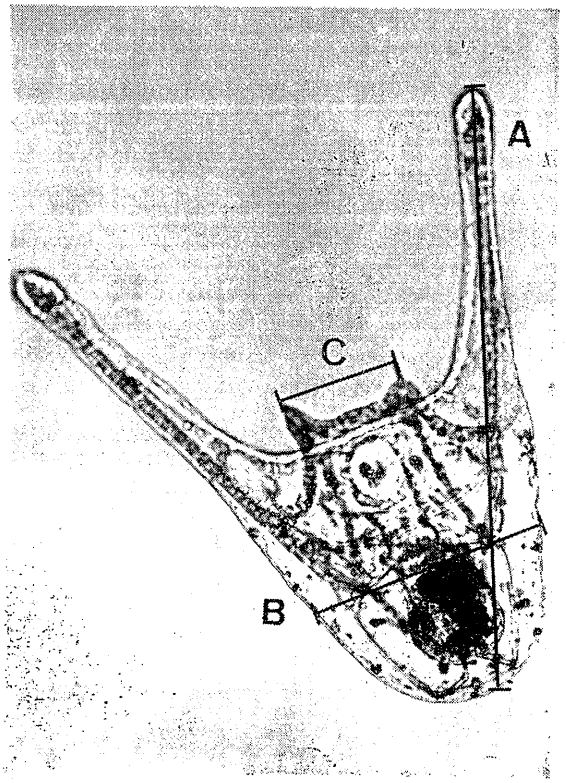

Fig. 1. The measurement of body length and body width of *Tripneustes gratilla* larva. A: body length, B/C: body width.

In order to find the appropriate feeding concentration of the alga *Isochrysis* aff. *galbana*, 5-day old larvae at a density of about 2 larvae per ml were fed with *I.* aff. *galbana* at one of the six concentrations: 5×10, 10^2, 5×10^2, 10^3, 10^4, 10^5 cells/ml. Seawater was changed and enriched with the algae each day. At the 5th and 10th day, the body length (A) of larvae in each group was measured.

In order to find the effect of algal culture medium on larval growth, 12-day old larvae at a density of 0.2 larvae per ml were fed with *Isochrysis* aff. *galbana* with or without the algal enrichment medium, Guillard's medium f/2 (Fox, 1983). Four to 8 ml of the algae culture were centrifuged (1000 g) for 5 min. The algae cells were resuspended either in the supernatant or in filtered seawater. After 5 days, the body length (A) of larvae in each group was measured.

RESULTS

The larvae of *Tripneustes gratilla* which fed on *Chaetoceros gracilic*, *Isochrysis* aff. *galbana* and *Tetraselmis chuii* for 5 days had a mean body length of 428 (SD=35.1), 446 (SD=58.8) and 325 (SD=54.9) μm, respectively (Fig. 2). Larvae which fed on *I.* aff. *galbana* had a larger body width than those fed with *C. gracilic* (Fig. 3). Therefore, *I.* aff. *galbana* is the best food for larval growth among the three algal species. The mean body length of larvae fed with *I.* aff. *galbana* at different concentrations did not differ significantly at the 5th day, but did significantly at the 10th day (ANOVA, $p\ll 0.01$): the larvae fed at the concentration of 10^4–10^5 cells/ml was larger (Fig. 4) and had full guts. The mean body length of larvae fed with or without algal culture medium did not differ, i. e., 595 (SD=117,

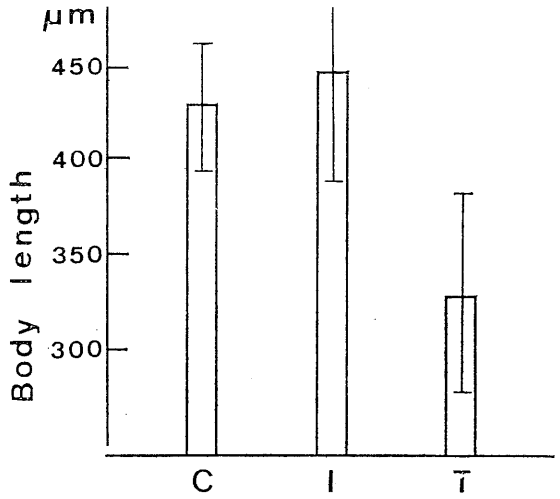

Fig. 2. Effect of algal species on body length of larvae *Tripneustes gratilla*. C: *Chaetoceros gracilic*, I: *Isochrysis* aff. *galbana*, T: *Tetraselmis chuii*. (Mean and 1 SD are given, n=16).

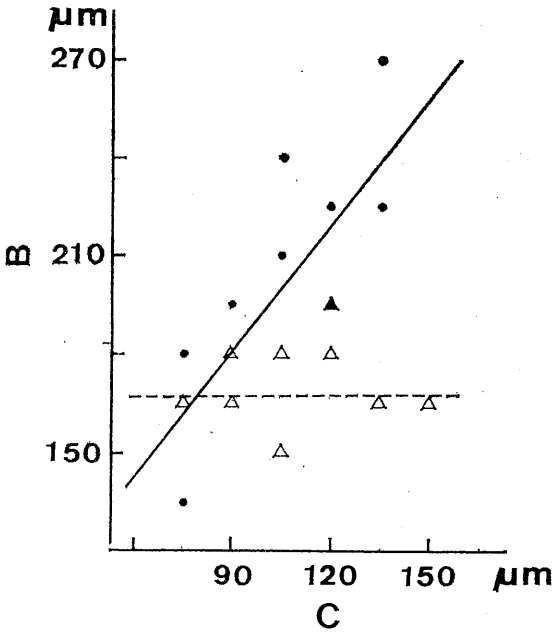

Fig. 3. Effect of algal species on body width (B/C) of larvae *Tripneustes gratilla*. ●: *Isochrysis* aff. *galbana*, △: *Chaetoceros gracilic*. The measurement of B and C is defined in Fig. 1. The lines were drawn based on linear regressive equations.

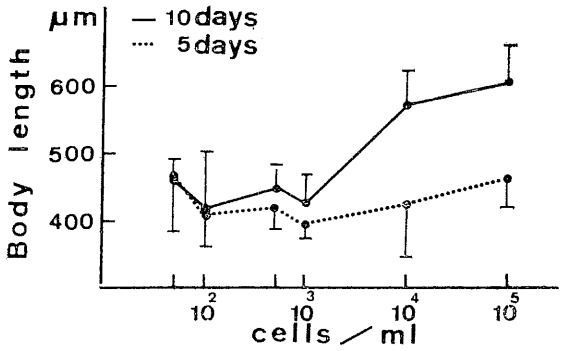

Fig. 4. The body length of the plutei of *Tripneustes gratilla* fed on the alga *Isochrysis* aff. *galbana* in different concentrations for 5 and 10 days. Mean and 1 SD are given. The observation is 5 and 10 for 5th and 10th day, respectively.

TABLE 1
Chronology of the larval development of *Tripneutes gratilla* at 25°C

Stage	Time
4-cell	2.5 h
8-cell	4 h
blastula	9 h
gastrula (triradiate spicules)	22 h
prism	30 h
red pigment	33 h
pluteus	2 d
4 arms	5 d
6 arms	15 d
8 arms	18 d
vestibule	23 d
urchin rudiment (tube feet and pedicellariae)	27 d
inductive metamorphosis	30 d
spontaneous metamorphosis	47 d

n=30) and 592 (SD=111, n=30) μm, respectively.

Table 1 shows the chronology of larval development at 25°C. The blastula stage was reached at 9 h after fertilization and the swimming larva stage was reached by 16 h. The gastrula possessed triradiate spicules at 22 h and gradually became a prism with some red pigments at 33 h (Fig. 5-1). At 2 d, the larva became a pluteus (Fig. 5-2, 3)

and was able to feed on algae at 4 d. At 5 d, two pairs of arms appeared in the pluteus (Fig. 5-4). Four pairs were present by 18 d (Fig. 5-5). The vestibule appeared in the left side of the pluteus at 23 d (Fig. 6-1), and the pedicellaria and tube feet were observed at 27 d (Fig. 6-2, 3). At about 30 d, the pluteus became competent to metamorphose (Fig. 6-4, 5). At this time, the rudiment with 5 tube feet existed in the vestibule, and 1 to 3 pedicellariae existed outside the body. Old competent larvae, even without pedicellariae, metamorphosed spontaneously after 47 d.

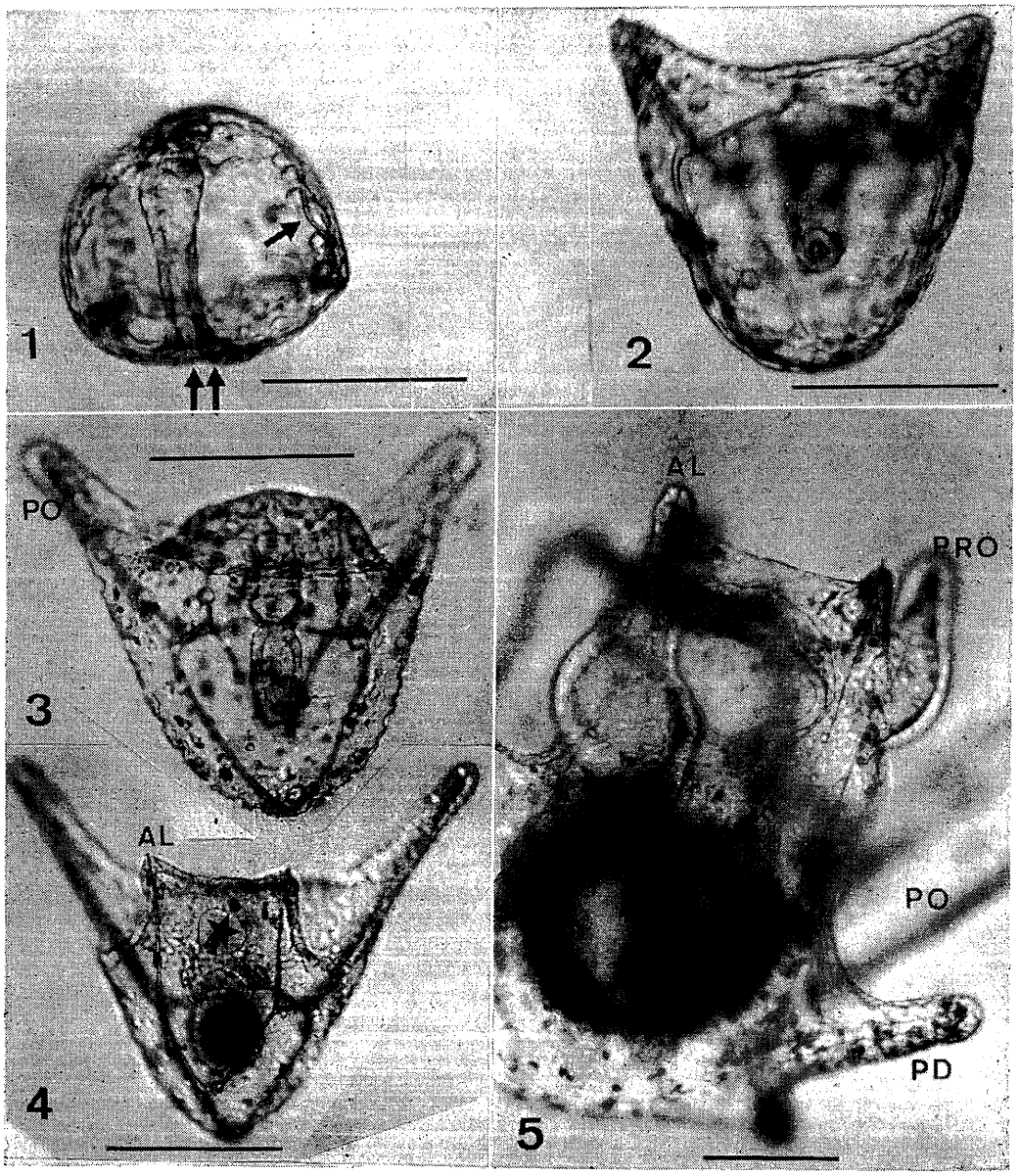

Fig. 5. Larval development of *Tripneustes gratilla*. (1) 30 h, the prism stage, arow: triradiate spicule, doule arrows: blastopore; (2) 2 day, young pluteus; (3) 2.5-day old pluteus, arms beginning, digestive tract differentiated, PO: postoral arm; (4) 5 days, 4-arms pluteus, dorsal view, AL: anterolateral arm, *: mouth; (5) 18-day old, 8-arms pluteus, dorsal view; PRO: preoral arm, PD: posterodorsal arm. Bar indicates 100 μm.

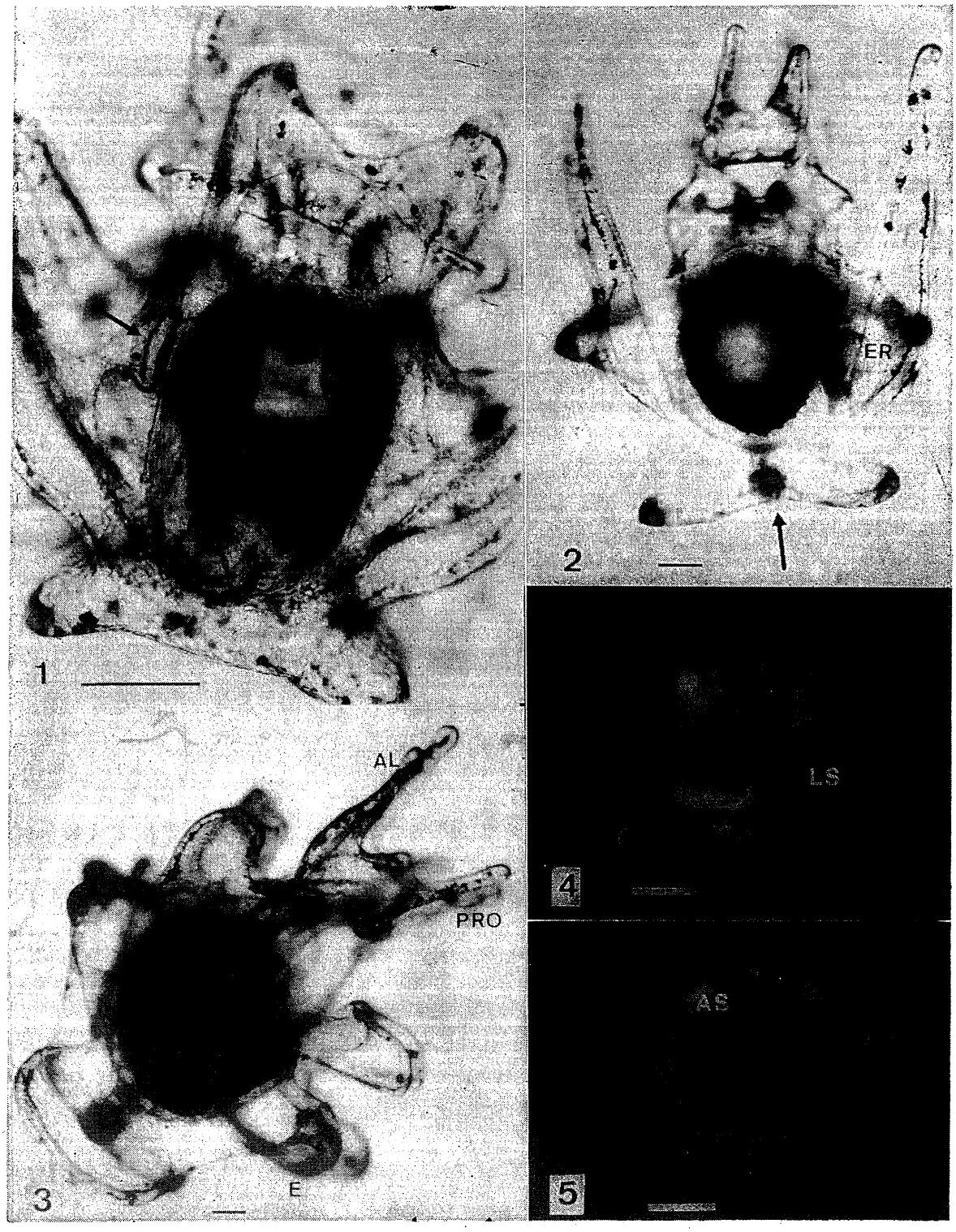

Fig. 6. Larval development of *Tripneustes gratilla*. (1) 23-day old pluteus, dorsal view, arrow: vestibule; (2) 27-day old pluteus, ventral view: pedicellaria, ER: echinus rudiment, ventral view; (3) 27-day old pluteus, E: epaulette, lateral, view; (4) 30-day old metamorphosing larva, T: tube feet, LS: larval spicule withdrawing; (5) 2-day old juvenile after metamorphosis, AS: adult spine. Bar indicates 100 μm.

DISCUSSION

By using the culture methods set up in this study, the larvae of *Tripneustes gratilla* can be reared through metamorphosis to juveniles in a large quantity. Thus, artificial seeding of the sea urchin is promising in near future.

The alga *Isochrysis* aff. *galbana* has been used as food successfully in rearing larvae of the polychaete *Serpula vermicularis*, the mussel *Mytilus californianus* and the oyster *Crassostrea gigas*, but not in the ophiuroid *Ophiopholis aculeata*, the sand dollar *Dendraster excentricus* (Paulay *et al.*, 1985), and the sea urchins *Arbacia punctulata* and *Lytechinus pictus* (Hinegardner, 1969). However, we successfully reared the larvae of *Tripneustes gratilla* and other two species of echinoderms (the sand dollar *Arachnoids placenta* and the asteroid *Archaster typicus*, unpublished data) with *I.* aff. *galbana* only. In addition, we proved that the algae containing the culture medium can be used directly.

Because agitation of larval cultures by air generally slows development and reduces the length of larval spines, sea-urchin larvae are usually raised in gently stirred seawater (Hinegardner, 1969; Leahy, 1986). However, while seawater being stirred, the larvae of *Tripneustes gratilla* are easily to tangle and interlock with other individuals due to its relative long spines compared to other species (McEdward, 1986). In fact, due to its large epauletts, its beating effect of cilia band, and the low viscosity of seawater in tropical regions, the larvae of *T. gratilla* float evenly within a stillwater column. The stillwater culture method is also proper in some other tropical species such as the sea urchin *Tripneustes ventricosus* (Scheibling, personal communication) and the sand dollar, *Arachnoids placenta* (unpublished data).

In addition, for supplying oxygen and removing metabolic wastes such as carbon dioxide and ammonia in the still water, planktonic algae are added to the culture even before the larvae being able to feed, and light was provided constantly for algal growing. However, because intensive fluoresent light (above 1000 lux) is lethal to the larval culture (personal observation), dim light about 500 lux is enough.

The larvae of *Tripneustes gratilla* in this study take 30 days to begin inductive metamorphosis and 47 days to begin spontaneous metamorphosis. However, the larvae reared by Mortensen (1937) in Red Sea conducted metamorphosis only at 18 days. This faster development may be due to food efficiency, i. e., natural mixed algae, or other environmental factors such as high salinity (40–44‰ S in the Red Sea) or even due to different subspecies (Dafni, 1983). Therefore, the factors and the effect of various growing rate are merit further study.

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馬糞海膽(*Tripneustes gratilla* (L.))幼生的育成及其胚胎發育

陳章波　阮靜觀

以 0.5 M 氯化鉀注射海膽，促使分別排出精與卵。人工授精後，受精卵以 25°C，35‰ S 的過濾海水飼養，不打氣，24 小時均照有微光（約500 lux）。海膽胚胎口形成之後，以每毫升 4 隻胚胎的密度，養於 500 ml 的燒杯中，飼以藻類，每 4～5 天換水一次。在試驗的三種藻裏，以 *Isochrysis* aff. *galbana* 爲最適宜，此藻的餵食濃度以 10^4～10^5 cells/ml 爲宜。在此濃度，藻液中的培養基對胚胎的成長沒有影響。胚胎的發育過程爲：受精 2 小時後四分裂，4 小時後八分裂，9 小時後達囊胚期，22 小時達原腸期，30 小時後形成三角錐體，33 小時後具有色素，並於二天後發育成長腕蛹(pluteus)，可以攝食。受精五天後有 4 個腕，15 天後 6 腕，18 天後 8 腕，27 天後左側體內有成體原基 (adult rudiment) 出現，並逐漸長出管足和鋏棘 (pedicellaria)，到了 30 天後可經誘引變態。但晚期幼生也可在燒杯中自然變態。