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First report of internal brooding and larval development of Actiniarian sea anemone (*Anthopleura handi*) from India

Smitanjali Choudhury* and C. Raghunathan[1]
Regional Centre of Zoological Survey of India, Andaman and Nicobar Islands - 744 102, India.
[1]Zoological Survey of India, M-Block, New Alipore, Kolkata - 700 053.

*Correspondence e-mail: evsmita@gmail.com

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Original Article

Abstract

Anthopleura handi, a widely distributed, actiniarian sea anemone, was raised for the first time from India under laboratory conditions and observed three consecutive internal brooding in a year at room temperature. At first brooding, 36 individuals of planulae were expelled; while during the second and third broodings the number of planulae released were 22 and 45 respectively. The survival rate of planulae showed high (80%) at third brooding whereas a rapid decrease in percentage of survival occurred during first brooding (27.77%) due to the infection with protozoans and parasitic flatworms. The cultured planulae successfully maintained and monitored for 60 days.

Keywords: *Sea anemone, internal brooding, larval development, India*

Introduction

Sea anemones (Cnidaria: Anthozoa) are solitary hexacorals belonging to the order Actiniaria, are a group of invertebrates showing diverse variants of sexual and asexual reproduction (Chia, 1976). Many researchers have carried out prolonged research in a variety of reproductive strategies in *in situ* and *in vitro* in the temperate regions (Weis *et al.,* 2002; Davy and Turner, 2003; Isomura *et al.,* 2003; Chen *et al*., 2008). Little information is available on the sexual and asexual reproductive biology of sea anemones in tropical species (Dunn, 1982; Scott and Harrison, 2009).

In India, the sea anemone is not well studied with respect to reproduction and life cycles except that of asexual reproduction of a sea anemone, *Anthopleura nigrescens* (Verrill, 1928) and the post-larval development of *Phytocoetes gangeticus* (Mathew, 1979; Panikkar, 1937). *Anthopleura handi* Dunn, 1978 was reported for the first time from subtidal zone at a depth of 15m from Pongibalu, South Andaman (Raghunathan *et al*., 2014). In the present study, the sea anemone *A. handi* was reared in laboratory and documented the brooding and developmental stages of planula larvae. The present report on the larval development of *A. handi* was first of its kind in India, not only for this species but also for entire sea anemone group.

Material and methods

Collection site

Seventeen individuals of *A. handi* of different sizes were collected from Garacharma (Minnie Bay) South Andaman, Andaman and Nicobar Islands (Lat.11˚37.144'N; Long.92˚42.340'E) at a depth of 0-2m on 3 June 2014. Identification of the species was based on morphological traits (Dunn, 1978).

Maintenance of sea anemones in laboratory

In the laboratory, all the sea anemones were maintained in acclimatisation tank with 10 litres of UV treated filtered seawater. The salinity, dissolved oxygen (DO), pH and temperature of seawater were maintained at the level of 30-34 ppt, 6-7 mlO_2/L, 8.0-8.3 and 27-29°C respectively. In order to maintain biological rhythm, the specimens were kept indoors with a 12-h light: 12-h dark regime. The anemones were fed with freshly hatched brine shrimps (*Artemia* sp. Nauplii) in every morning hour. For the first month, 50% of seawater of top layer changed once or twice in a day; subsequently this was done every 2 to 3 days. After the period of 3 months' acclimatization, only two individuals of sea anemones were survived and were transferred into breeding tank (40 cm diameter and 30 cm height). The seawater parameters in breeding tank were maintained similar to the level of acclimatization tank. However, only two specimens of *A. handi* with body size group (8.1-10 mm) were survived in the breeding tank and kept as the brooders.

Morphological study of the experimental specimen

The pedal disc diameter of each specimen (brooder) was measured under Sterozoom microscope (Leica M205A) and their body weight was also measured for 330 days.

Analysis of larvae

The internal brooding period of the brooder was recorded in 330 days and the released larvae were collected and counted from each of the brooding time of the brooder. Within 20 min of release, each larva was placed on a petri plate and then digitally photographed (Leica M205A). Subsequently, for all larvae, the number of tentacles were recorded and the height of the body; the longest and shortest diameters of the pedal disc were measured under a stereo zoom microscope. The morphological events were monitored from early gastrula stage to juvenile transition upto late polyp development under a Stereo zoom microscope. The planulae were examined at one-day interval to determine their development. Photographs were used to measure the size of larvae (cross sectional area) with image processing software Leica M205A. The size of cylindrical planulae was measured on the basis of surface area (A)

$A = 2\pi rh + 2\pi r^2$
Where, A = Surface area
h = length of column (mm)
r = Pedal disc diameter + oral disc diameter / 4

The size of spherical planulae (gastrula stage) was measured on the basis of surface area (A)

$A = 4\pi r^2$
Where, A = Surface area
r = gastrula diameter / 2

Survival rate of planulae

The survival rates of planulae of each brooding time were determined using a modified method of Harrison (2006). After each brooding, all the planulae of each brooding were pooled into 2 groups on the basis of their sizes and were placed in different containers. The gastrula (small sized) larvae were kept in Petri plate and each containing 10 ml of seawater without aerator. The seawater is changed thrice in a day. After the period of one week, the gastrula sized larvae were transferred to glass containers and each containing 1000 ml sea water and was aerated to maintain dissolved oxygen levels; provide circulation, and ensured natural lighting and photoperiod. Similarly, the large sized planulae were kept in glass containers with aerator. The planulae were fed with freshly hatched brine shrimps (*Artemia* sp. Nauplii) in every morning hour. The number of planulae surviving in each container was counted at one-day interval to determine planulae survival rates. The water within each container was changed to maintain suitable water quality. Utmost care was taken to isolate the surviving planulae.

Results

Size of brooders

On the basis of body size (pedal disc diameter), all the brooders were categorised into 5 classes *viz.* 5.0 - 6.0 mm, 6.1-8.0 mm and 8.1-10.0 mm represented with 8, 4 and 3 individuals respectively, whereas 4.0-5.0 mm and >10.1 mm were having one individual each. However, after 3 months, only two brooders of *A. handi* with body size group (8.1-10 mm) were survived in the breeding tank. Out of which, only brooder I was survived for 330 days and brooder II was disintegrated after 120 days due to stress condition. It was observed that between 120 days to 180 days, the brooder size of brooder I was comparatively larger

than the rest of the days (Fig. 1). In the current observation, the brooder size was ranging in pedal diameter from 14.758 to 15.942 mm as well as body wet weight from 3.654 to 5.105 gm during the peak brooding time of 120 to180 days. At 180th day, the body weight as well as pedal disc diameter of brooder I was extremely high as compared to the rest of other days. The brooder size was grander at 180 days with the value of pedal disc dimeter 15.942 mm and body weight 5.105 gm whereas, at 150th day the brooder size was less with the value of pedal disc dimeter 14.758 mm and body weight 3.654 gm. At 120th day, brooder size was with the value of pedal disc dimeter 14.876 mm and body weight 3.989 gm.

Observation of brooding time

It was observed that only one brooder was survived for 330 days in the breeding tank and it displayed consecutively 3 internal brooding in a year in laboratory conditions. The first internal brooding was occurred at 120 days through which 36 number of brooded planulae were released. Further, the second internal brooding was occurred at 150 days and the number of brooded planulae were 22. Similarly, the third brooding was occurred at 180 days and the number of brooded planulae were 45 (Fig. 2). The brooder size was bigger during 180 days being brooded a greater number of individuals. Whereas, the brooder size was very small during 150 days being brooded a smaller number of planulae. Various types and sizes of planulae were expelled from the gastro vascular cavity of the brooder in each brooding time.

Types of planulae

The sizes and types of planulae or propagules expelled from the mouth of brooder were quite variable. Various types of the planulae *viz.* gastrula, pear shaped planula and planula with tentacle bud; planulae with different tentacle numbers such as 6 tentacles (6T), 7 tentacles (7T), 8 tentacles (8T), 10 tentacles (10T), 12 tentacles (12T) and 13 tentacles (13T) were released during the three different brooding times. There were 9 types of planulae during the third brooding; 7 types of planulae from first brooding time; and 5 types planulae from second brooding time by the same brooder. Further, the size of all planulae was measured on the basis of surface area. A maximum of 23 numbers gastrula stage of planulae with mean size of 0.93±0.4 mm^2 in the third brooding whereas, the second brooding was shown with a smaller number of gastrulae of 12 with mean size of 0.89±0.34 mm^2. The gastrula stage of planula was with 21 numbers having mean size of 0.91± 0.3 mm^2 in the first brooding. Two larger size of planulae with 13 tentacles were observed only in third brooding time with mean size of 3.64±0.08 mm^2. Various types and sizes of planulae were clearly depicted in Fig. 3 and Table 1.

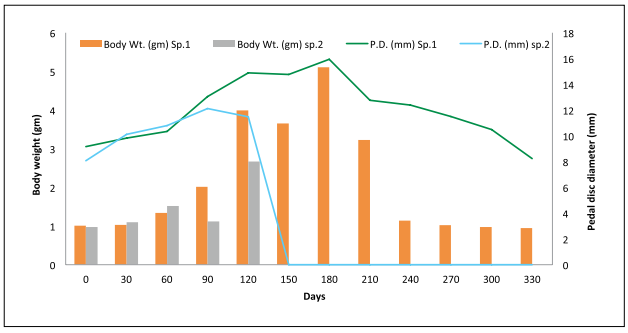

Fig. 1. Body weight and size of brooders during the study period.

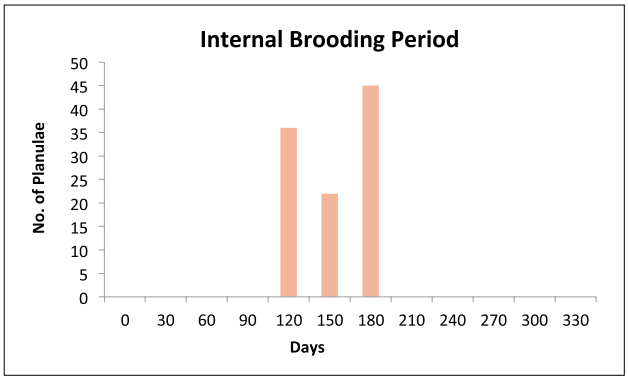

Fig. 2. Internal brooding period and Planulae released during the study period of the brooder

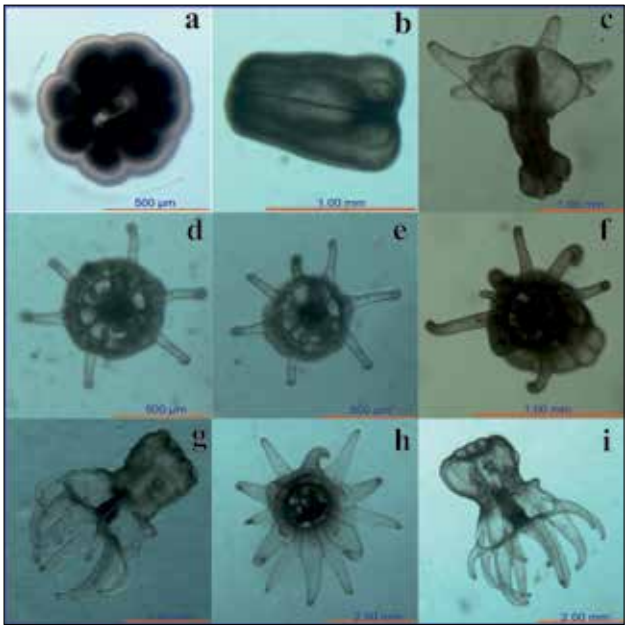

Fig. 3. Types of planulae released during brooding time

[a: Gastrula stage (0T); b: Pear shaped (0T); c: Planulae TB (0T); d: Planulae with six tentacles (6T); e: Planulae with seven tentacles (7T); f: Planulae with eight tentacles (8T); g: Planulae with ten tentacles (10 T); h: Planulae with twelve tentacles (12T); i: Planulae with thirteen tentacles (13T).]

Table 1. Mean size and types of planulae during the three brooding times

Types of Planulae	1st Brooding		2nd Brooding		3rd Brooding	
	No. of offspring	Mean size (SA) mm²	No. of offspring	Mean size (SA) mm²	No. of offspring	Mean size (SA) mm²
Gastrula (0T)	21	0.91± 0.3	12	0.89±0.34	23	0.93±0.4
Pear shaped (0T)	1	2.98	2	2.85±0.025	2	3.08±0.05
Planulae TB (0T)	1	3.29	0	0	1	3.32
Planulae (6T)	3	2.41± 0.32	4	2.51±0.24	4	2.60±0.25
Planulae (7T)	5	2.53± 0.2	3	2.86±0.06	4	2.87±0.06
Planulae (8T)	0	0	0	0	3	2.93±0.06
Planulae (10 T)	3	2.81± 0.07	0	0	5	3.00±0.11
Planulae (12T)	2	3.18± 0.03	1	3.42	1	3.48
Planulae (13T)	0	0	0	0	2	3.64±0.08

Planulation

After an immediate release from the brooder in each brooding time, the small or gastrula sized or pre metamorphic larvae were allowed to acclimatize for about 2h. The planula larvae were examined every hour for the first 6 h and then at 8, 12, 16, 18, and 24 h. Further, the morphological event of small sized larvae was examined in the laboratory. The specimens were monitored once in a day until the larvae reaches 60 days old. Herein, the morphological events were monitored from early gastrula stage to juvenile transition to polyp development. A summary of the development and experimental observations was given in Table 2 and Figs. 4- 8. The body sizes of planulae were found to increase consistently from day 1 to day 22 of transitional stage of planulae. A sudden decrease in size at 24th day due to stress condition was observed. Gradually an increase of in size was noticed along with number of days. At day 60, the size of planulae reached at its maximum of 60.97 ± 7.3 mm². At day 22, the planulae consisted 12 number of long tentacles with light band and also mesenteries with more coiled with size 39.69 ± 5.8 mm² which was ten times bigger than the size of (12T) planulae with small tentacles without bands released immediately from the brooder (3.18 -3.48 mm²).

Further, the scatter plot provided a clear picture of the relationship between numbers of tentacles and size of planulae with increasing days (Fig. 9). There is significant positive relationship between number of tentacles and size of planulae with increasing days. Pearson's correlation r (24) =0.84, the coefficient of determination, $R^2 = 0.70$, *df* =24 and the *p* value, 0.03 is less than the significance level of $\alpha = 0.05$. The linear regression equation was denoted by No. of tentacles = 0.4125* size + 2.883 (Fig. 9).

Survival rate

The survival rate of planulae was monitored in every 24 hours and it was observed that it suddenly dropped down to 47.2% at day 25 in first brooding whereas, the proportion of survival of planulae was remarkably high with 88.8% in third brooding and 86.4% in second brooding at day 25. During third brooding, the survival rate was more consistent. At day 60, the survival

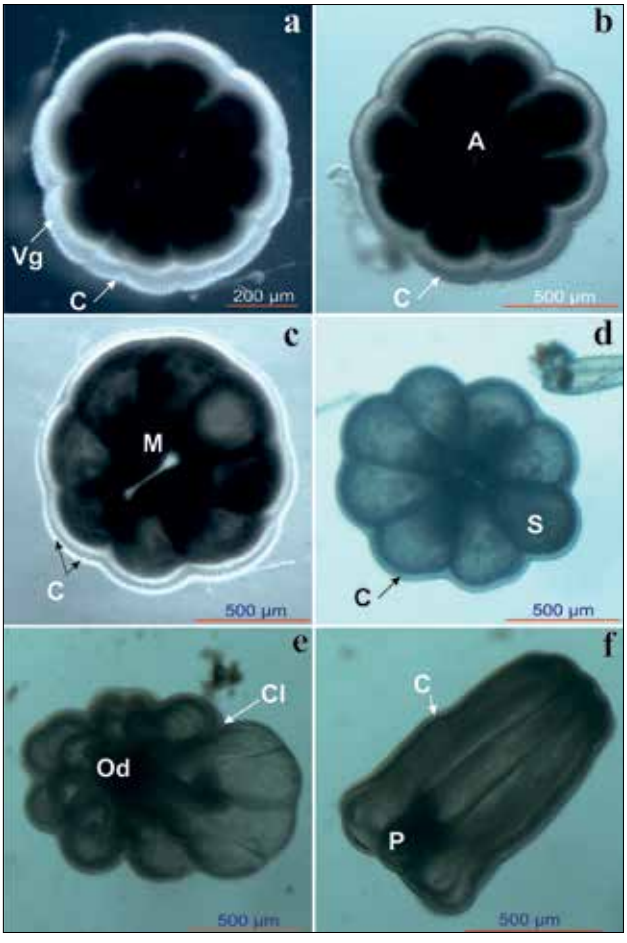

Fig. 4. Morphological events of planulae

[a. 1 day; b. 2 days; c. 3 days; d. 4 days; e. 5 days; f. 6 days. A: archenteron, C: Cilia, Cl: Cleavage, M: Mouth; P: pharynx; S: Septa; OD: Oral disc; Vg: vegetal plate]

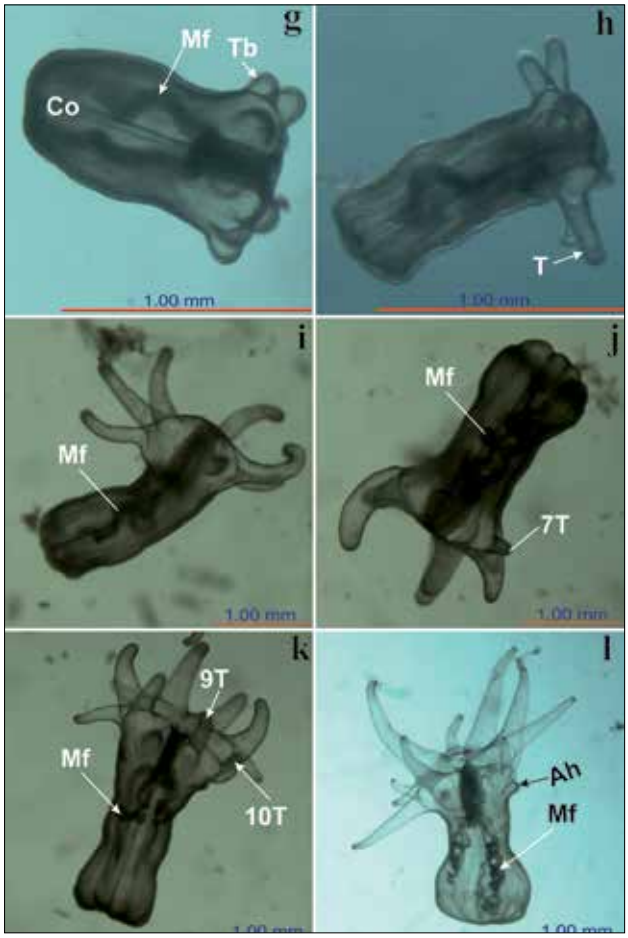

Fig.5. Morphological events of planulae

[g. 7 days; h. 8 days; i. 9 days; j. 10 days; k. 12 days; l. 14 days. Ah: Acrorhagi; Co: Coelenteron; Mf: Mesenterial filaments; Tb: tentacle bud, T: tentacles; 7T: 7th tentacle; 9T: 9th tentacle; 10 T; 10th tentacle.]

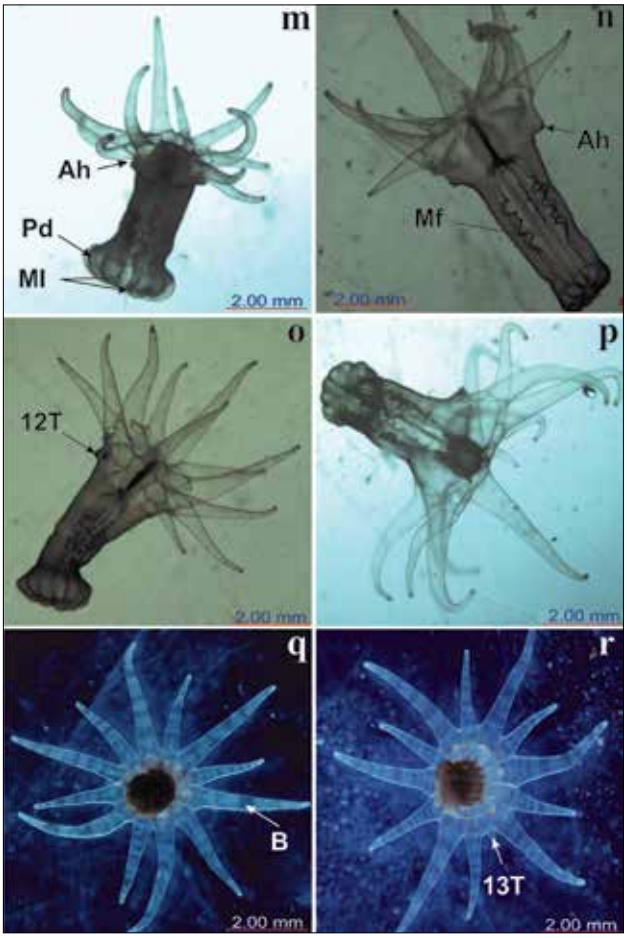

Fig.6. Morphological events of planulae

[m. 16 days; n. 18 days; o. 20 days; p. 22 days; q. 24 days; r. 26 days. Ah: Acrorhagi; B: Band; MI: Mesenterial insertion; Mf: Mesenterial filaments; Pd: Pedal disc; 12T: 12th tentacle; 13T: 13th tentacle]

Table 2. Morphological characteristics of planula from Day 1 to Day 60

[PD =Pedal disc; OD = Oral disc; C =Column; TB =Tentacle bud; TL =Tentacle]

Period	Morphological Characteristics	Size					Surface Area (mm^2) (n=6)
		PD (mm)	OD (mm)	C (mm)	TB (mm)	T (mm)	
Day 1 (Fig. 4a)	Early gastrula stage; vegetal plate is more prominent; cilia formation with sparse, not settled in the substratum		0.687	NP	NP	NP	1.48 ± 0.14
Day 2 (Fig. 4b)	Late gastrula stage; the cilia is numerous with uncoordinated; a central cavity, called the archenteron is formed; endodermal cells arise by ingression; motile in nature		0.719	NP	NP	NP	1.62 ± 0.19
Day 3 (Fig. 4c)	Gastrulation stage is over; ciliary beating coordinated; movement is vigorous; vegetal plate is still present.		0.805	NP	NP	NP	2.04 ± 0.17
Day 4 (Fig. 4d)	Longitudinal depression visible; formation of 8 septa; sphere like appearance; vegetal plate disappeared; cilia are still present.		1.008	NP	NP	NP	3.19 ± 0.13
Day 5 (Fig. 4e)	Planula began to settle; longitudinal depression or cleavage was more prominent; elongation with its vertical axis; tapering towards posterior end; oral disc is slightly formed.		1.094	0.346	NP	NP	3.15 ± 0.14

Day 6 (Fig. 4f)	Planula was pear shaped; pharynx was visible; basal was developed at aboral region; cilia is visible.	0.541	0.898	1.143	NP	NP	3.4 ± 0.07
Day 7 (Fig. 5g)	Six tentacle rudiments were seen; coelenteron (gut cavity) formed with a pair of mesentery with less coiled.	0.632	0.82	1.158	0.012	NP	3.47 ± 0.2
Day 8 (Fig. 5h)	6 tentacles were appeared with transparent colour; cilia disappeared.	0.999	0.435	1.161		0.257	3.42 ± 0.23
Day 9 (Fig. 5i)	No changes in morphology; only size became bigger; Mesenterial filaments little coiled	0.524	0.845	1.306		0.574	3.63 ± 0.25
Day 10 (Fig. 5j)	Planula with 7 tentacles; tentacles were transparent; mesenterial filaments starts coiling.	1.046	1.026	1.622		0.722	6.99 ± 0.35
Day 12 (Fig. 5k)	Planula with 8 tentacles; three mesenteries; 9th and 10th tentacles appeared	0.836	1.231	2.07	0.014& 0.203	0.817	8.4 ± 1.7
Day 14 (Fig. 5l)	Planula with complete 10 tentacles; tentacle length slightly increased; mesenteries little more coiled; acrorhagi are slightly visible.	1.356	1.234	1.978		1.779	10.68 ± 1.6
Day 16 (Fig. 6m)	Planula with 11 tentacles; only tentacle lengths were increased; bands appeared lightly in the tentacles; pedal disc properly developed; mesenterial insertion is slightly visible; acrorhagi is prominent.	1.9	1.622	2.083		2.204	16.4 ± 2.09
Day 18 (Fig. 6n)	4 mesenteries completely formed and 5th and 6th were slightly developed; dark lines hazily appeared at basal part; acrorhagi was prominent; no changes in tentacle number.	1.508	2.475	3.89		2.472	30.65 ± 3.4
Day 20 (Fig. 6o)	6 Mesenteries with more coiled mesenterial filaments; no changes in the tentacle number; 12th tentacle rudiment was appeared; only light band was formed in the tentacles; column was becoming darker.	2.246	2.537	3.119		3.628	32.06 ± 3.7
Day 22 (Fig. 6p)	12th tentacle was fully developed; mesenteries were more coiled; the length of tentacles increased.	1.92	2.8	4.55		3.758	39.69 ± 5.8
Day 24 (Fig. 6q)	Planula became shrivelled; no changes in tentacle number; dark band was properly noticeable in the tentacles.	1.599	1.817	2.721		3.375	21.67 ± 3.6
Day 26 (Fig. 6r)	13th tentacle was appeared; column was becoming thicker and darker like adult polyp	1.843	1.976	2.834	0.147	3.956	22.99 ± 4.0
Day 28 (Fig. 7s)	14th tentacle was appeared; the mesenterial filaments are more coiled	1.833	2.273	2.574	0.205	3.25	23.22 ± 4.3
Day 30 (Fig. 7t)	The number of tentacles became 15; tentacle bands are more prominent.	1.891	2.793	2.243	0.186	3.033	25.12 ± 2.8
Day 35 (Fig. 7u)	16th tentacle was appeared; mesenterial filaments were more coiled	2.312	2.181	2.993	0.114	2.871	29.05 ± 2.5
Day 40 (Fig. 7v)	17th and 18th tentacles were formed at the margin of oral disc; mesenteries were no more visible due to dark column.	1.675	2.415	2.754	0.027& 0.193	2.875	24.26 ± 2.2
Day 45 (Fig. 7w)	The tentacle number became 18; dark band in the tentacles was more prominent; only dark lines began to appear in the oral disc; epithelial cell becoming darkened	1.522	2.549	2.856		2.077	25.54 ± 1.8
Day 50 (Fig. 7x)	The number of tentacles became 20; dark radial lines were more prominent with small white patches is visible in oral disc.	1.693	2.738	2.611	0.289	2.204	25.88 ±3.7
Day 55 (Fig. 8y)	The number of tentacles became 21; 22nd, 23rd and 24th tentacles were under developed; planula was more shrunk and tentacles were more stubby.	1.658	2.849	2.659	0.212 & 0.127	2.278	26.8 ± 2.4
Day 60 (Fig. 8 z, za &zb)	The number of tentacles were 24; dark radial lines were fully developed with small white band in the oral disc; dark bands were more prominent in the tentacles; mesenterial insertions are visible through the pedal disc as dark lines; column is dark in colour.	2.503	5.232	3.298		6.952	60.97 ± 7.3

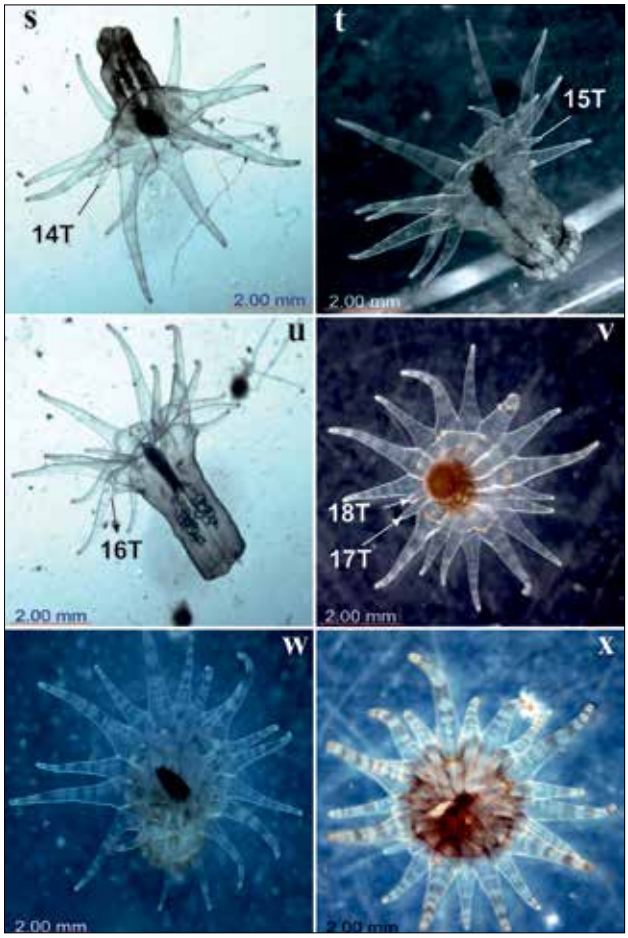

Fig.7. Morphological events of planulae

[s. 28 days; t. 30 days; u. 35 days; v. 40 days; w. 45 days; x. 50 days. 14T: 14th tentacle; 15 T: 15th tentacle; 16 T: 16 T: 16th tentacle; 17 T: 17th tentacle; 18 T: 18th tentacle]

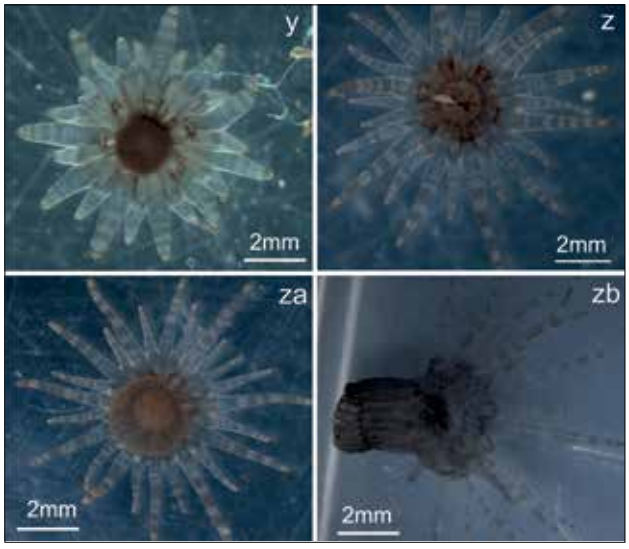

Fig.8. Morphological events of planulae

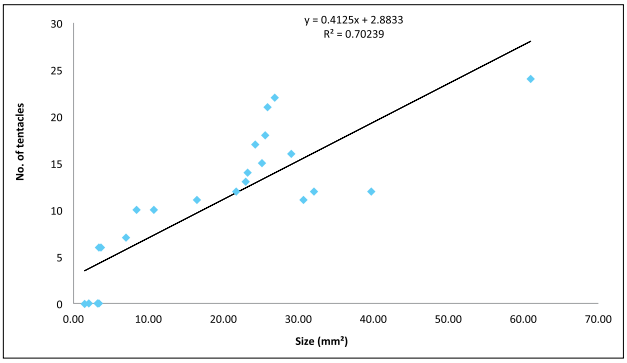

Fig.9. Relationship between larvae size and number of tentacles with increasing days

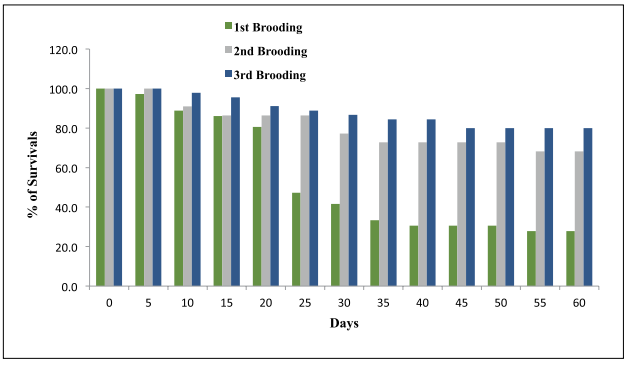

Fig.10. Survival rate of the planulae during the brooding times

rate was 80% during third brooding time and 68.2% during second brooding time. Whereas, the proportion of survival of planulae of the first brooding was very less with a value of 27.7 % at day 60. The mortality rate of planulae of first brooding was very high in comparison to second and third broodings (Fig. 10). During first brooding, the planulae maintained in cultured tank were contaminated with the growth of ciliates and parasitic flatworms. Hence, after 20 days, the mortality rate was increased. The survival rate was suddenly dropped down from 80.55% to 33.33% due to severe infection. After 40 days the survival rate was retained with consistency. Whereas, the mortality rate of planulae of second and third brooding was observed due to the stress condition.

Discussion

The present study revealed cellular events from gastrula stage to late planula or adult polyp development. It has been documented for the first time in India. It was difficult to observe the gametogenesis from early blastula to early gastrula stage, since, this species exhibited internal brooding and all the events of fertilisation, cleavages and blastula stages of gametogenesis were occurred inside the gastro vascular cavity. Moreover, the specimens were limited to conduct the experiment on the brooder to monitor gametogenesis. The characteristics of late gastrula

or early planula of the present brooder was quite similar with the gastrulation of the temperate sea anemone *Anthopleura ballii* (Cocks, 1851). Early development was observed in the temperate symbiotic sea anemone *A. ballii* (Davy and Turner, 2003). During planulation, they could not culture and keep the planula larvae alive for more than 7 days and so the settlement was not observed till polyp form. Herein, the settlement was observed till late planula or polyp form (60 Days). During planulation, it was observed that the development rate was very high from day 1 to day 7. From day 8 to day 22, the rate of development was moderate and from day 24 to day 60, the rate of development was gradually decreased. The result of present study indicate that the size and development of planulae is very slow within the parental care as compared to outside the parent. From the scatter plot, the value of the correlation coefficient, r, is 0.84 which indicates a very strong positive correlation between number of tentacles and the size of planulae. The coefficient of determination, r^2, has a value of 0.70. This indicates that about 70% of the relationship is the result of the size which is the factor being considered for the development of tentacles.

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References

Chen, C., K. Soong and C. A. Chen. 2008. The Smallest Oocytes among Broadcast-Spawning Actiniarians and a Unique Lunar Reproductive Cycle in a Unisexual Population of the Sea Anemone, *Aiptasia pulchella* (Anthozoa: Actiniaria) *Zool. Stud.,* 47(1):37-45.

Chia, F. S. 1976. Sea anemone reproduction: patterns and adaptive radiations. In GO Mackie, *Coelenterate ecology and behaviour*, New York: Plenum, p. 261-270.

Davy, S. K. and J. R. Turner. 2003. Early development and acquisition of Zooxanthellae in the temperate symbiotic sea anemone *Anthopleura ballii* (Cocks). *Biol. Bull.*, 205: 66-72

Dunn, D. F. 1978. *Anthopleura handi* n. sp. (Coelenterata, Actiniaria), an internally brooding, intertidal sea anemone from Malaysia. The Wasmann *J. Biol.*, 35(1): 54-64.

Dunn, D. F. 1982. Sexual reproduction of two intertidal sea anemones (Coelenterata: Actiniaria) in Malaysia. *Biotropica,* 14(4): 262-271.

Harrison, P. L. 2006. Settlement competency periods and dispersal potential of scleractinian reef coral larvae. *Proceedings of the 10th International Coral Reef Symposium,* Japanese Coral Reef Society, Tokyo, Japan, p 78-82.

Isomura, N., K. Hamada and M. Nishihira. 2003. Internal Brooding of Clonal Propagules by a Sea Anemone, *Anthopleura* sp. *Invertebrate Biol.*, 122(4): 293-298.

Mathew, K. 1979. Studies on the biology of a sea anemone, *Anthopleura nigrescens* (Verrill) from the south west coast of India. *Bulletin of the Department of Marine Sciences* University of Cochin, p 1075-158.

Panikkar, N. K. 1937. A study of the actinian *Phytocoetes gangeticus* Annandale, with an account of the post-larval development and the occurrence of neoteny in the anemone. *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere,* 62: 395-422

Raghunathan, C., R. Raghuraman, S. Choudhury and K. Venkataraman. 2014. Diversity and distribution of sea anemones in India with special reference to Andaman and Nicobar Islands. *Records of Zoological Survey of India*, 114(2): 269-294.

Scott, A. and P. L. Harrison. 2009. Gametogenic and reproductive cycles of the sea anemone, *Entacmaea quadricolor. Mar Biol.,* 156: 1659-1671.

Weis, V. M., E. A. Verde, A. Pribyl and J. A. Schwarz. 2002. Aspects of the larval biology of the sea anemones *Anthopleura elegantissima* and *A. artemisia. Invertebrate Biol.*, 121: 190-201.