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LARVAL DEVELOPMENT OF BLACK SEA URCHIN, *DIADEMA SETOSUM* (LESKE, 1778)

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Abstract

Diadema setosum is one of the common marine forms of 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. The sea urchin aquaculture is mainly based on the production of marketable gonads, which are valuable seafood product in Asian and European markets. The investigation on the developmental basis of morphological changes in larvae of *D. setosum* was conducted in a controlled Wet laboratory at the College of Aquaculture and Fishery (CAF) of Can Tho University, Vietnam, during July to August, 2017. The fertilized eggs of sea urchin were obtained by the induced spawning. Induced spawning was done using 1.5 to 2 ml of 0.5 M Potassium Chloride (KCl,) on their oral (mouth) surface. The daily larval developmental stages were studied in the wet laboratory from 10 days after fertilization to 49 days after fertilization. During investigation, the larvae reached metamorphic competence within 43 days after fertilization. This study represented the successful investigation on larval and early juvenile development of *D. setosum*. The findings would be helpful towards the development of breeding and seed production techniques for aquaculture of sea urchins.

Keywords; sea urchin, *Diadema setosum,* induce spawning, larval development

Introduction

Sea urchins are members of the phylum Echinodermata, They are typically spiny, globular animals, echinoderms in the class Echinoidea. About 950 species inhabit all oceans and zones from the intertidal to 5,000 meters (16,000 ft) deep (Animal Diversity Web, 2012). Sea urchins can be found in all climates, from warm seas to polar oceans (Andreas, 2010*).*

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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 (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). In Myanmar *D. setosum* are called Than Pa Chup and they are found along the coastal region especially Bouldern Island at Myeik (Mergui) Archipalago, Adman Sea and Rakhine coastal region.

This species is nocturnal and 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. There is no external morphological difference between male and female sea urchins. The sex can be determined only after gamete shedding has begun or following biopsy of the gonads (Hinegardner 1967, 1975).

Several months are needed for the larva to complete its development; the change into the adult form beginning with the formation of test plates in a juvenile rudiment which develops on the left side of the larva, its axis being perpendicular to that of the larva. Soon, the larva sinks to the bottom and metamorphoses into a juvenile urchin in as little as one hour (*Ruppert, et al.*, 2004). In some species, adults reach their maximum size in about five years (*Barnes, 1982*) The purple urchin becomes sexually mature in two years and may live for twenty years (Alisa , 2001*).*

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).

Sea urchin is one kind of seafood that has high nutritional value and beneficial effects on health. Their 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)], they 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).

According to the importance of *D. setosumin* in the field of nutritional food and medicine, the information of the early life history is also important for enhancing large scale seed production, culture and management. Therefore, the present study was conducted to study the detailed larval and early juvenile development of *D. setosum* in a captive lab-rearing condition.

Materials and Methods

Study site

The present study was conducted in a controlled wet laboratory at the Collage of Aquaculture and Fishery (CAF) of Can Tho University, Vietnam.

Study Period

The recent study was lasted from July to December, 2017.

Specimen collection

The matured urchins *Diadema setosum* was collected from Kien Giang Province, Viet Nam during their natural breeding season in July. The collected specimens were transported to the Laboratory of CAF and then maintained in the recirculation system with well aerated sea water at salinity of 30 ppt.

Induced spawning

Induced spawning was done by injection of 2.0 ml of 0.5 M KCl (Potassium Chloride) into the coelomic cavity from the oral disc. About 10-15 min after injection, fertilized eggs were siphoned via scoop net (125 µm) then waste carefully 2-3 times news clean seawater. Then fertilized eggs were placed into the aerated larval rearing tank.

Larval rearing

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 with a larval density of 1 individual/ml. 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 larva were daily observed from the pluteus larval stage until they reached metamorphic competence.

The morphometric measurements of ten individual larvae were made on freshly prepared specimens. After that, it was observed and finally measured and photographed under the compound microscope.

(A) Preparation for induced spawning

(B) Algae culture for larval feed

(C) Larval rearing tanks

(D) Larval and juvenile rearing tanks

Plate 1. Wet laboratory, induced spawning and larval rearing tanks

Results

Systematic position of *Diadema setosum*

Kingdom: Animalia
Phylum: Echinodermata
Subphylum: Echinozoa
Class: Echinoidea
Subclass: Euechinoidea
Super order: Diadematacea
Order: Diadematoida
Genus: *Diadema*
Species: *setosum*

Diadema setosum (Adult)

Larval development

The present study was observed from late four arms pluteus larva at 10 day after fertilization (daf). Two long post oral arms and two short anterolateral arms were prominent at this stage. Mouth, oesophagus and stomach were prominent (Plate 3A). The anus was formed in the lower half of the aboral side. Post oral arms (POA) elongated larval stage was recorded from 12 daf to 14 daf (Plat 3B). In this stage red pigmentation occurs throughout the body especially more concentrated around the oral hood and at the tip of the arms. The rest of the body was transparent. Basal portion of the larval was nearly pointed. Arms muscle visible at the near of stomach, arms skeleton (fenestrated rod) prominent and the larval was moveable using with them. Body transverse rod was prominent at the aboral surface. At 14 daf the beginning of larval arms degeneration was occurred in recent study.

During the three weeks after fertilization (15–21daf), the larval development was continuous observed. Body transverse rod was more prominent and become rounded and more pigmentation. Basal portion of the larval was gradually blunt and enlarged to form the body rod. All of the arms were gradually degenerate. Adult rudiment was visible at the left side of the larval body. Ventrolateral process and body transverse rod were more prominent. The appearance of the larva was not changed, except for the arms degeneration until the 18 daf. At 19 daf arms become shorter and the tip becomes the club shape. The pigments were concentrated in dark at the tip of the arms. Larval skeleton was visible on both aboral and oral sides at third week after fertilization (Plate 3C).

During four weeks after fertilization (22-28 daf), the premature larval stage was recorded. The pigment spot more accumulated on the body during this stage. Stomach was become folded and contracted during the study period. Long arms tip were continuously degenerate together with the arm epidermis and pigmented sport was more concentrated in this area. Short arms were completely reduced as appear only the oral hood of mouth. Body rod was more prominent and the basal portion with the pigmented arches. Larval skeletons were appeared on the mouth and body. Larval spicules were visible inside the larval body. Body rod was prominent instead of basal disc. Larval skeleton was more prominent on the lower half of the aboral portion (Plate 3D).

The arms absorption was continuously occurred during five weeks after fertilization (29- 35 daf). Short arms become reduced together with larval mouth tissue. Larval tissue began to regress from the mouth and other parts of the body, forming a globoid structure. The pigments become concentrated around the oral hood and at the end of the arms. The rest of the larva body was transparent. Body transverse rod and body rod were more prominent and rounded. Fusion of these two portions was resembled nearly global shape (Plate 3E).

Competent larva was recorded within the sixth week after fertilization (36 -42 daf). Star shape muscles were prominent on the oral hood in this stage. Adult body rudiment growing and gradually accumulation of larval tissue on the rudiment was appeared. Arms become more and more short and body of larval size was small because of larval tissue resorption was formed in this stage. The arms degenerated muscles were accumulated at the base of the arms and muscle of arms were more prominent. Furthermore a pair of muscle fiber was occurred that attached to the posterior end of the stomach. Arms rods and epidermis were together receded toward the body. The larval arms and tissue degeneration was nearly completed during this week (Plate 4A). In this stage, pedicelleriaes were emerged and larval body was started to circle from the mouth as center point to form the adult globoid body and tissue more compact on the adult rudiment.

The arms were completely degenerated and the larva started metamorphosis to build up the juvenile form during seventh week after fertilization (43-49 daf). The accumulation of resorptive arms muscle was found as dark color portion on the each side of the larval body. Mouth opening with five radial discs and anus were more prominent (Plate 4B). In this stage the larval movement was more rapidly rotated around the body side to form the adult globoid and compact body. The larval size was too small about 5µm in diameter. Larval was appeared pentagonal shape and purple pedicellariae were emerged around the body in 44 days after fertilization. At the same time, larval movements were slow and start to settle at the bottom to do the metamorphosis into juvenile form. At the 45 days after fertilization, larval body shape was more rounded. The body surface of larval had a lot of prominent purple pedicelleriaes and translucent tube feet were occurred at the end of this stage (Plate 4C). Oral disc more prominent and body was formed the globoid shape like the adult form except the long spines. Larval was absolutely settle at the bottom and gradually do the metamorphosis into Juvenile stage at the end of this week (Plate 4D)

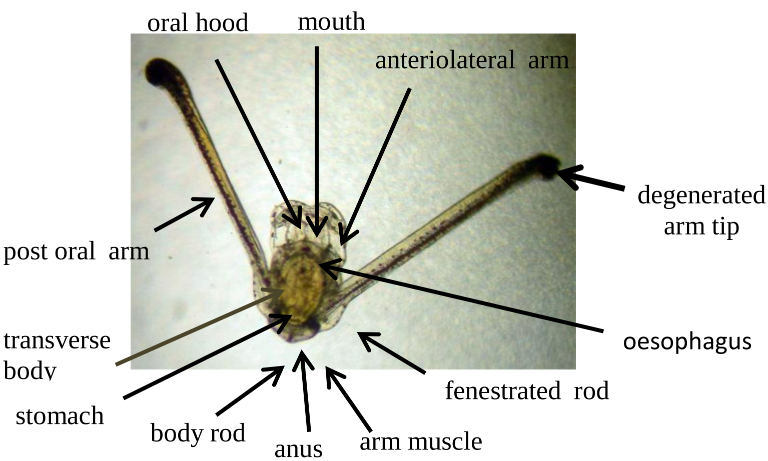
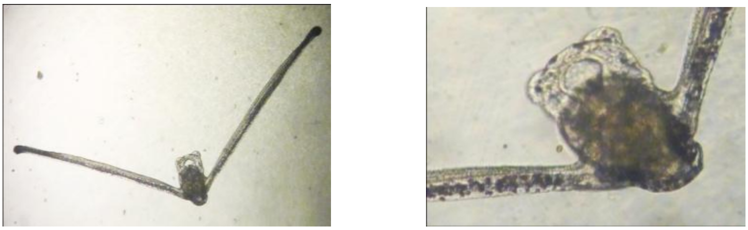
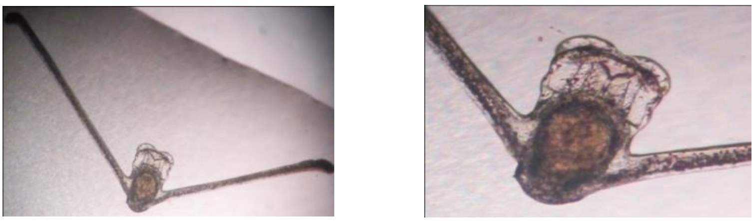


Plate 2. Distinctive characters of the larva (aboral view) (32daf)

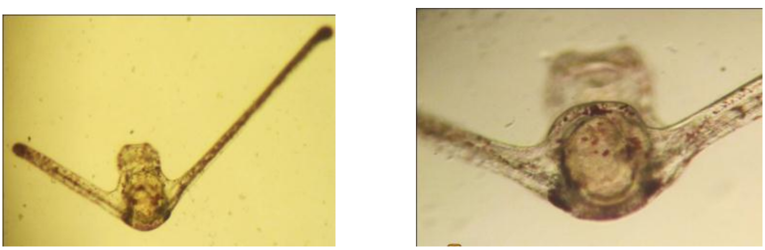
(A) Late four arms pluteus larva (40X) (B) Two weeks old larva (100X)



(40X) (C) Three weeks old larva (100X)

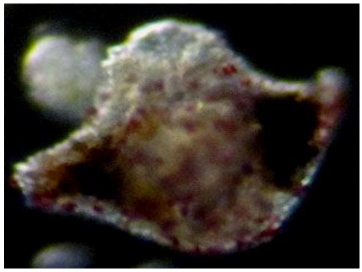


(40X) (D) Four weeks old larva (100X)

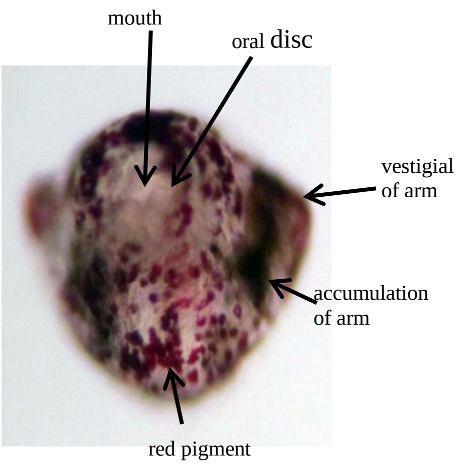


(40X) (E) Five weeks old larva (100X)

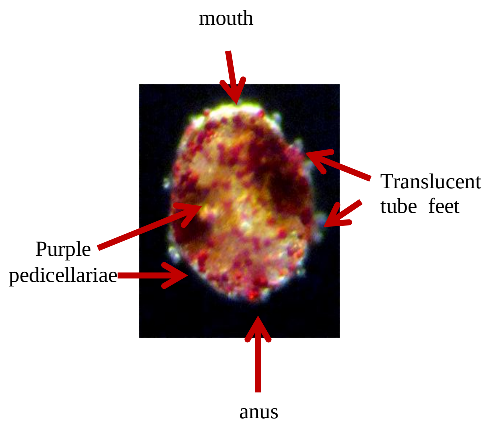
Plate 3. Weekly developmental stages of sea urchin (*Diadema setosum*) larval



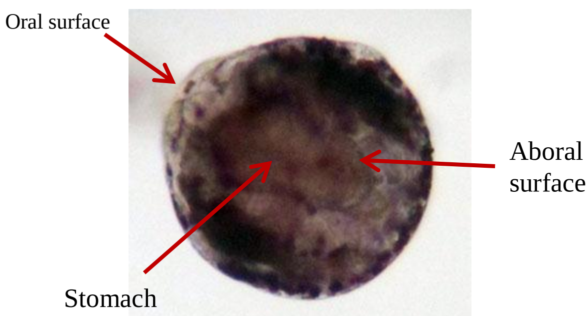
(100X) (A) Six weeks old (100X)



(B) 43days old metamorphosing larva (100x)



(C) 45days old metamorphosing larva (100x)



(D) 49 days old juvenile (100x)

Plate 4. Developmental stages of sea urchin larval to start of juvenile

Measurements of sea urchin larvae

In present study, the total lengths of larvae were highest in number (952.85 ±138.77µm) during three weeks after fertilization, follow by two weeks (904.28± 147.40 µm), four weeks(627.14 ± 169.38 µm) and five weeks (522.85 ± 147.84 µm) old larval respectively. After five weeks old larval stage, total lengths of larvae were quickly slowdown until the end of larval stage. Therefore the lowest numbers of larval length were recorded in six weeks (58.5 7 ± 12.14 µm) after fertilization.

The body lengths of larvae were also recorded in highest numbers during two weeks (101.42 ± 8.99 µm) after fertilization, followed by three weeks (98.57 ± 12.14 µm) , four weeks (94.28 ± 9.75 µm) and five weeks (91.42 ± 10.69 µm) old larvae respectively. The lowest numbers of larval body length were observed during seven weeks (5.85 ± 1.21 µm) after fertilization.

During the study period, the body widths of larvae were a little changed during the two (51.42 ± 6.90 µm) to three weeks (50 ± 5.77 µm) old larval stages. The larval widths were not changed until the end of larval stage (50 ± 0 µm).

Table 1. Measurements of sea urchin larvae during study period

Period (daf)	Larval stage	Total length (µm)	Body length (µm)	Body width (µm)
14 daf	POA elongated stage-1	904.28± 147.40	101.42 ± 8.99	51.42 ± 6.90
21 daf	POA elongated stage-2	952.85 ±138.77	98.57 ± 12.14	50 ± 5.77
28 daf	Pre-competent	627.14 ± 169.38	94.28 ± 9.75	50 ± 0
35 daf	competent	522.85 ± 147.84	91.42 ± 10.69	50 ± 0
39 daf	metamorphosis	258.57 ± 121.16	77.14 ± 4.87	50 ± 0
44 daf	Early juvenile	58.5 7 ± 12.14	58.57 ± 12.14	50 ± 0

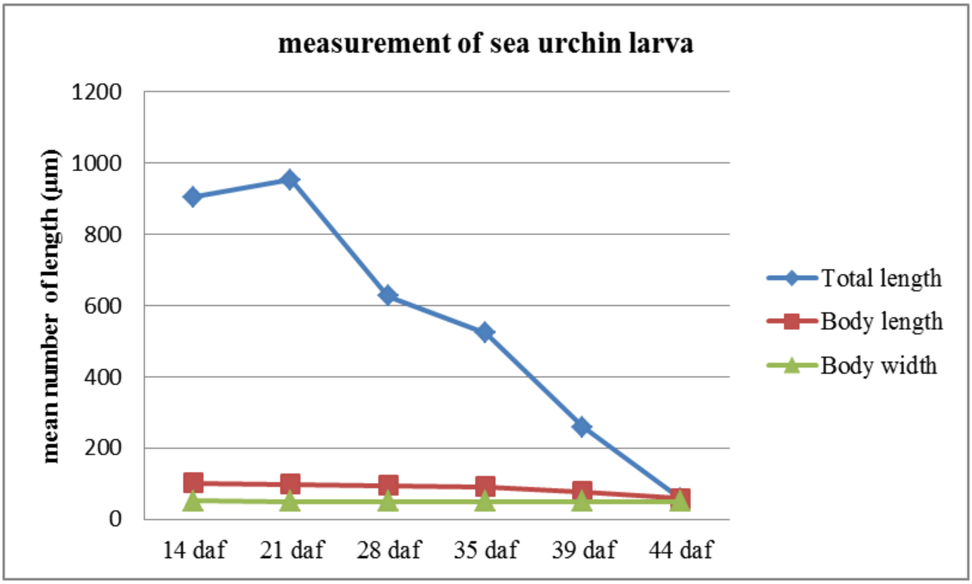


Figure 1. Measurements of sea urchin (*Diadema setosum*) larva during study period

Discussions

In recent study, *Diadema setosum* larval developments were achieved until they reach to the juvenile stage. Many morphological changes of larval were occurred during studying period. Within first two week old larva, postoral arms (POA) of larval were conspicuously elongated from the four arms pluteus to postoral arms (POA) elongated stage 1 (904.28 ± 147.40 µm) .

At three weeks old larva was (POA) elongated stage 2, the total lengths of larvae were highest in number (952.85 ±138.77 µm) in present study. But during first week old larva could not measure in the recent study because of this larval stage was too small and difficult to measure.

This record was more or less similar with the report of Rahman *et. al*, 2015, who also stated that the measurement of POA elongated stage 1 larva (1011.76 ± 15.44 µm) and POA elongated stage 2 larva (1186.67 ± 18.39 µm) was the highest number in their observation.

According to the Rahman *et. al*, 2015, they stated that the premature (precompetent) larval stage started to form 28 days after fertilization. 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. The measurement of this stage was 894.28 ± 14.82 µm.

This report supported to the recent study except the emerging of pedicelleriaes, during four weeks (28 daf) old larva stage, the premature larval stage like that the pigment spots accumulation in basal portion, stomach activation, arms epidermis degeneration, larval skeletons and larval spicules formation were recorded in their body. The mean number of total larval length was 627.14 ± 169.38 µm.

Mean length of larval were continuously reduced in both result of Rahman *et. al*, 2015 and present study because the larval arms were gradually degenerated until absolutely disappear at the end of larval stage. Therefore total lengths of larvae were slowdown to end of larval stage (258.57 ± 121.16 µm). The lowest mean numbers of larval length were recorded in early juvenile stage (58.5 7 ± 12.14 µm.)

Rahman *et al*., 2015, pointed out that 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), *Strongylocentrotus fanciscanus* (Miller and Emlet, 1999) and *S. sphaeroides* (Rahman *et al*., 2012b), whereas pedilellariae of *S. purpuratus* appear sometime after metamorphosis.

The present observation was more or less similar to those reported in competent larva within the sixth week (42 daf) old larva. Larval tissue resorption and accumulation on the rudiment, arms rods and epidermis completely degenerated, pedicelleriaes were emerged.

This finding was also agreed with the report of Rahman *et. al*,(2015), they stated that the larval arms in newly metamorphosed juvenile of *D. setosum* were completely absorbed together with the skeleton and epidermis.

But the duration of armless metamorphosed juvenile formation was different with their report (approximately 35 daf) and the present study (42 daf). The time variations of developmental stages were suggested that it may be different situation of fertilization and larval rearing methods. Their experiment was done by small skill using with the glass beaker and petridish in the laboratory, but the recent study was done by large skill using with the tank at room temperature in the wet laboratory.

Furthermore larval body circulation was occurred in the recent observation. It behavior was assumed that the global shape of their juvenile body formation and to resorption and compact their larval tissue on the adult rudiment together with their vestigial of arms were formed. No any reported concern with their circling behavior during metamorphosis stage.

Later, the larva was started to metamorphosis and settle at the bottom to build up the juvenile form during seventh week old larva. Like the juvenile forms, mouth with five radial discs pentagonal shape, purple pedicellariae and translucent tube feet were emerged around the globoid shape body.

The body lengths of larvae were also recorded in highest numbers during two weeks after fertilization (101.42 ± 8.99 µm). In this stage, larval body was look like the biggest size among the larval developmental period. During four weeks after fertilization, larvae body lengths were gradually reduced (91.42 ± 10.69 µm) because of the larval body tissue absorption was began together with arms epidermis. The lowest numbers of larval body length were observed in seven weeks after fertilization (58.57 ± 12.14 µm) because of the larval tissue absorption was completed.

During the study period, the mean body widths of larvae were a little changed during the two (51.42 ± 6.90 µm) to three weeks (50 ± 5.77 µm) old larval stages. After that larval width was no more changed until the end of larval stage and early juvenile stage (50 ± 0 µm). Their stability of larval width suggested that the adult rudiment was developed as the frame of their adult body shape like the globoid body.

The findings emerged from the present study would be useful towards the understanding of ontogeny and life-history strategies of sea urchin, which will ultimately help to seed production and aquaculture techniques of commercially important sea urchins in captive rearing conditions.

Summary

The larval development of *D. setosum* was observed from four arms pluteus to early juvenile stage during the recent study. The present study was achieved the understanding of ontogeny and part of life-history events of sea urchin. The competent larval circling behavior to form the compact and globoid juvenile body was recorded during metamorphosis stage. This recorded behavior was not reported in all of sea urchin research field.

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