



## Note

### Induced spawning and larval rearing of sea urchin *Echinometra mathaei* (de Blainville, 1825)

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#### Abstract

Induced spawning of the sea urchin *Echinometra mathaei* was accomplished by injecting 0.5 M KCl. The larval developmental stages were studied in the laboratory for 22 days by which time the larvae reached competency. The survival of larvae was  $79.0 \pm 2.08\%$  when fed with microalgae *Isochrysis galbana* in combination with *Chaetoceros calcitrans*. The survival was  $77.0 \pm 1.73\%$  when fed with *I. galbana* diet. Comparatively low survival rate of  $60.0 \pm 2.52\%$  and  $40.0 \pm 2.0\%$  were observed while feeding with *C. calcitrans* and *Nannochloropsis* sp. respectively at the competent stage.

The sea urchin aquaculture is mainly based on the production of marketable gonads, which are valuable seafood product in Asian and European markets (Buitrago *et al.*, 2005). The sea urchin gonads contain the dominant carotenoid echinenone, which the urchins synthesise from  $\beta$ -carotene. The intense gonad colour depends upon the level of echinenone, which in turn influences the market value (George *et al.*, 2001; Shpigel *et al.*, 2005). The global catch of sea urchins decreased due to increased fishing effort and great demand. This evinced keen interest in culturing them (FAO, 2000; Kelly *et al.*, 2000). Research is focused to improve the desirable gonad colour (bright yellow orange) through combination of algal feed and prepared diets. Shpigel *et al.* (2005) reported that improved gonad colour and quality were achieved in the European sea urchin, *Paracentrotus lividus* fed with a mixed species of macroalgal feed and a formulated diet. The quality of the gametes and feeding during the larval period are important in determining the survival rate and it turn out to be critical for obtaining large numbers of competent urchin larvae (Carcamo *et al.*, 2005). *Echinometra mathaei* is a common in-

habitant of shallow sea burrows, which are affected by strong wave action (Rahman and Uehara, 2001; Rahman *et al.*, 2005). The feeding behaviour, ecology and use of growth lines for the ageing of sea urchins especially *E. mathaei* were well studied by Ebert (1982, 1988). This paper reports the larval development of *E. mathaei* up to 22 days in confined conditions during 2005. The metamorphosis of larvae was achieved in 20 to 22 days after fertilization.

#### Materials and Methods

**Sample collection and maintenance:** The sea urchin *Echinometra mathaei* was collected from the rocky beaches of Vizhinjam, southwest coast of India. Specimens were collected by diving as well as hand picking from rocks at depths ranging from 2.5 to 3.0 m. The urchins were stocked in plastic containers in seawater. The specimens were immediately transported to laboratory and stocked in 500 l FRP tanks provided with sea water and live rocks collected from the natural habitat. As suggested by Buitrago *et al.* (2005) and Shpigel *et al.* (2005), the sea urchins were fed *ad libitum* with the green macroalga, *Ulva fasciata*. After 3 to 4

days of acclimatization, the urchins were selected for induced spawning according to their weight.

**Induced spawning, fertilization and larval development:** Adult sea urchins (weight: 40 to 85 g) were selected for induced spawning. After washing in 0.5% of Ampicillin (Himedia) for 10 minutes, the animals were injected using 1.5 to 2 ml of 0.5 M Potassium Chloride (KCl, pH 6.1) on their oral (mouth) side using 2 ml syringe (Dispo van U-40) and gently shaken (Fig.1A). The injected urchins were immersed in 1 l glass beaker filled with disinfected seawater. The disinfected seawater was prepared by treating the water with 10 ppm Hypochlorite, then de-chlorinated and filtered through 0.2  $\mu$ m cartridge filter. The urchins started to shed their gametes after 40 to 60 seconds of injection. Sperms were released from dorsal side as creamy white mass and eggs were spilled through the aboral side as orange colour solution. After the completion of the shedding of gametes, the urchins were removed from the beakers. The beakers were gently rotated in clockwise and anti-clockwise direction for uniform mixing.

The sperms and eggs were collected and transferred to a 5 l glass beaker containing disinfected seawater for fertilization at room temperature (28.0 to 32.0 $^{\circ}$  C). Mild aeration was provided and the beaker was partially covered with black cloth to reduce the light penetration. Free-swimming blastula stages obtained after 18 hours of fertilization were transferred to 50 l glass aquarium tank filled with disinfected seawater at a stocking density of 5 larvae/ml.

One day after fertilization, 4 triplicate sets with 100 numbers of 'prism stage' larvae stocked in each set in separate plastic troughs of 15 l capacity were monitored. From the second day of post-fertilization when the larvae were at '2-arm echinopluteus' stage, micro algal feeds such as *Isochrysis galbana*, *Chaetoceros calcitrans*, *Nannochloropsis* sp. and a mixture of *I. galbana* and *C. calcitrans* (1:1 ratio) were provided to the larvae @ 50,000 cells/ml/ 2 days. Water exchange was carried out at the rate of 50% in two days using suitable filter and the tanks were covered using black cloth. The antibiotic Ampicillin was applied to the larval rearing tank @ 2 mg/l on

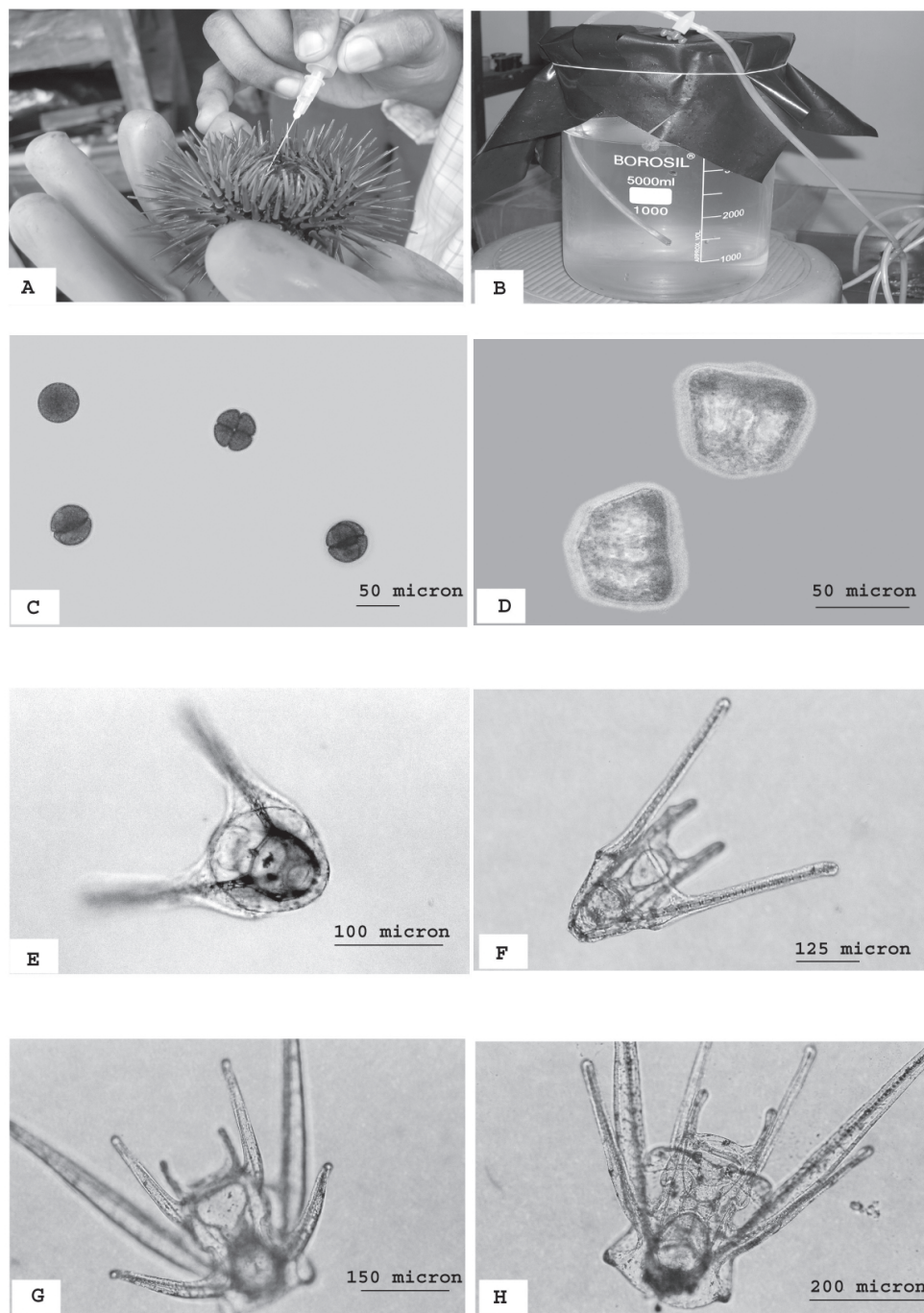
alternate days. When the larvae reached '4-arm stage', the stocking density was reduced to 2 larvae/ml as suggested by Buitrago *et al.* (2005).

## Results

The larvae were in free-swimming prism stage from 18 to 20 hours of post-fertilization. The following larval stages viz., 2-arm, 4-arm, 6-arm and 8-arm stages were observed on the 2<sup>nd</sup>, 3<sup>rd</sup>, 15<sup>th</sup> and 20<sup>th</sup> day of post-fertilization. The larval size and observations of development are depicted in Fig.1 A to H. From the second day after fertilization, the larval growth and survival were almost similar in the four sets. All the larvae reached competency in 20 to 22 days when they were in 8-arm stage. Competent larvae were identified by their tendency to stay near the bottom of the beaker. There was no significant difference in the larval survival rate among the different feeds used during the initial days. Higher percentage (79.0  $\pm$  2.08%) of survival was observed in the combined algal feed of *Isochrysis galbana* and *Chaetoceros calcitrans* (1:1 ratio). This was followed by the *I. galbana* (77.0  $\pm$  1.73%) diet, *C. calcitrans* (60.0  $\pm$  2.52%) and *Nannochloropsis* sp. (40.0  $\pm$  2%) diets.

## Discussion

The sea urchins, apart from value of gonads, are important resources for biologists due to their large-scale availability of gametes. The spawning by injecting 0.5 M KCl stimulated their gonad wall to contract and as a result the ripe gametes emerged from the gonophores surrounding the anus on the aboral side of the sea urchin. The larvae attained competency after 22 days which is similar to the observations made by Rahman and Uehara (2001) on *E. mathaei*. The types of feed available influence the morphology, growth and duration of the larval development (Jimmy *et al.*, 2003). In this experiment, the larval feed concentration and its influence on the larval survival rate were assessed. Increased survival rate of 79.0  $\pm$  2.08% was observed in the combination diet of *Isochrysis galbana* and *Chaetoceros calcitrans*. Survival up to 82.3  $\pm$  9.0% was achieved in another species, *Loxechinus albus* by providing a combined diet of *C. calcitrans* and *I. galbana* (Carcamo *et al.*, 2005). Algal feed consisting *Dunaliella tertiolecta*,



A. Induced spawning using 0.5 M KCl B. Fertilization C. Fertilized egg and developing embryo. D. Prism stage E. 2-arm pluteus F. 4-arm pluteus G. 6-arm pluteus H. 8-arm pluteus

Fig. 1. Induced spawning, fertilization and larval development of sea urchin *Echinometra mathaei*

*Rhodomonas lens* and *C. muelleri* also induced early metamorphosis of the sea urchin *Lytichinus variegates* (Edward and Herrera, 1999; Buitrago *et al.*, 2005).

The stocking density of 1 larva/ml was found to improve the survival of *Lytichinus variegates*. Low mortality reduces the cost of production (Buitrago *et al.*, 2005). Successful production of a hybrid variety of Genus *Echinometra* was also achieved in the early settlement of larvae so as to make the sea urchin Genus *Echinometra* aquaculture as a viable one (Rahman and Uehara, 2001; Rahman *et al.*, 2005). The results of high survival and faster competency of larvae suggest scope for sea urchin aquaculture in India.

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