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Isolation, identification and culture of the marine rotifer *Colurella adriatica* Ehrenberg, 1831 (Family: Lepadellidae) from Andaman & Nicobar Islands: A promising live feed for larval rearing of high value shellfishes and finfishes

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Original Article

Abstract

An extremely small rotifer was isolated from the micro zooplankton samples collected during February, 2014 from Havelock islands of Andaman and Nicobar Islands. The species was identified as *Colurella adriatica* Ehrenberg, 1831 (Family: Lapadellidae). Its lorica length under culture period ranged from 47.530 to 98.868 μm and width from 34.308 to 56.277 μm. The size of the eggs, neonates and adults are also documented. Comparison of size of *C. adriatica* with *Brachionus plicatilis* (L type) and *B. rotundiformis* (S and SS type) revealed that *C. adriatica* is smaller in length and width than the SS-type rotifer which is currently used as a first feed in marine tropical fish larval rearing. However, the larvae of many marine food fishes including groupers and high value marine ornamental fishes are unable to consume the SS-type rotifers as a first feed due to their extremely small mouth gape. The culture of *C. adriatica* was carried out using *Nannochloropsis oculata* Diet-I), *N. oculata* and yeast (0.01g/litre) (Diet-II), Yeast (0.01g/litre) alone (Diet-III). Average population density of *C. adriatica* with these diets reached a maximum of 1000 nos. of individuals /ml on 10th day of culture on feeding with Diet-I; 950 nos. /ml on 14th day (Diet-II) and 650 nos. /ml on 15th day of culture (Diet-III). Diet I &II and Diet II &III did not show any significant difference ($P>0.05$) whereas, Diet I and III showed significant difference ($P<0.01$). Preliminary studies of *C. adriatica* as a feed to the larvae of *Stenopus hispidus, Lysmata amboinensis* and *Pomacentrus caeruleus* showed better survival than larvae fed with *B. routundiformis* during first phase of larval rearing. The study proved that *C. adriatica* can be used as a promising starter live feed for the larval rearing of marine fishes. This is the first record of *C. adriatica* from Andaman and Nicobar Islands and the sequences were submitted to GenBank with the accession no KX387633.

Keywords: *C. adriatica, rotifer, live feed, culture, population density.*

Introduction

Micro live feeds play a pivotal role in the larval rearing and seed production of marine finfishes and shellfishes as the larvae depend on live prey at their first feeding (Tocher *et al.*, 1997). As the live food organisms are able to swim in the water column and are constantly available to the larvae rather than formulated diets which congregate or sink to the bottom of tanks, the dependence on live feed organisms is increasing day by day. Moreover, the jerking movement of live feed in the water column also help to stimulate feeding response in larvae. The newly hatched larvae are of two types precocial and altricial. Most of the marine fish larvae are altricial type.

Hence a major impediment in larval rearing is the first feeding stage, when the larvae shift from endogenous yolk reserves to exogenous feeding (Turingan *et al*., 2005). Most of the hatcheries commonly use species of rotifer *Brachionus plicatilis* (L type), *B. rotundiformis* (S and SS Type) and brine shrimp (*Artemia* sp.) as live feed organisms due to their small size, fast multiplication rate and ease in culture methods (Dhert, 1996). However, larvae of many of the marine food fishes including groupers and high value marine ornamental fishes are unable to consume these live foods as their first feed due to their extremely small mouth gape. It is also established that prey capture success and percentage of successful feeding strikes is low at first feeding by marine fish larvae (Hunter, 1981) but rises rapidly during early development (Houde and Schekter, 1980). At this stage provision of suitable size and nutritionally adequate enriched live feed in high density is one of the important factors for their survival as the larvae will be able to accept only small sized prey organism due to the small mouth gape, and if they do not encounter and successfully capture food before depleting their energy reserves, the larvae may starve and it will eventually leads to mortality. As the mouth gape of most of the fish larvae is between 50- 120 μm, the larvae need to be fed with live feeds measuring less than their mouth gape for active feeding, indicating that size of live diet is an important prerequisite in larval nutrition.

The species belonging to the genus *Colurella* are extremely smaller rotifers in the order Ploima in which *Brachionus* species is also present. Being a filter feeder, its nutritional quality can also be enhanced with proper enrichment. Occurrence of *Colurella* sp. are reported from wide range of habitat ranging from freshwater to brackishwater (De Smet, 1994), marine environment (De Smet, 2006); temperate and tropical regions (De Ridder and Segers, 1997; Baribwegure and Segers, 2001; Leszek and Ellison, 2003). The occurrence of *Colurella* sp. from the backwaters of the Delhi segment of the Yamuna River was reported by Arora and Mehra (2003).

The success of captive production of marine fishes depends on the ability to produce large numbers of good quality larvae that successfully metamorphose to juveniles (Holt, 2003). Eventhough a large number of marine species undergo gonadal maturation and spawn in captivity, prey capture success in the first stage of feeding and subsequent larval developments and metamorphosis still remains as a critical bottleneck in their production (Holt, 2003) due to the non availability of suitable sized and nutritionally adequate live food organisms. As the species belonging to the genus *Colurella* are much smaller in size than *B. plicatilis* and *B. rotundiformis*, its isolation and development of culture techniques may be useful for the larval rearing of marine fish species for which the currently used live prey organisms are too large. Hence the present study was undertaken to isolate smaller species of rotifer from marine environments from India and to investigate the feasibility of developing stock and mass cultures for evaluating its suitability as first feed in larviculture.

Material and methods

Micro zooplankton samples were collected by towing plankton nets (20 and 50 μm) during February, 2014 from the littoral and sub littoral areas of coral reef ecosystem of Havelock island of Andamans and Nicobar islands where an assemblage of early juvenile stages of different varieties of marine ornamental fishes were noticed. In the laboratory, the zooplanktons were sorted in live condition using zooplankton sorter of different mesh size, and observed under trinocular microscope. The sorted species were then transferred to 100 ml test tubes containing 50 ml sterilized seawater having 34 ppt, and fed with 5 ml of microalgae *Chaetoceros calcitrans*, *Nannochloropsis oculata* and *Isochrysis galbana* and yeast in separate test tubes. The test tube racks were placed in front of light source at 1000 to 1500 lux for 24 hours photoperiod under room temperature (28ºC). Every three days, the samples were observed for a period 15 days with addition of feed and then the culture was further purified by transferring to new culture media. This method of isolation was continued until a pure stock of organism was obtained. Once a pure stock was obtained, the culture was carried out in 250 ml conical flask for stock culture maintenance using *N. oculata* as feed. The species were identified using standard identification characters (Hauer, 1924; Sorenson and Kristensen, 2000; Jersabek and Leitne, 2013) and the different shapes of occurrence were photographed under Trinocular microscope (Leica DMLB-2 attached with camera 450C) at 20x and 100x magnification and the measurements *viz*., length and width of lorica and eggs, and length of foot and toes from 100 live specimens were documented using software LAS and its average were taken. The size of *C. adriatica* was also compared with other rotifer species *B. plicatilis* (L-type), *B. rotundiformis* (S and SS type) available in the marine hatchery after measuring 100 specimen each. DNA extraction was carried out using a standard phenol/chloroform extraction protocol. A 650bp region of the Cytochrome C oxidase 1 (CO1) was amplified using universal primer (Folmer *et al*., 1994) in *Colurella adriatica* collected from Indian waters. Purification of the PCR products was carried out using Qiagen PCR purification kit and sequencing was carried out with BigDye Terminator Sequencing Ready Reaction v3.0 kit (Applied Biosystems) using the primers.

Microalga *N. oculata* was grown in 15 litre buckets as batch cultures at 20 to 22ºC under continuous photoperiod of

1500 to 2500 lux light intensity in the marine hatchery. The influence of three feeds *N. oculata* ($10x10^4$ to $20x10^4$ cells/ml) (Diet-I), *N. oculata* ($5x10^4$ to$10x10^4$ cells/ml) +Yeast (0.01g/l) (Diet-II), and yeast alone (0.01 g/l) (Diet-III), on its multiplication was carried out in 15 litre cylindrical perspex container with lid to avoid evaporation, and three replicates were maintained. Each container was filled with 10 litres of respective feed, and stocked with 5 Nos. of rotifers/ml. All the containers were kept under 28ºC temperatures, 34 ppt salinity, 1000 to 1500 lux light intensity at 24 h photoperiod with continuous moderate aeration to provide dissolved oxygen level at 3.2 to 3.5ml/l. The total duration of culture experiment was 17 days. Every day 5 ml of samples from each culture vessels were randomly taken to determine the population density. Before counting, one drop of neutral buffered formalin was applied through the side of cover slip of rotifer counting chamber to anaesthetise the organism. The total number of rotifers in each ml were calculated and expressed as average of three replicate in each culture container and the number of rotifers were expressed as average number of individuals/ml. The density of algae in each culture container were determined with a haemocytometer, and adjusted every 3 days during the culture experiment. Data was analysed using ANOVA to determine the effect of various feed on multiplication of *C. adriatica*. A probability of 0.01 and 0.05 was utilized to account for the statistical difference between the means.

Feeding Trial

The larvae of blue *Caerulean* damselfish *Pomacentrus caeruleus*, marine ornamental banded coral shrimp *Stenopus hispidus* and scarlet cleaner shrimp *Lysmata amboinensis* were fed with *C. adriatica* at 5 to 10 nos/ml up to 10days of post hatch and compared with the survival obtained in the larvae fed with *B. rotundiformis* (control) up to 10 days of post hatch. Larval rearing of *P. caeruleus* was carried out in 500 l FRP tanks using micro algae *I. galbana*, *N. oculata* and *Chlorella salina* at 1:2: 2 proportion at cell density $5x10^5$ cells/ml whereas larval rearing of *S. hispidus* and *L. amboinensis* was carried out in 500 l black coloured cylndroconical tank using microalgae N. oculata, *I. galbana*, and *C. gracilis* at 1:2: 2 proportion at cell density $5x10^5$ cells/ml. Each tank was stocked with 100 nos of larvae. Three replicates were maintained for each treatment. On 10th day of rearing, the larvae survived in each thank were accounted to record the survival.

Results and discussion

Identification characteristics

The species was identified as *C. adriatica* belonging to the Phylum: Rotifera, Class Eurotatoria, Order: Ploima, Family: Lapadellidae. The different shapes of its occurrence are presented in the line drawings (Fig.1 A-D). The species was identified based on the morphological features as suggested by Hauer (1924); Sorenson and Kristensen (2000); Jersabek and Leitne (2013) and the following characteristics were recorded after observing live

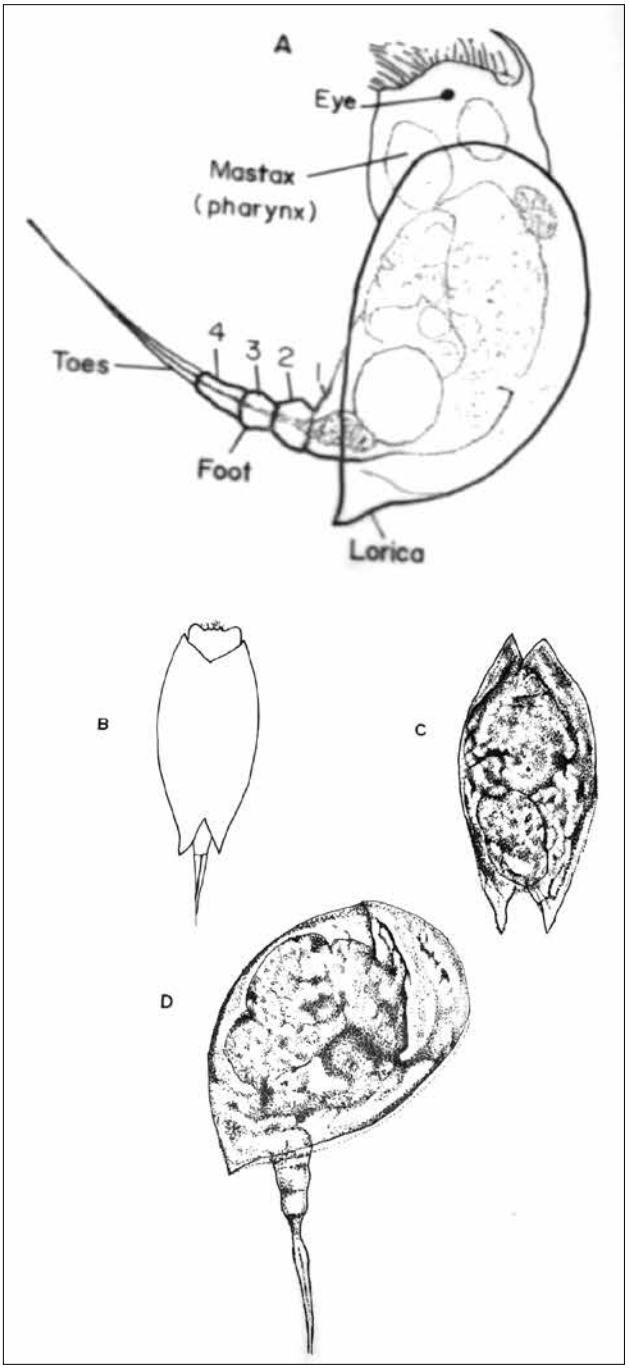

Fig. 1. Line drawing of *C.adriatica* : A: Adult female showing segmented foot with long toes, eyes and internal organs (Lateral view); B: Dorsal view, C : Mussel shaped side view; D. Semi-circular shield dorsal to the corona retracted completely within lorica

specimens. Lorica is very variable in size and shape with characteristic divergence of posterior margin which has a pointed and acute shape (Fig. 2A). Lorica composed of two lateral plates, strongly compressed laterally and it open along anterior, ventral and posterior margins. So it appears mussel-shaped in side view (Fig. 2B). Frontal head hood present and the head carries a small, retractable, semi-circular shield dorsal to the corona which is retractable completely within lorica (Fig. 2C). The foot consists of four segments and two slender toes which are longer than the length of foot (Fig. 2C). There are two lateral eyes which are red in colour and can be distinguished easily (Fig. 2D). Sorenson and Kristensen (2000) also reported that the species in the genus *Colurella* are solely recognized by lorica characteristics and the trophi plays no taxonomical role in systematic studies. Pejler (1962) reported a transition morph between *C. adriatica* and *C. colurus*. Different ecotypes have been described in relation to the size and shape of the lorica, as well as length of the foot. Hauer (1924) reported that both species increased their size with salinity. *C. adriatica* is often confused with *C. colurus*. However, *C. colurus* is relatively smaller than *C. adriatica*, and it can be distinguished by the morphology of the posterior margin of the lorica, which is rounded.

Though *C. adriatica* is reported as euryhaline and eurytopic species occurring in current water in the littoral zone among macrophytes, and are considered cosmopolitan (De Ridder and Segers, 1997), it was also recorded from freshwater, brackishwater bodies (De Smet, 1994), temperate, tropical regions (De Ridder and Segers, 1997; Baribwegure and Segers, 2001; Leszek and Ellison, 2003), and from the marine littoral environment of Reunion I (21ºS, 55.30ºE) which is one of the Mascarene island lying in the Indian ocean, 800 km east of Madagascar (De Smet, 2006). The comparison of the trophi of marine specimens from Mascarene island of the Indian ocean with that of freshwater specimens revealed no significant differences

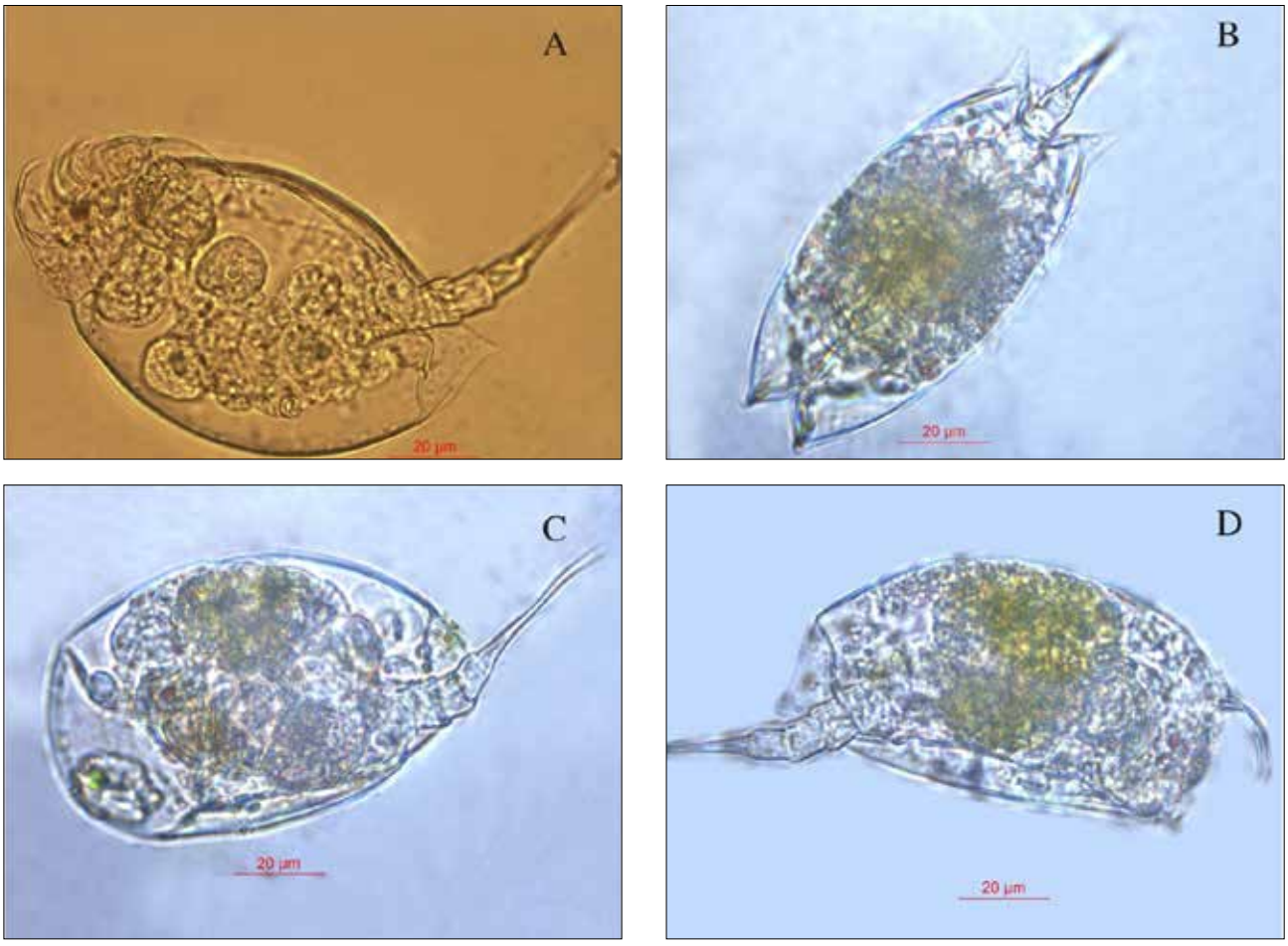

Fig. 2. Microscopic view of live *C. adriatica* showing morphological characteristics (Magnification: 100x): 2A. Adult female (Lateral view); 2B. Mussel-shaped side view showing two lateral plates; 2C. Semi-circular shield dorsal to the corona retracted completely within lorica, showing four segmented foot and two long slender toes; 2D. Lateral view of adult *C. adriatica* showing red eyes

(De Smet, 2006) indicating that it can tolerate wide range of salinity fluctuation, and habitats or ecological conditions as reported by De Ridder and Segers (1997). Though occurrence of *Colurella* sp. from the backwaters of the Delhi segment of the Yamuna river was reported by Arora and Mehra (2003), and annotated a checklist of 42 Indian species of Lepadellidae from northeast India (Shrama and Sharma, 2015), the present study is the first report of occurrence of *C. adriatica* from marine environment, and its isolation and culture in India. The partial sequence of *Colurella* sp. CO1 was deposited in GenBank with the accession no: KX387633.

Measurements of C. adriatica under captive conditions

The lorica length of the isolated species under culture period ranged from 47.530 to 98.868 μm and its width ranged from 34.308 to 56.277 μm. The size of the eggs ranged from 53.821 to 62.432 μm length and 37.543 to 44.176μm width. The lorica length of the neonates ranged from 47.530 to 50.004 μm and their width ranged from 34.308 to 36.702 μm (Fig. 3). Size of the adults usually ranged from 70.454 to 98.868 μm lorica length and 54.243 to 56.277μm lorica width. The length of foot ranged from 25 to 30 μm and the two toes present were slender, long and separated which measured 30 to 40 μm (Fig. 4).

Comparison of size of C. adriatica with common rotifers

In aquaculture, the most widely used species of rotifer are *B. plicatilis* (L-type) and *B. rotundiformis* (S-type and SS-type), and the results of comparison of their lorica length with that of *C. adriatica* are given in Table 1. The observation revealed that *C. adriatica* the Super Minuscule Rotifer (SMR) is smaller than the super small *B. rotundifromis*. The microscopic photographs of these species documented at 20x magnification are presented in Fig. 5 A-D. The efficacy of SS type rotifer which is presently used as the first feed is questioned as many of the high value fishes are unable to consume this rotifer due to the extremely small mouth gape of larvae (Holt, 2003). The lorica length and width of the common species *B. plicatilis*, *B. rotundiformis* (S and SS type) used in the present study are also in agreement with data recorded in various studies (Lim,1993; Dhert, 1996; Molly Varghese, 2003). Various studies also pointed out that variation in size and growth can be observed due to geographical location (Hagiwara *et al*., 1995; Rimper *et al*., 2008), prevailing culture conditions like temperature, salinity, feed type, feed concentration, etc. (Snell and Carrillo, 1984; Somamihardja and Bart, 2008). Knuckey *et*

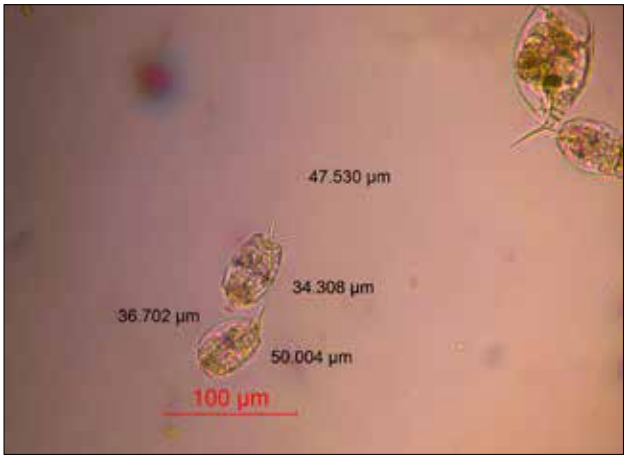

Fig. 3. Microscopic view of neonates of *C. adriatica* (Magnification 20x).

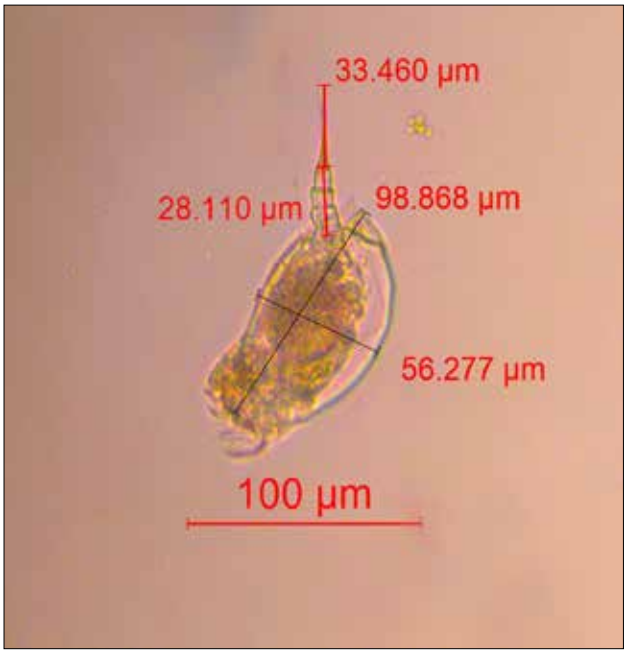

Fig. 4. Measurements of *C. adriatica* showing shorter foot and long slender toes (Magnification 20x).

Table 1. Comparison between *Brachionus* species and *Colurella adriatica* in terms of size

Rotifer species	Lorica length (μm)	Lorica width (μm).
C. adriatica	47.530- 98.868	34.308–56.277
B. plicatilis (L type)	130- 340	116–146
B. rotundiformis (S type)	100- 210	98–121
B. rotundiformis (SS type)	58-120	58 -100

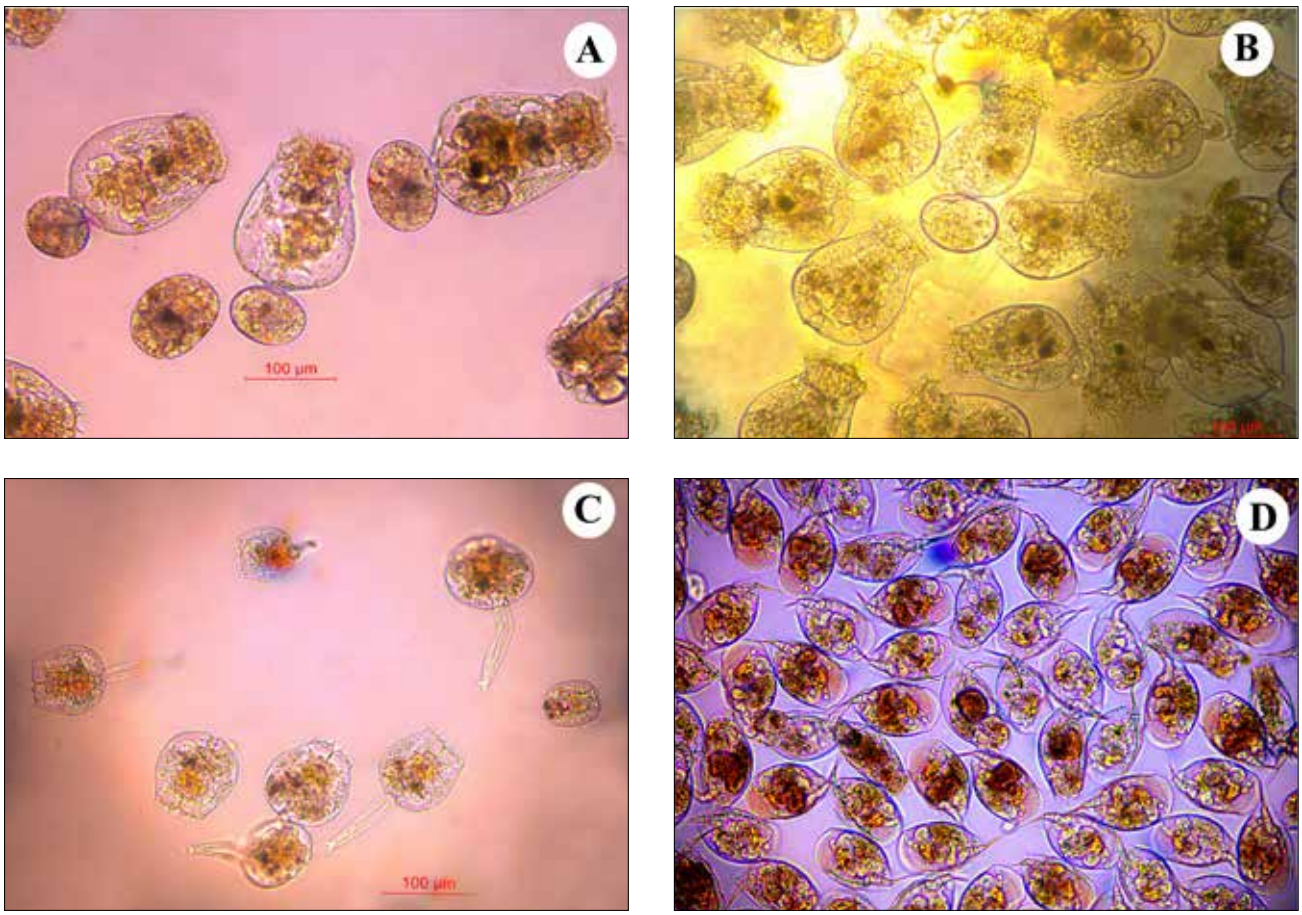

Fig. 5. Comparison of size of different Rotifers (Magnification 20x): 5A. *Brachionus plicatilis* (L-type), 5B. *Brachionus rotundiformis* (S-type), 5C. *Brachionus rotundiformis* (SS Type),5D. *Colurella adriatica*

al. (2004) stated that particle size of diet can also influence the degree of morphological plasticity in size.

Population density in the culture experiments

The culture experiments showed that *C. adriatica* can be multiplied under laboratory condition providing optimum conditions and suitable feed. The population density of rotifer during culture experiments are presented in Fig. 6. The determination of number of rotifers present in the culture vessels during the 17 days of culture period showed that the treatment fed with *N. oculata* (Diet-I) had attained a population density of 1000 nos. of individuals/ml on 10th day of culture. In the treatment fed with *N. oculata* and Yeast (Diet-II) a peak population density of 950 nos. of individuals /ml was noticed on 14th day and the culture fed with yeast alone (Diet-III) had reached peak density on 15th day of culture with 650 nos. of individuals /ml. A hike in the number of individuals/ml was noticed from the 6th day of culture onwards. Comparison of population density of rotifer in different diets revealed that between Diet I &II and Diet II &III did not show any significant difference ($P>0.05$). However, Diet I and III showed significant difference ($P<0.01$). The study revealed that for mass multiplication of *C. adriatica*, the microalga *N. oculata* can be effectively used as feed. However after attaining peak, unless the culture is harvested or replaced with new algae, the culture showed a collapse. In general, the life span of rotifers has been estimated to be between 3.4 to 4.4 days at 25°C. Generally, the neonates become adult after 0.5 to 1.5 days and females thereafter start to lay eggs approximately every four hours. It is believed that females can produce ten generations of offspring before they eventually die (Dhert, 1996). The multiplication rate of *C. adriatica* is reported as 0.765 ± 0.017 offspring/female/day), whereas it was 0.23-1.15 offspring/female/day, in *B. plicatilis* and 0.54-1.37 offspring/female/day in *B. rotundiformis* (Dhert, 1996; Stottrup, and Mc Evoy, 2003). The present study also revealed that partial harvesting from 10th day of culture and with replacement of harvested volume with fresh algae can sustain the culture for a period of 17 days. The harvested biomass of *C. adriatica* was yellowish orange in colour.

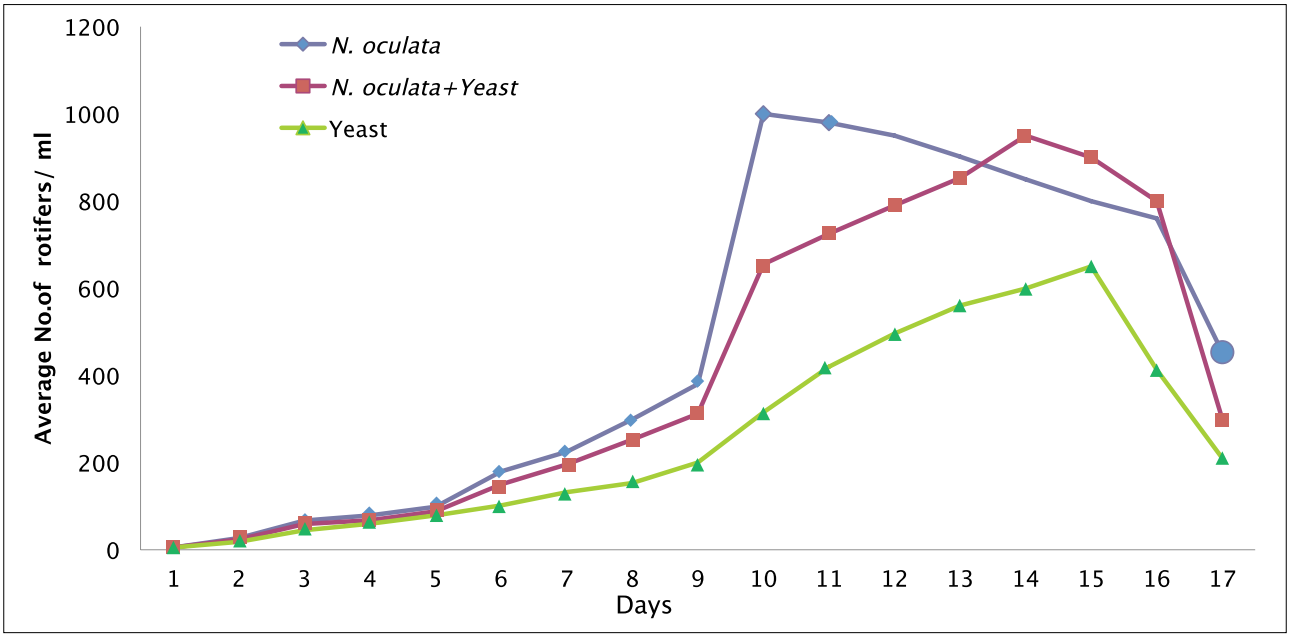

Fig. 6. Population density of *C. adriatica* fed with different feed at 34 ppt salinity

Table 2. Survival in larvae of marine ornamental species fed with *C. adriatica* as first feed up to 10 day of post hatch (dph)

Species	Larval survival (%) upto 10 dph fed with *C. adriatica*	Larval survival (%) upto 10 dph fed with *B. rotundiformis*
Pomacentrus caeruleus	80	20
Stenopus hispidus	60	10
Lysmata amboinensis	45	5

Preliminary studies of C. adriatica as a feed to the larvae

Preliminary studies on feeding the larvae of marine ornamental banded coral shrimp *S. hispidus*, scarlet cleaner shrimp *L. amboinensis* and blue *Caerulean* damselfish *P. caeruleus* with *C. adriatica* showed that the larvae had ingested it. The survival obtained in larvae fed with *C. adriatica* and *B. rotundiformis* up to 10 days of post hatch are presented in Table 2 and revealed that the larval survival is significantly higher ($p<0.05$) when fed with *C. adriatica* than *B. rotundiformis*. Thus the study revealed that *C. adriatica* can be used as first feed in larval rearing of high value fin and shell fishes before weaning them to larger sized live feed organism. The newly hatched larvae are of two types precocial and altricial. Precocial larvae are those which appear as mini adults, exhibits fully developed fins and mature digestive system with functional stomach and also can able to ingest and digest formulated diets. However, in altricial larvae, when the yolk reserve is exhausted, they remain relatively in an undeveloped state with rudimentary digestive system and lacks stomach (Stottrup and Mc Evoy, 2003). In such case the protein digestion is being taken place in the hind gut epithelial cells (Govoni *et al.*, 1982), hence unable to accept formulated feeds. Therefore, altricial larvae require suitable sized live feeds. Most of the marine fishes have altricial type larvae and hence need to be fed with suitable sized live feed organism as first feed. Recent report also revealed that use of *C. adriatica* in grouper larval rearing was successful (Wullur *et al.*, 2013). Hence the isolation and culture of *C. adriatica* which is smaller than *B. rotundiformis* (SS type) is a major breakthrough in larval rearing of marine fishes.

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