Embryonic, larval and juvenile development of tropical sea urchin, *Diadema setosum*

Aminur Rahman M.[1*]; Yusoff F.M.[1,2]; Arshad A.[1,2]

Abstract

Diadema setosum (Leske, 1778), is one of the common echinoids widely distributed in the Indo-West Pacific Ocean, where it occurs from the Red Sea, Persian Gulf and the east coast of Africa to Japan, Australia and Malaysia. To investigate the developmental basis of morphological changes in embryos and larvae, we documented the ontogeny of *D. setosum* in a controlled laboratory condition at the Institute of Bioscience, Universiti Putra Malaysia, during July–September, 2012. Matured gametes were obtained from the adult individuals and the eggs fertilized at limited sperm concentration (10^{-5} dilution). The obtained embryos were then reared at 24-25ºC. First cleavage (2-cell), 4-cell, 8-cell, 16-cell, 32-cell and multi-cell (Morulla) stages were attained at 01.20, 02.14, 02.44, 03.09, 03.32 and 03.54 h after fertilization, respectively. Blastulae with a mean length of 111.47±1.88 µm (mean±SD) hatched 08.45 h after sperm entry. Gastrula formed 16.36 h post-fertilization and the archenteron extended constantly, while the ectodermal red-pigmented cells migrated synchronously to the apical plate. The pluteus larva started to feed unicellular algae (*Chaetoceros calcitrans*) in 2 d, grew continuously, and finally attained metamorphic competence within 35 d after fertilization. Induction of metamorphosis took approximately 1 h 30 min from attachment on the substratum to the complete resorption of larval tissues and the development of complete juvenile structure with adult spines, extended tubefeet and well-developed pedicellaria, the whole event usually took place within 1 d post-settlement. The newly formed juvenile (473.16 ± 6.96 µm, *n*=30) with a complete adult structure then grew on coralline algae to 3-month old juvenile, which represents the "sea urchin seed" for stocking in grow-out culture. This study represents the first successful investigation on embryonic, larval and early juvenile development of *D. setosum*. The findings would greatly be helpful towards the development of breeding and seed production techniques for aquaculture of sea urchins.

Keywords: Sea urchin, *Diadema setosum*, Embryo, Larva, Juvenile, Development, Pulau Pangkor

1 - Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

2 - Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author's email: aminur1963@gmail.com

Introduction

D. setosum (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae), is one of the common echinoids widely distributed in the Indo-West Pacific Ocean, where it occurs from the Red Sea (Gulf of Suez, Gulf of Aqaba, Northern and Southern Red Sea), and the east coast of Africa to Japan and Australia (Lessios *et al*., 2001). It has distinctively long black spines and five white spots on its aboral side. The orange ring around its anal cone completes the special visual features of this species. It is an omnivorous scavenger and detritus feeder, ingesting loose substrate and scraping films off hard surfaces.

This cryptic species is commonly observed around reefs and shallow rocky habitats (1–6 m depth), where it hides in crevices and under overhangs by day, and forages at night, at a distance of a few meters away from its daytime hideout. *Diadema* spp. has a wide range of diet, which includes algae, coral polyps and encrusting animals (Grignard *et al*., 1996). In the Gulf of Suez, gametogenesis begins in April–May, when the water temperature rises above 25°C and spawning takes place between June and September (Pearse, 1970).
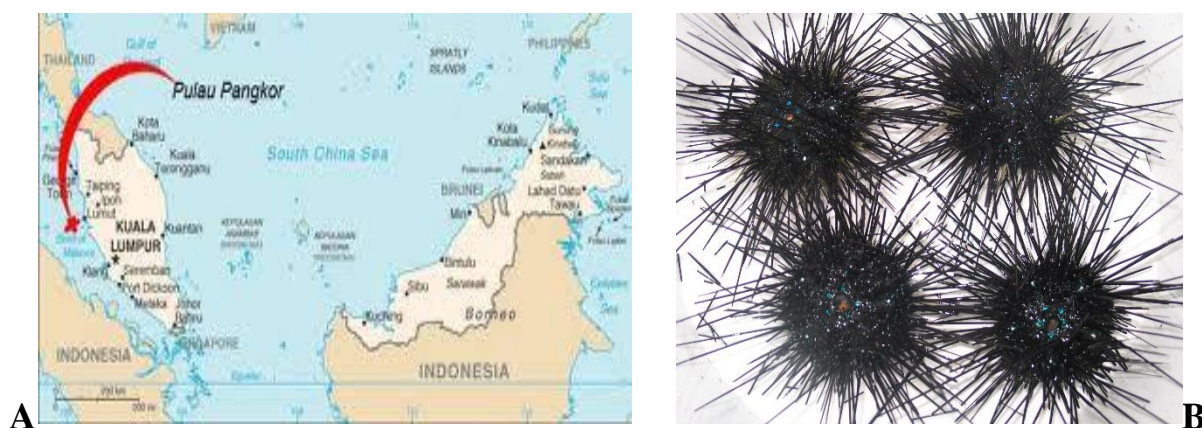
Sea urchins are important objects of research in different fields of biology, ecology, biodiversity and aquaculture. At the same time, they are used as raw material to produce foodstuff, in particular, the product of processing gonads known as "Sea urchin Roe or Uni" (Kaneniwa and Takagi, 1986; Oshima *et al*., 1986; Ichihiro, 1993). The roe is considered a prized delicacy in Asia, Mediterranean, and Western Hemisphere countries such as Barbados and Chile (Lawrence *et al*., 1997). People of the Asian Pacific Region have also used sea urchin gonads for many years as a remedy for improving general body condition, treatment for a number of diseases and strengthening of sexual potency of men (Yur'eva *et al*., 2003). Gonads of sea urchins have long been a luxury food in Japan (Shimabukuro, 1991). Although, *D. setosum* has not yet been used as a commercially edible species in Malaysia, it has been reported that in Sabah, an indigenous tribe known as "Bajau Laut" eats sea urchin roe with rice. The bodies of sea urchins are cleaned and the roes removed. The clean test is then filled with rice and roe and after adding spices; the concoctions are steamed and then served to guests and customers (Rahman *et al*., 2012a). Sea urchin gonads are also rich in valuable bioactive compounds, such as polyunsaturated fatty acids (PUFAs) and β-carotene (Dincer and Cakli, 2007). PUFAs, especially eicosapentaenoic acid [EPA, C20:5) (n-3)] and docosahexaenoic acid [DHA C22:6 (n-3)], have significant preventative effects on arrhythmia, cardiovascular diseases and cancer (Pulz and Gross, 2004). On the other hand, the high levels of arachidonic acid (AA) and EPA recently detected in *D. setosum* supported the development of aquaculture of this urchin (Chen *et al*., 2010), since PUFAs are important for human nutrition (Lawrence, 2007).

Due to the emerging importance of *D. setosum*, information of the early life history is an indispensable prerequisite for enhancing large scale seed production, culture and management. A few studies on its abundance, distribution and population

characteristics have recently been carried out (Grignard *et al.*, 1996; Lessios *et al.*, 2001; Kee, 2003; Rahman and Yusoff, 2010; Rahman *et al.*, 2012a), but no systematic studies have yet been conducted to optimize larval development, growth and survival. Therefore, an attempt was made to study the detailed embryonic, larval and juvenile development of *D. setosum* in a captive lab-rearing system.

Materials and Methods

Sea urchin collection and maintenance
In total, 30 matured of *D. setosum*, weighing from 80 to 150 g, were collected from the inter-tidal reef of Pulau Pangkor, Peninsular Malaysia (Fig. 1) during their natural breeding season in 16 July–18 September, 2012. The collected specimens were transported to the Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia and maintained in well aerated sea water tank before use for the experiment.

Figure 1: A. Location map of sampling site at Pulau Pangkor in Peninsular Malaysia and B. Matured adults of collected sea urchin, *Diadema setosum*.

Reproduction and fertilization
The Aristotle's lantern from the matured urchins was removed by using scissors and forceps and rinsed thoroughly with 2.0 µm filtered sea water (FSW). Gametes were obtained from each sea urchin by injecting 2.0 ml of 0.5 M KCl solution into the coelomic cavity. Eggs were collected by inverting female urchins over a glass beaker filled with FSW. "Dry" sperm were pipetted off the genital pores and kept in a refrigerator at 4–5°C for not more than 3 h before use. Fertilization was done by mixing two drops of a diluted sperm into a petri dish containing 15ml egg suspensions.

The sperm concentration was maintained at 10^{-5} dilution of "dry" sperm (Rahman *et al.*, 2000; 2005; 2012b; 2013). Sperms were then allowed to remain with the eggs for 10 minutes and then extra sperms were removed by 3–4 consecutive washes with FSW. Six replicates fertilization experiments were performed using fresh gametes from each new female and male individual in each time. The first 100 eggs encountered were classified as "fertilized" if they had reached the 2–4 cell stage 1.25-1.50 h post-insemination (Rahman *et al.*, 2001; 2005; 2012b).

Embryonic and larval development

Approximately, 2500-3000 fertilized eggs were transferred to each 500-ml glass beaker and incubated in FSW at ambient room temperature (24–25ºC) until they attained free swimming blastula stage at 9-10h post-fertilization, which was determined by continuous checking under a compound microscope. They were then transferred to glass bottles containing 500ml sterile filtered sea water (SFSW), which was stirred constantly by 10 rpm rotating motors. Larval densities up to the four-armed pluteus stage were maintained at 2–3 individuals/ml, following the methods described by Rahman *et al*. (2000, 2005, 2012b). When the larvae attained feeding stage (four-armed pluteus), they were cultured in the same system (500 or 1000 ml glass bottles) with a larval density of 1 individual/ml. About 90% of the culture water was removed by filtration/siphoning every 4–5 days and replaced with fresh FSW. Larvae were supplemented with a laboratory cultured phytoplankton, *C. calcitrans* at concentrations of 6,000-8,000 cells per ml (Rahman *et al*., 2000). All the developmental stages of embryos and larva were observed at regular time intervals after insemination until they reached metamorphic competence. At each stage, 30 specimens were fixed in 10% formalin for more details. Observations on both living and fixed specimens, provided information on the times required for embryos to attain specific developmental stages. In each experiment, the times after insemination for 50% of the embryos to develop to 2-cell, 4-cell, 8-cell, blastula, gastrula, prism, 2-arm, early 4-arm, late 4-arm, POA-elongated pluteus and competent stages were estimated, following Fujisawa (1993) and Rahman *et al*. (2002, 2012b)

Induction of metamorphosis and rearing of juveniles

When the developed larvae attained metamorphic, competent were used for settlement induction. Competence was indicated by the presence of large juvenile rudiments and a high rate of metamorphosis (>80%). Induction of metamorphosis was performed on coralline algal extracts+ *Chaetoceros* diatom (50:50) in the petri dishes (9.0×3.0cm) containing FSW. Larval density at this stage was maintained at 1 individual/2ml FSW following the method of Rahman and Uehara (2001) and Rahman *et al*. (2012b). In each experiment, petri dishes were used as replicates and percent metamorphosis was estimated within 24-32 h post-settlement in the same environmental conditions as larval cultures. The newly formed juveniles were then reared on encrusting coralline red algal (*Amphiroa fragilissima*) substratum in small aerated glass aquaria (25×20 ×10 cm) until 3 months by which they attained stocking-sized seeds for culturing in grow-out system.

Morphometric measurements

All morphometric measurements of embryo, larvae and juveniles were made on freshly prepared specimens, following McEdward (1984) and Rahman *et al*. (2004, 2012b) with slight modifications. Larvae were first killed in 5% formalin in FSW and were concentrated by settling to the bottom of a vial. A few drops of formalin-seawater containing 10-12 larva

were put under an elevated coverslip on a microscope slide. After that, it was observed and finally measured and photographed under the compound microscope (Zeiss Axioskop 2) fitted with a software (Spot Advanced Verson 3.4). Each sample was observed four times to identify the developmental stages (Rahman *et al*., 2012b).
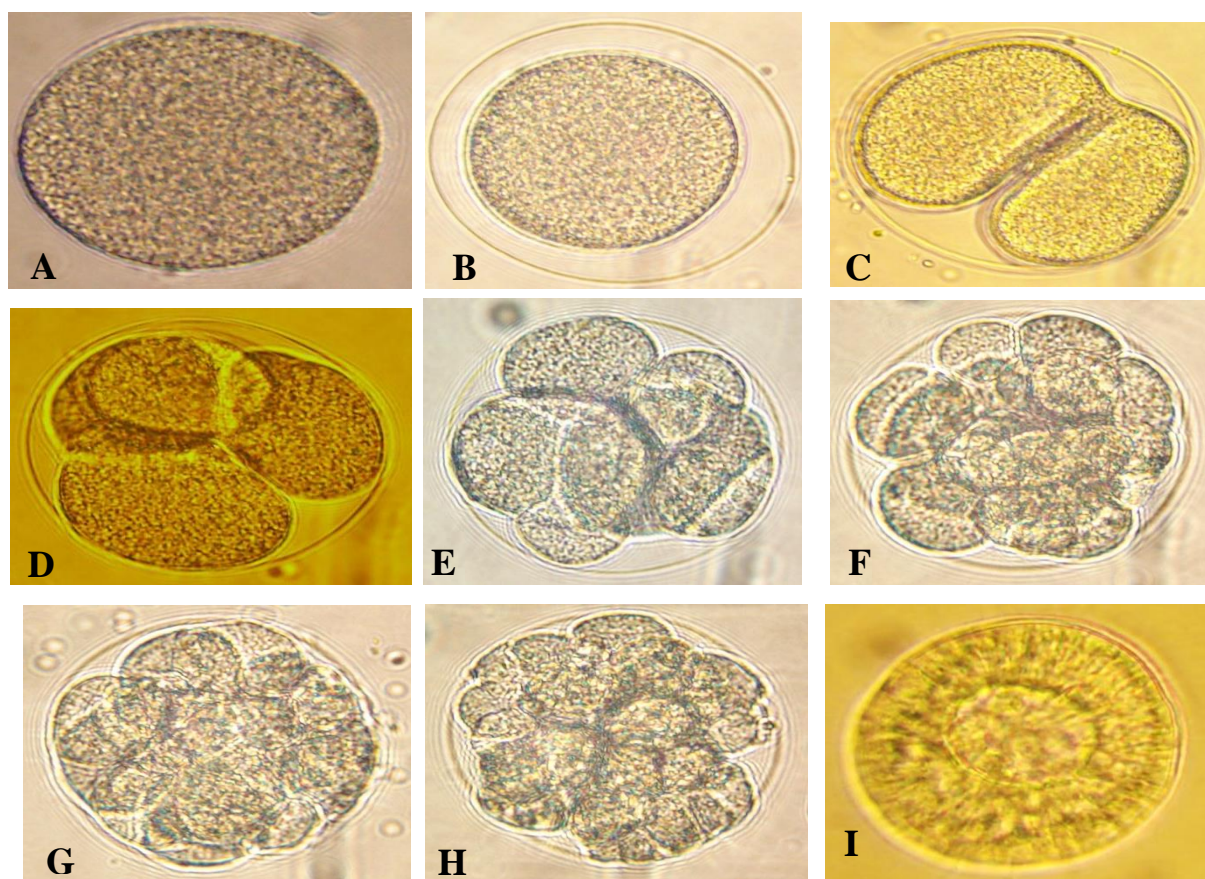
Results

Embryonic development

The detailed morphological events occur during the embryonic development of *D. setosum* are presented in Table 1, while the developmental stages are shown in Fig. 2. The mean diameter of the unfertilized eggs of *D. setosum* was 82.20-88.63 μm (85.75 ± 3.38 μm, *n*=30). The fully developed eggs are transparent, spherical in shape, non-adhesive and yellowish in color. The vitelline membrane of the egg was raised after 40-50 sec of sperm entry and the fertilization membrane began to form (Fig. 2A). On the other hand, the complete formation of fertilization envelope took place within 5 min of insemination (Table 1, Fig. 2B). Soon after sperm entry, the male pro-nucleus was moved forward by microtubules towards the centre of the egg and the female pro-nucleus was rapidly pulled towards the male pro-nucleus when touched by microtubules. However, this sperm-egg fusion took place within 10-12 min after sperm entry. In the course of the fertilization events, the cytoplasmic

movements increased and the cell surface attained an irregular feature. Nearly before starting the first cleavage, the membrane ceased the vibration, the cell surface became regular, and the hialine layer thickened.

First cell division was holoblastic (Fig. 2C) and occurred 01.20 h after fertilization (Table 1). Second cleavage started 2.14 h post-fertilization (Table 1) and was meridional, dividing the embryo into 4 equal blastomeres (Fig. 2D). The third cleavage occurred at 2.44 h and was equatorial, separating animal and vegetal blastomeres with 8 cells (Table 1; Fig. 2E). In the course of the 4th division, micromeres originated equally from vegetal blastomeres, while 8 mesomeres were formed by a meridional cleavage of animal blastomeres (Fig. 2F) at 3.09 h post-fertilization (Table 1). Equatorial division of mesomeres, meridional division of macromeres, and unequal micromere division formed embryos with 32 cells at 3.32 h after fertilization (Table 1; Fig. 2G). After 3.54 h succeeding fertilization, the seventh cleavage occurred without micromere division and the embryos formed Merulla with 108 cells (Table 1; Fig. 2H). In blastula stage, cells acquired a polygonal shape during the consolidation of the epithelium. Immediately before hatching, the vegetal plate thickened and cilia were formed on the perimeter within 9.15 h post-fertilization, (Table 1; Fig. 2I).

Table 1: Embryonic developmental events of *Diadema setosum*. Three replicates fertilization experiments were conducted and for each developmental stage, 10 embryos from each replicate were used for the observation and measurement of embryos.

Time after insemination	Developmental stages	Diameter (μm)
00.01 h	Fertilized eggs with the formation of fertilization membrane	88.82 ± 2.93
00.05 h	Fertilized eggs with complete fertilization membrane	93.30 ± 3.88
01.20 h	2-cell stage	99.13 ± 7.17
02.14 h	4-cell stage	103.47 ± 1.76
02.44 h	8-cell stage	105.96 ± 1.71
03.09 h	16-cell stage	107.60 ± 1.52
03.32 h	32-cell stage	109.05 ± 1.63
03.54 h	Multi-cell (Morulla) stage	110.50 ± 2.24
09.15 h	Hatching blastula	111.47 ± 1.88

Figure 2: Embryonic developmental stages of *Diadema setosum* under a compound microscope. A. Fertilized egg showing fertilization membrane, B. Fertilized egg with complete fertilization membrane, C. 2-cell stage, D. 4-cell stage, E. 8-cell stage, F.16-cell stage, G. 32-cell stage, H. Morulla stage enclosed with fertilization membrane, I. Blastula. See Table 1 for the measurements of embryonic stages.

Larval development

The morphological events taking place during the larval development of *D. setosum* are summarized in Table 2, while the developmental stages are depicted in Fig. 3. The ciliated gastrula formed 16.36 h post-fertilization (Table 2). At the commencement of this stage, larva

experienced with primary mesenchyme cells (PMC) which were detached from the vegetal pole, became spherical, and aggregated in a unipolar manner on the vegetal pole. PMC then migrated through the blastocoel forming a ring connected by thin pseudopodia on the posterior end. During this stage, red-pigmented cells were first observed on the vegetal pole and then migrated through the epithelium, simultaneously with PMC, towards the apical plate. Secondary mesenchyme cells (SMC) originated on the vegetal pole, extending cytoplasm projections towards the blastocoel during archenteron invagination. SMC on the archenteron then reached the anterior pole while red-pigmented epithelial cells reached the anterior pole, when the blastocoel was occupied by SMC (Fig. 3A). The prism stage started in 22.53 h after fertilization (Table 2). Epithelial red-pigmented cells were not present on the ventral (oral) region of the embryo at prism stage. In the course of the complete development of prism larva, the surface of the embryo was covered by cilia with an apical tuft on the anterior pole and a ciliated ring around the anus (Fig. 3B).

The 2-arm pluteus stage was developed 34.35 h post-fertilization (Table 2; Fig. 3C). In this stage, the mouth opened, but the larva were unable to feed, while microalgae captured by the larval arms were carried towards the mouth, but were deflected away possibly by an opposing current. Although, the gut had three portions identified as esophagus, stomach, and intestine but was not functionally active. During this event, muscles of the esophagus also began to contract; the stomach grew in diameter,

while its epithelium became thinner. The 4-arm pluteus larva was formed with two well-developed postoral arms 48.30 h following fertilization (Table 2; Fig. 3D). In this stage, pluteus larva experienced with complete digestive tract and was able to feed on unicellular algae. The distinct anus was formed in the lower half of the larva. The tips of larval arms and the arched oral lobe behind them represented the leading front of the swimming larva under the oral lobe which directed algae into the mouth. The mean length of larva at this stage was 242.88 ± 12.25 µm. The 4-arm pluteus larva was further grew and developed into late-4 arm pluteus (Fig. 3E), POA (postoral arms)-elongated stage-1 (Fig. 3F), POA-elongated stage-2 (Fig. 3G) by increasing the overall larval length of 580.11 ± 13.52, 1011.76 ± 15.44 and 1186.67 ± 18.39 µm in 10, 16 and 22 days after fertilization, respectively (Table 2).

The premature (precompetent) larval stage started to form 28.00 d after fertilization (Table 2). During this stage, the basal portion of the larva was enlarged and the pigmented arches appeared to form, and the pedicellaria was encircled with a ciliated ring (Fig. 3H). Increased differentiation of adult tissue accounted for the dense appearance of the interior portion of the larva. In developed (competent) larval stage, the rudiment developed tubefeet and spines, which became active still inside the larval body (Fig. 3H). No pedicellariae were formed on the surface of the larval body, as commonly observed in competent larvae of regular echinoids. A continued degeneration of larval tissue and arms accompanied by the emergence of the adult spines and tubefeet may be seen

slightly below the left corner of the larva. Competent larvae exhibited a typical substrate-test behavior which consisted of swimming close to the bottom. In this stage, well-formed spines and extended tubefeet were evident. Larval structures were discarded or absorbed at this point (Fig. 3I). Under the temperature of 24–25ºC, competent stage was reached at approximately 35 days post-fertilization (Table 2).

Table 2: Larval developmental events of *Diadema setosum*. Three replicates fertilization experiments were conducted and for each developmental stage, 10 larvae from each replicate were used for the observation and measurement of larvae.

Time after insemination	Developmental stages	Length (µm)
16.36 h	Gastrula	117.58 ± 1.79
22.53 h	Prism	122.64 ± 2.62
34.35 h	2-arm pluteus	207.07 ± 10.48
48.30 h	4-arm pluteus	242.88 ± 12.25
10.00 d	Late 4-arm pluteus	580.11 ± 13.52
16.00 d	POA-elongated stage-1	1011.76 ± 15.44
22.00 d	POA-elongated stage-2	1186.67 ± 18.39
28.00 d	Pre-competent larva	894.28 ± 14.82
35.00 d	Competent larva	752.26 ± 13.95
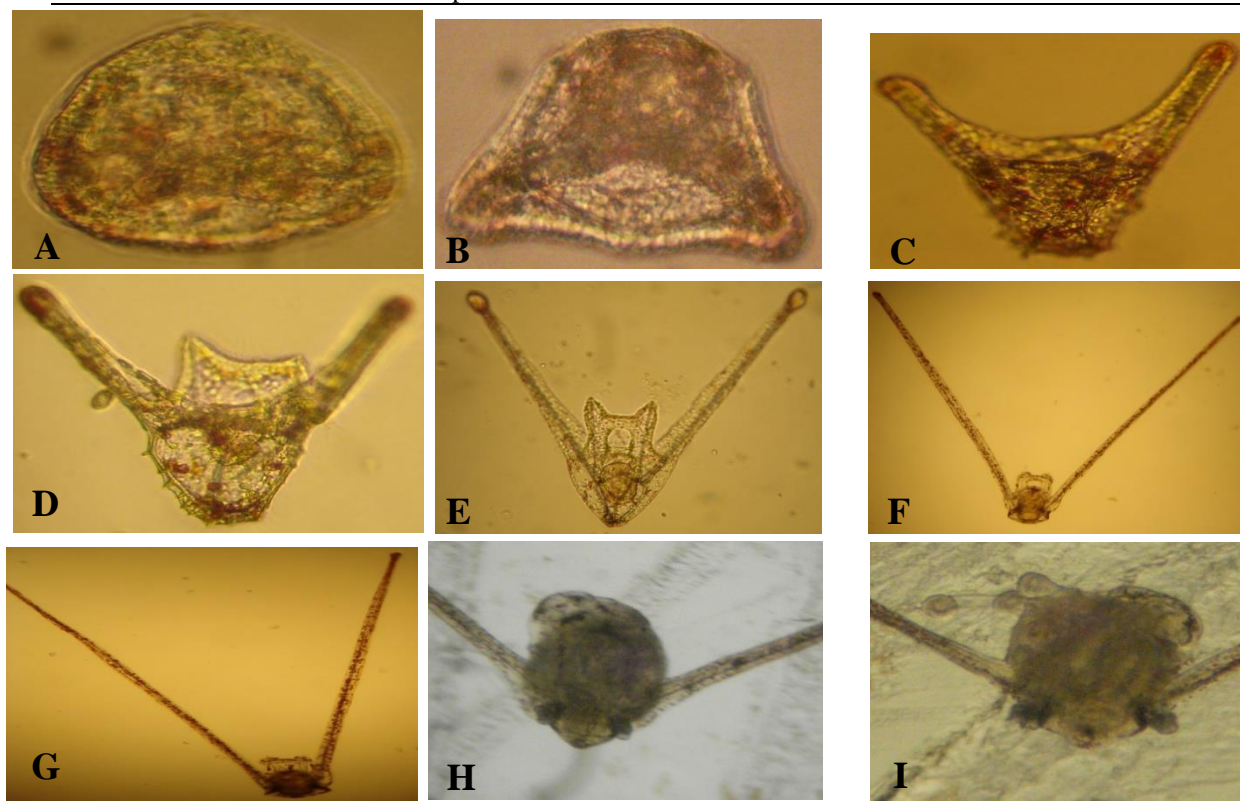
Figure 3: Larval developmental stages of *Diadema setosum* under a compound microscope. A. Gastrula, B. Prism, C. 2-arm pluteus, D. 4-arm pluteus, E. Late-4 arm pluteus, F. POA-elongated stage-1, G. POA-elongated stage-2, H. Pre-competent larva with growing rudiment, I. Competent larva with complete rudiment growth. See Table 2 for the measurements of larval stages.
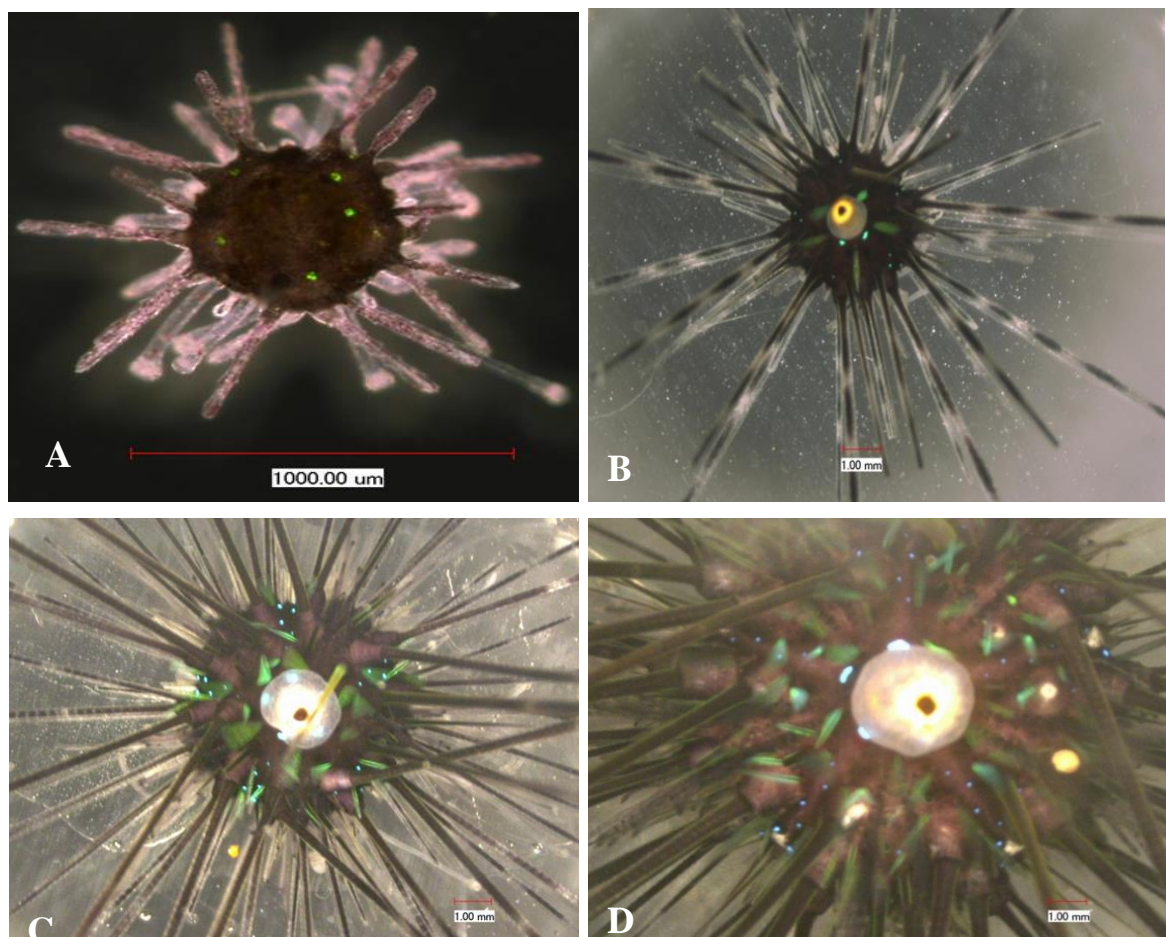
Metamorphosis and juvenile development

Metamorphosis occurred when larvae attached firmly to the bottom with the protruding tubefeet and the larval tissues began to regress and accumulate on the aboral surface of the rudiment. During this process, larval spicules became exposed and broke off and the larval tissues accumulated on the aboral surface of the rudiment forming a globoid structure. Metamorphosis took approximately 1 h 30 min from attachment to the complete regression of the larval tissues.

Metamorphosis was followed by the resorption of larval tissues and the development of complete juvenile structure with adult spines, extended tubefeet and well-developed pedicellaria (Fig. 4A) and the whole event usually took place within 1 d post-settlement (Table 3). Early postlarval juveniles had no skeleton on the aboral surface, except for the remnants of larval rods. The gut was not yet formed neither mouth nor anus was present. During the resorption of larval tissues, the rudiments of Aristotle's lantern and teeth were visible in the oral region under polarized lights. The newly formed juvenile with a complete adult structure (mouth, gut, anus, spine, tubefeet etc) then grew on coralline algae to 1-month (Fig. 4B), 2-month (Fig. 4C) and 3-month old juvenile (Fig. 4D) by increasing the overall juvenile body, spine and tube foot lengths (Table 3). The 3-month old juvenile produced through the above developmental and growth stages represents the "sea urchin seed" (Fig. 4D) for stocking in grow-out culture.

Table 3: Juvenile developmental events of *Diadema setosum*. Three replicates fertilization experiments were conducted and for each developmental stage, 10 juveniles from each replicate were used for the observation and measurement of juveniles.

Developmental stages	Body length (mm)	Spine length (mm)	Tube foot length (mm)
Juvenile (1 day after metamorphosis)	0.47 ± 0.01	0.48 ± 0.02	0.51 ± 0.03
Juvenile (1 month after metamorphosis)	3.91 ± 0.24	14.29 ± 0.40	4.98 ± 0.32
Juvenile (2 month after metamorphosis)	6.22 ± 0.31	28.81 ± 2.54	6.15 ± 0.51
Juvenile (3 month after metamorphosis)	9.52 ± 0.51	42.88 ± 3.81	7.27 ± 0.62

Figure: 4. Juvenile growth and developmental stages of *D. setosum* under a compound microscope. A. Juvenile (1 day after metamorphosis), B. Juvenile (1 month after metamorphosis), C. Juvenile (2 months after metamorphosis), D. Juvenile (3 months after metamorphosis). See Table 3 for the measurements of juvenile stages.

Discussion

The cleavage and development of embryo and larva of *D. setosum* were more or less similar to those reported in other echinoids with planktotrophic larvae (Emlet, 1986; Strathmann, 1987; Pearse and Cameron, 1991; Wray, 1997; Thet et al., 2004; Vellutini and Migotto, 2010; Rahman et al., 2012b) except for some differences at later stages through which larva grow with only two very long and well-developed postoral arms (instead of the typical 6- and 8-arm stages), until attaining metamorphic competent (McEdward and Miner, 2007). The developmental timing of hatching blastulae took longer period (9.15 h at 24ºC) than those in *Lytechinus variegatus* (6 h at 23ºC; Strathmann, 1987), *Clypeaster subdepressus* (7 h at 26ºC; Vellutini and Migotto, 2010) and *Salmacis sphaeroides* (8.45 h at 24ºC). Developmental timing of later stages followed the same trends but slightly differed from those of Caribbean species of *L. variegatus* (Strathmann, 1987) at 23ºC and the Pacific species of *Colobocentrotus mertensii* (Thet *et al*., 2004) at 27ºC.

Gastrulation occurs with the correlation between the types of gastrulation and the pattern of migration of red-pigmented cells

in *D. setosum*, as that also reported in *S. sphaeroides* (Rahman *et al*., 2012b). Red-pigmented cells originate on the vegetal pole and migrate through the ectoderm to the apical plate while the archenteron elongation is continuous. The same phenomena were observed during the beginning of gastrulation in tropical sea urchins, *Echinometra mathaei* (Takata and Kominami, 2004), *S. sphaeroides* (Rahman *et al*., 2012b), and sea biscuit *Clypeaster subdepressus* (Vellutini and Migotto, 2010). Red-pigmented cells can have regulatory role and are recognized to trigger gastrulation in *E. mathaei* (Takata and Kominami, 2004). These cells might participate in the morphological changes during the formation of prism and early axis specification of pluteus larvae (Thet *et al*., 2004; Vellutini and Migotto, 2010). Moreover, the triradiate spicules, the first sign of larval skeleton, were formed during gastrulation in *D. setosum*, which were more or less similar to those witnessed in other regular echinoids (Thet *et al*., 2004; Vellutini and Migotto, 2010; Rahman *et al*., 2012b).

The competent larvae of *D. setosum* demonstrated substrate-test behavior similar to those documented in other echinoid species (Caldwell, 1972; Burke, 1980; Gosselin and Jangoux, 1998; Nunes and Jangoux, 2007; Vellutini and Migotto, 2010; Rahman *et al*., 2012b). While early juveniles resemble regular urchins with a spherical body, bilateral symmetry could be identified soon after the resorption of larval tissues and was probably determined during rudiment formation as those recently observed in sea biscuit (Vellutini and Migotto, 2010) and short-spined white

sea urchin (Rahman *et al*., 2012b). Larval arms in newly metamorphosed juvenile of *D. setosum* were completely absorbed together with the skeleton and epidermis (Fig. 3A), as similar to those reported in *S. sphaeroides* (Rahman *et al*., 2012b). Quite the reverse, in *Eucidaris thouarsi* (Emlet, 1988) and *Paracentrotus lividus* (Gosselin and Jangoux, 1998), tissue resorption is achieved by the retraction of only epidermis resulting in the naked skeleton. The naked skeletal rods will eventually be broken down. However, such type of discrepancy may be related to the species differences (Thet *et al*., 2004).

Subsequent to the induction of settlement and complete metamorphosis, *D. setosum* juveniles had 4 primary spines per interambulacrum (20 totals), similar to those documented in *P. lividus* (Gosselin and Jangoux, 1998), *Strongylocentrotus purpuratus* (Miller and Emlet, 1999) and *S. sphaeroides* (Rahman *et al*., 2012b). The irregular echinoid *E. cordatum* has a greater number of primary spines per interqambulacrum after metamorphosis and also differs from *D. setosum* by the presence of secondary spines and a subanal facsciole and 4 primary spines (Nunes and Jangoux, 2007). The newly metamorphosed juveniles of *D. setosum* had one tubefoot per ambulacrum as similar to that reported in *S. fanciscanus* and *S. purpuratus* (Miller and Emlet, 1999), *P. lividus* (Gosselin and Jangoux, 1998), *E. cordatum* (Nunes and Jangoux, 2007) and *S. sphaeroides* (Rahman *et al*., 2012b). On the contrary, *C. subdepressus* uniquely displayed 3 podias after metamorphosis (Vellutini and Migotto, 2010). The mature (competent) larvae of

D. setosum had pedicellariae during the late larval period and after metamorphosis as those documented in other regular urchins, *P. lividus* (Gosselin and Jangoux, 1998), *S. fanciscanus* (Miller and Emlet, 1999) and *S. sphaeroides* (Rahman *et al*., 2012b), whereas pedilellariae of *S. purpuratus* appear sometime after metamorphosis. In contrast, competent larvae of *E. cordatum* do not exhibit spines or pedicellariae (Nunes and Jangoux, 2007), while *C. subdepressus* has spines but devoid of any pedicellariae (Vellutini and Migotto, 2010).

The newly metamorphosed young juvenile of *D. setosum* has neither a mouth nor anus and no guts either. Similar phenomenon was also observed in other sea urchins (Hinegardner, 1969; Mazur and Miller, 1971; Thet *et al*., 2004; Rahman *et al*., 2012b) and sea biscuits (Emlet, 1986; Vellutini and Migotto, 2010). In this stage, the dorsal half is essentially a rounded lump of larval tissue punctured by the three pedicellaria. The dorsal organs appear to develop out of this tissue. For the first 2 days, the larval tissue can easily be picked off the urchin. The digestive system and probably other internal organs appear 4-5 days after settlement and then the urchin begins to feed and passes through subsequent juvenile stages, as those documented in *L. pictus* (Hinegardner, 1969), *P. lividus* (Gosselin and Jangoux, 1998), *Colobocentrotus mertensii* (Thet *et al*., 2004) and *S. sphaeroides* (Rahman et al., 2012b). On the contrary, the juveniles of *C. subdepressus* and *C. rosaceus* start feeding 7 and 10 days after metamorphosis, when the Aristotle's lantern and mouth become

functional (Emlet, 1986; Vellutini and Migotto, 2010).

This study exhibits the first successful investigation on the embryonic, larval and post-metamorphic juvenile development until 3-month-old seeds of *D. setosum* under a controlled laboratory condition. The findings emerged from the present study would greatly be useful towards the understanding of ontogeny and life-history strategies, which will ultimately help us in the development of breeding, larval rearing, seed production and aquaculture techniques of commercially important sea urchins in captive rearing conditions.

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