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Population growth and mass production of brackish water cladoceran *Eurycercus beringi* sp. nov. under different diet and salinity regime, and its role in *P. indicus* larval rearing

R. Aravind *, P.S. Shyne Anand, T.N. Vinay, I.F. Biju, K.P. Sandeep, J.A.J. Raymond, S. Rajamanickam, C.P. Balasubramanian, K.K. Vijayan

ICAR- Central Institute of Brackishwater Aquaculture, Santhome High Road, Chennai 600028, India

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ABSTRACT

The growth performance and mass production of *Eurycercus beringi* sp. nov., comes under cladocera isolated from Adyar creek, Chennai fed with different diets includes *Chlorella* sp, *Nannochloropsis* sp, *Chaetoceros* sp, *Thalassiosira* sp, *Tetraselmis* sp, *Dunaliella* sp and *Isochrysis* sp, rice bran and baker's yeast as monospecific and mixed diets (1:1) have been investigated. The species reproduce parthenogenetically attained a size range of 300- 1098 μm and tolerate salinity range of 3–35‰ with fecundity range of 2–7 numbers with minimum maturation period of 3–6 days depending on quality of feed with minimum aeration. Salinity found to play a significant role in survival with maximum survival at 25 ‰. There is a significant difference ($p<0.05$) in survival at lower and higher salinity. The highest fecundity was observed in the treatment fed with *Chlorella* and *Nannochloropsis* sp followed by *Chlorella* and *Thalassiosira* sp ($1\text{-}3\times10^5$ cells/ml) i.e. 8 and 7 number of individual/broodstock respectively where as the species attained maturity within 3 days in the treatment fed with *Chlorella* sp followed by combination of *Chlorella* and *Chaetoceros* sp (4 days) and *Chlorella* and *Thalassiosira* sp as monospecific diet (4 days each). Mass culture of *E. beringi* sp. nov. at 100 nos/L was carried out using microalgae, rice bran and baker's yeast revealed a maximum density 5887 ± 109 nos/L recorded at 30 ‰ within 7 days when fed with mixed diets of *Chlorella* and *Nannochloropsis* sp followed by rice bran with baker's yeast (4586 ± 167 nos/L). Partial replacement of *Artemia* nauplii with *E. beringi* sp. nov. (1:1) with highest survival (65 ± 3 %) during early post larval stages of *Penaeus indicus* revealed its potential to use as live feed in larval rearing. The study highlighted the scope of *E. beringi* sp. nov. to use as live feed during the larval rearing of brackishwater finfish and shellfishes.

1. Introduction

Zooplanktons are considered as the living capsules of nutrition for larval rearing of commercially important finfish and shell fishes. They are riches source of vital nutrients such as proteins, lipids, carbohydrates, vitamins, minerals, aminoacids, fatty acids and carotenoids (New, 1998; Hernandez Molejon and Alvarez-Lajonchere, 2003; Rajkumar et al., 2008). Live feeds are reported to be excellent alternative to artificial feed owing to its multiple qualities and versatilities over artificial feed (Wang et al., 2005).

Several classes of planktonic organisms include rotifers, brine shrimp, copepods, cladocerans and amphipods occupy a major position in the aquatic food web of tropical counties. Cladocerans are important planktonic crustaceans, recorded as a most abundant primary consumer group found in water bodies ranging from freshwater to marine with wide tolerance to wide salinity and temperature (5 to 30 °C) with pH ranges of neutral to slightly alkaline water (Ivleva, 1973). There are more than 700 species reported worldwide, though most of them are parthenogenetic, when undergone stress, they produce males and ephippia (Smirnov, 2017).

Cladocerans are rich in essential nutrients and get easily ingested and digested by larvae, fulfil the larval dietary requirements and improve the water quality by minimizing the need of artificial feeding (Tamaru et al., 1991; Sorgeloos and Lavens, 1996; He et al., 2001). The population density of cladoceran can be enlarged by increasing the fecundity and decreasing the reproductive period by manipulating the quantity and quality of feed which have used (Hakima et al., 2013) including protein and amino acid (Koch et al., 2011), fatty acid (Wacker and Creuzburg, 2007) and vitamin composition (Mehdipour et al., 2011). Several authors suggested that use of mixed diets meets the nutritional requirements of target species compare to monospecific diets (Cai

* Corresponding author.
E-mail address: aravind.pillai@icar.gov.in (R. Aravind).

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et al., 2007; Puello-Cruz et al., 2009). In most of the cladoceran species, cholesterol plays a major role to improve the somatic growth while Poly unsaturated fatty acid (PUFA) improves reproduction rate, growth performance and survival rate (Fereidouni et al., 2013). Most of the species can tolerate low oxygen and reach maximum densities in a short duration. The nutritional quality of some species of cladocera is superior to commercially important newly hatched *Artemia* nauplii (Sorgeloos and Lavens, 1996; He et al., 2001). The application of cladoceran group in aquaculture point of view, it can be used as live food in hatchery seed production is widely recognized, although papers concerning mass scale culture are scarce. There is little information concerning the biology of this particular species that would help to develop a controlled production for aquaculture practices. The applications of feed at the right time directly influence the survival and growth rate. *Eurycercus beringi* sp. nov. comes under family Eurycercidae are euryhaline species reproduce parthenogenetically and minimum maturation period will vary depending on the quality of feed diets (Bekker et al., 2012) and have a little information about biological aspects. This study addresses the population growth and mass production of *E. beringi* sp. nov. and its efficacy to use as live feed fed larval rearing of finfish and shellfish.

2. Material and methods

Zooplankton sample were collected from Adyar estuary at Chennai district, Tamil Nadu (13°1′30″N and 80°16′45″E) using plankton net (200 μm) at 15‰ salinity during summer season and inoculated into fresh culture medium with least disturbance in order to get the pure isolate of cladoceran species. Identification through molecular tools (Partial sequencing using cytochrome oxidase sub unit I) showed that there is no matching sequences available in Genbank and hence identified with morphological tools (Bekker et al., 2012) revealed similarity to *Eurycercus beringi* sp. nov. comes under family Eurycercidae. Six salinity treatments (0, 3, 5, 15, 25 and 35‰ were prepared in triplicate by volumetrically mixing natural dechlorinated cartridge filtered (1 μm) UV treated (3 lamp) sea water with dilution water. The dilution water was prepared using refractometer (MASTER-S/Mill α, Atago Co. Ltd.) by mixing dechlorinated tap water with distilled water so that it closely matched characteristics of the water in the culture tanks. 24 h old neonates (100 nos) were randomly transferred to test salinities and used *Chlorella* sp as feed diet ($1–3\times10^5$ cells/ml) and survival rate estimated using Sedgewick Rafter counting chamber after 3 days as the minimum maturation period of *E. beringi* sp. nov. was 3 days.

The microalgae such as *Chlorella* sp (CHL), *Nannochloropsis* sp (NAN), *Chaetoceros* sp (CC), *Thalassiosira* sp (THL), *Tetraselmis* sp (TET), *Dunaliella* sp (DUN) and *Isochrysis* sp (ISO) obtained from microalgal culture laboratory of Muttukadu Experimental Station (MES) of ICAR- Central institute of Brackishwater Aquaculture (CIBA), Chennai, India were used in monospecific and mixed diet combination (1:1) besides commercially available rice bran (RB) and baker's east (BY) to find out fecundity range and minimum maturation period of *E. beringi* sp. nov.. The microalgae cultures were kept at indoor unit at 24 °C under continuous light intensity of 4000 lux using F/2 nutrients media in 3 L conical flask. The algal density was maintained in an indoor algal culture lab at a cell density range of $1–3\times 10^5$ cells/ml and regularly enumerated the cell number using haemocytometer. The algal cultures were carried out under aseptic conditions. In order to reduce the variability of the research output, a selection procedure was followed to isolate the cladoceran populations: the females containing embryos were isolated from pure culture stock. All experiments were carried out at room temperature at 29 ± 1 °C using dechlorinated water at a pH of 7 and photoperiod of 12 h light and 12 h dark. Proximate composition of harvested pure culture biomass of *E. beringi* sp. nov. was analysed as per the standard method (AOAC, 1997). Lipid was extracted by using chloroform and methanol (2:1) by Folch et al. (1957) method and the respective fatty acid methyl esters (FAMEs) were prepared and extracted into petroleum ether. The quantity of fatty acids (g/100 g) was calculated according to Aziz et al. (2012). The sample was digested using microwave digestion method (Anton Par microwave system) for mineral analysis with a combination of nitric acid and hydrogen peroxide in inert polymeric microwave vessels. Ash content was determined by combusting sample at 550 °C for 24 h in pre-weighed porcelain 265 crucibles.

Population density experiments were conducted with equally sized juvenile of *E. beringi* sp. nov. (300 μm) placed individually in 1 L conical flask fed with different microalgal species at regular intervals using a pasteur pipette. Survival and reproduction of *E. beringi* sp. nov. were monitored daily. Three replicates were performed for each treatment and experiments were continued until the hatching of first parthenogenetic progeny. Total numbers of neonates produced by each parthenogenetic female were recorded in order to find its fecundity. The total number of individuals occupied in a given volume of water will determine the population density of the cladocera. The initial age of reproduction was referred to as an average age (in days) at which a female started to produce first batch of its offspring. Longevity referred as the average total number of days the female survived during the course of experiment. The experiment was continued for two weeks to estimate log phase to record highest density per treatments and, decline phase in each treatment. To test the starvation tolerance of adult and neonates *E. beringi* sp. nov. (10 nos adult and neonates each were reared in conical flask in triplicate without feeding and observed the number of individuals alive in each treatment daily. Dead individuals were counted and discarded. The experiment was lasted for 3 days by which time most of the individual were died in the flasks.

The rate of population growth increase per day was derived using the exponential growth equation (Krebs, 1985):

$r = (\ln N_t - \ln N_0)/t$, where

r:- Rate of population growth,

N_0:- Initial population density (nos),

N_t:- Final population density (nos),

t:- time in days

Somatic growth rate of body length (g) of *E. beringi* sp. nov. under each treatments for a period of 3 days were estimated using the following formula (Lampert and Trubetskova, 1996)

$g = (\ln L_{fin} - \ln L_0)/t$ where,

L_{fin}:- linear size of an animal at the day preceding the hatching of first clutch below will be called the size of females at first reproduction (mm)

L_0:- Linear size of an animal at the beginning of the experiment (mm)

t:- Time period (days)

Mass culture of *E. beringi* sp. nov. carried out in an experimental FRP tanks of 200 L capacity arranged in triplicate (Fig. 1) were filled with 100 L of dechlorinated, cartridge filtered, UV treated seawater (30‰) fed with microalgae species such as CHL, NAN, THL (@ $1-3\times10^5$ cells/ml) and RB and BY (1 g/ton) individually, and in combination were fed in daily dosage for a period of 7 days. Population density was estimated during the mass culture by counting samples taken at random with 1 L beaker after mixing thoroughly the culture medium. Sub sample of 1ml taken from beaker and samples were immobilized using formalin (4%) and counting and body length measurement was carried out using Sedgewick raft plankton counting chamber using Nikon Eclipse Ci microscope (with 4X and 10X magnification) mounted with

Fig. 1. Experimental set-up for mass culture of *E. beringi* sp. nov. using different feed diet.

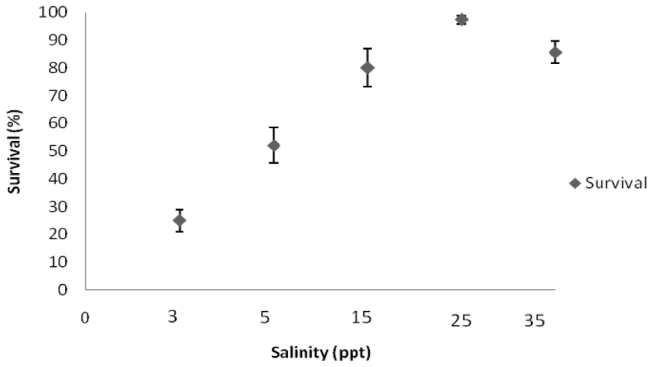

Fig. 2. Percentage survival of *E. beringi* sp. nov. (Mean ± SE) at different salinity range.

QEi Monochrome camera using ImagePro software. Population density was expressed as number of individuals/ L.

In order to check its suitability as live feed for crustacean larvae, an experiment was conducted using *Artemia* naupli and *E. beringi* sp. nov. as monospecific and mixed diet (1:1) at the rate of 5 numbers per larvae during the early larval stages of *P. indicus*. Mysis 3 (M_3) larvae was transferred to 5 L plastic tub and stocked at a density of 50 nos/L in triplicate. Salinity and temperature were maintained at 30 ± 1‰ and 29 ± 1 °C respectively during the experiment. A photoperiod of 12:12 h light: dark and constant aeration was maintained throughout the experiments with daily exchange of 80% water to eliminate molts, debris and uneaten food. Larvae were reared up to Post Larvae 12 (PL_{12}) following different feeding regimes.

Instantaneous Growth Rate (IGR) was estimated using the formula (Castille et al., 1993),

$IGR = 100 \times [(\ln W_f - \ln W_i)]/t$

where W_f:- Wet Weight at PL_{12}

W_i:- Wet Weight at M_3

t:-time in days

One way ANOVA was performed in each experiment in order to determine significant differences among treatments. If effects were found to be significant, Tukey's post hoc comparison was to identify significantly different treatments among them (Zar, 1996). SPSS 16.0 software program was used to conduct all statistical analysis during the study.

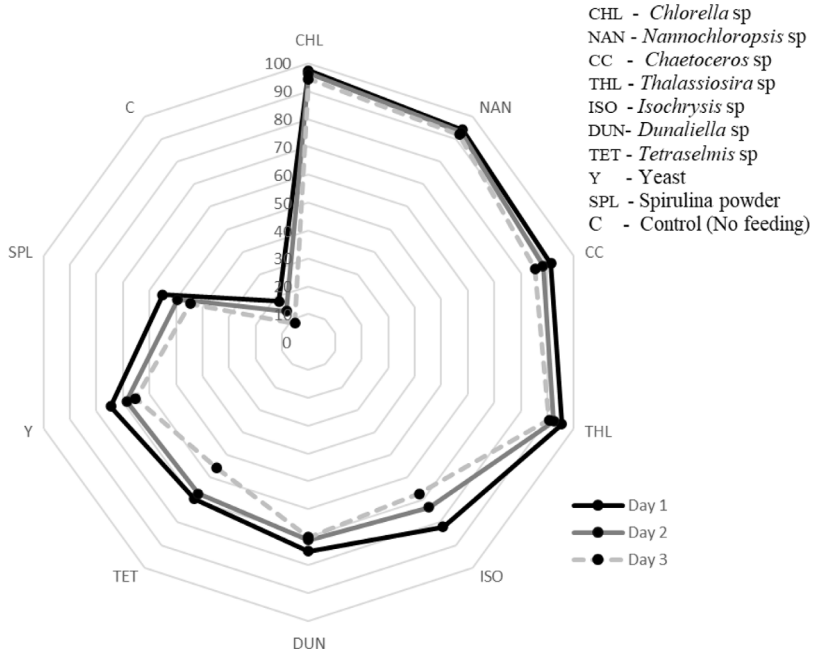

Fig. 3. Percentage survival change of *E. beringi* sp. nov. with culture days using different feed diets.

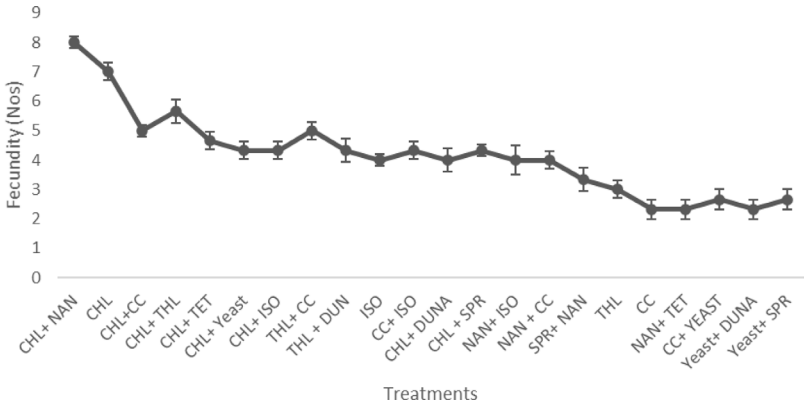

Fig. 4. Fecundity of *E. beringi* sp. nov. fed with different feed diets.

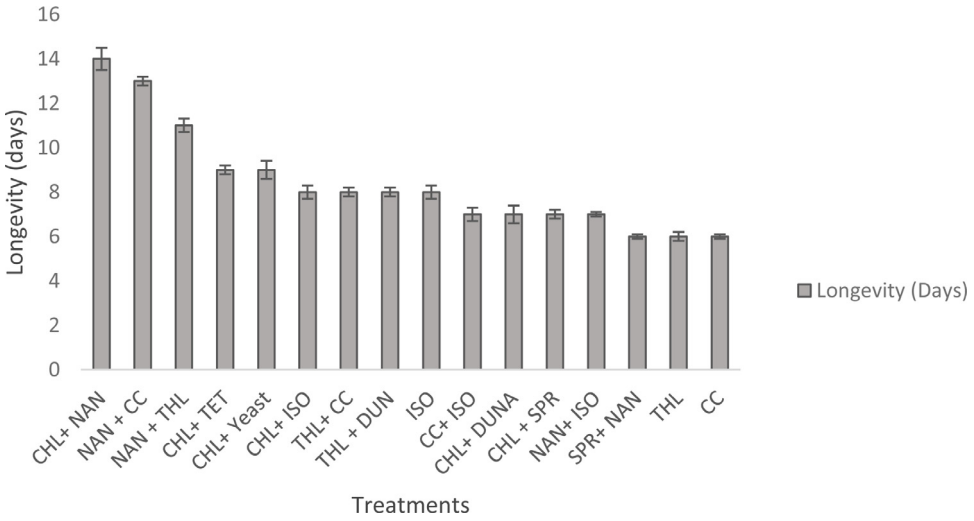

Fig. 5. Longevity (days) of *E. beringi* sp. nov. using different feed diets.

3. Result

E. beringi sp. nov. reproduce parthenogenetically with an average mean length of 300 ± 9.5 μm and reached maturity at length of 866 ± 32.7 μm with highest length of 1098 μm. An *in-situ* experiment for 3 days at different salinity range from 0 to 35‰ observed that *E. beringi* sp. nov. can tolerate a salinity range between 3 to 35‰ with highest survival (Fig. 2) was observed at 25‰ (97.3 ± 1.5%) and lowest at 3‰ (25 ± 4%). There was a significant difference in survival ($p < 0.05$) at lower (3‰) and higher salinity (25‰) treatments. The salinity tolerance study reveals *E. beringi* sp. nov. can survive both in brackish and marine water but not in freshwater.

The number of offspring produced ranges from 2 to 8 per broodstock depending on the type of feed provided during culture period. *E. beringi* sp. nov. fed with live microalgae includes CHL, NAN, THL, CC, TET, DUN and ISO resulted in higher population peak compared to control treatment without feeding, and treatments fed with BY and RB. Percentage change in survival of *E. beringi* sp. nov. fed with different feed diets showed highest in the treatment fed with CHL followed by NAN and THL and minimum survival was observed in the treatment fed with spirulina and yeast (Fig. 3). The result showed a significant difference in the survival ($p < 0.05$) in the treatment fed with different species of microalgae compared to other treatments. Moisture content of *E. beringi* sp. nov. was 93.3 g/100 g. Proximate compositions of *E. beringi* sp. nov. recorded crude protein, crude fat and total ash content 3.42. 0.46 and 1.77 g/100 g respectively (Table 1) and vary depending on the quality of diets provided.

Table 1
Proximate composition of *E. beringi* sp. nov. (per 100 g of frozen sample) with standard protocol.

Test/Parameter	Result	Unit
Energy	22.4	kcal/100 g
Total carbohydrates (by difference)	1.15	g/100 g
Crude protein	3.42	g/100 g
Crude fat	0.46	g/100 g
Total ash	1.77	g/100 g
Moisture	93.20	g/100 g

Microalgal species were used as either monospecific or in combination (1:1) as diets to study the fecundity range and minimum maturation period found that reproduction starts on third and fourth day and average time between clutches was 72 h. Clutch size followed a typical pattern, peaking in the middle of the life span during second to third reproductive events, regardless of the treatments. The highest fecundity was observed in the treatments (Fig. 4) fed with 1:1 combination of NAN and CHL (8 neonates/brood) followed by treatment fed with CHL (7 neonates/brood) with minimum maturation period of 3 days. There is no significant difference ($P < 0.05$) in fecundity range between the treatments. The maximum longevity was observed in the treatment (Fig. 5) fed with NAN + CHL (14 days) followed

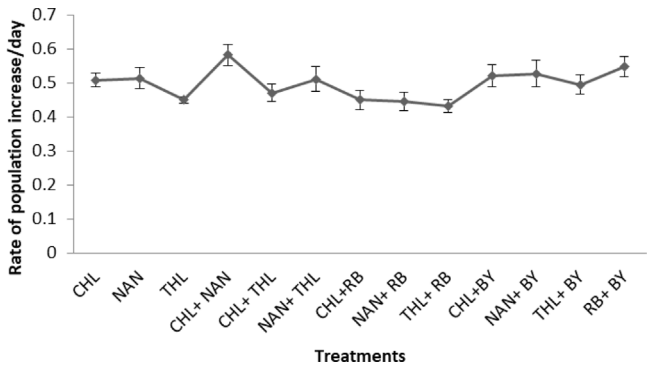

Fig. 6. Rate of population growth of *E.beringi* sp. nov. fed with different feed diets.

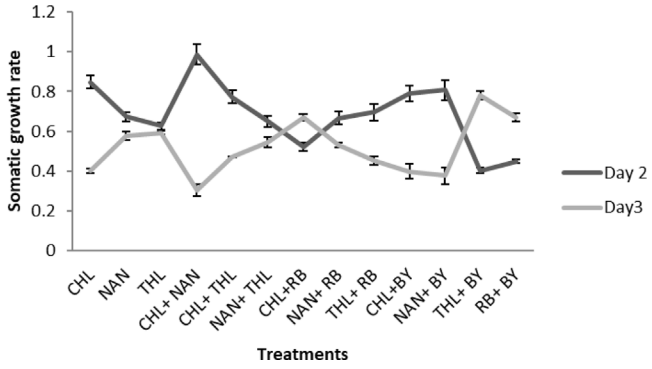

Fig. 7. Somatic growth rate of *E. beringi* sp. nov. on 2nd and 3rd day in different treatments.

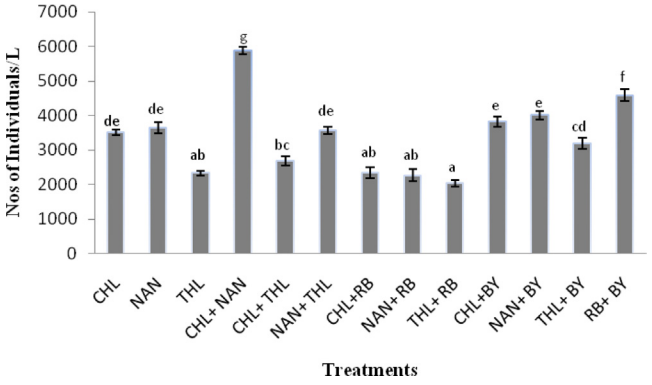

Fig. 8. Mass culture of *E. beringi* sp. nov. (mean ± SE) fed with different feed diets. (Alphabet above bardiagram denotes significant difference between treatments).

by NAN + CC (13 days) and NAN + THL (11 days). Rate of population growth of *E. beringi* sp. nov. per day recorded highest in the treatment fed with CHL + NAN (0.582) followed by RB + BY (0.547) and minimum growth rate was observed in the treatment fed with THL + RB and NAN + RB respectively (Fig. 6). Somatic growth rate of *E. beringi* sp. nov. on second and third day found that highest rate was observed on second day in all treatments except treatments CHL + RB, THL + BY and RB + BY where highest somatic growth rate was observed on third day (Fig. 7).

Based on the fecundity range and minimum maturation period, 4 species of microalgae, RB and BY as monospecific as well as mixed diet (1:1) were used for mass culture of *E. beringi* sp. nov., and maximum production was obtained with a density

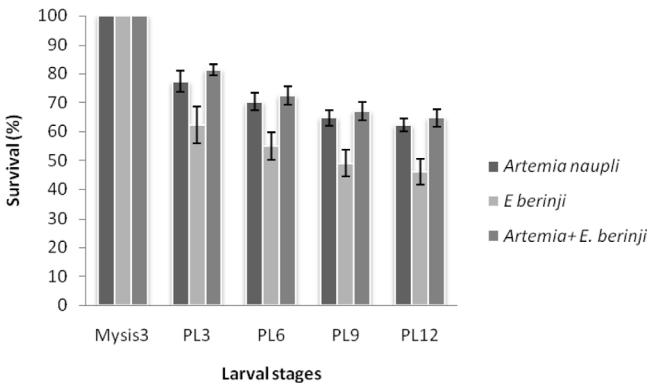

Fig. 9. Percentage survival of early larval stages of *P. indicus* fed with *E. beringi* sp. nov. and *Artemia* naupli.

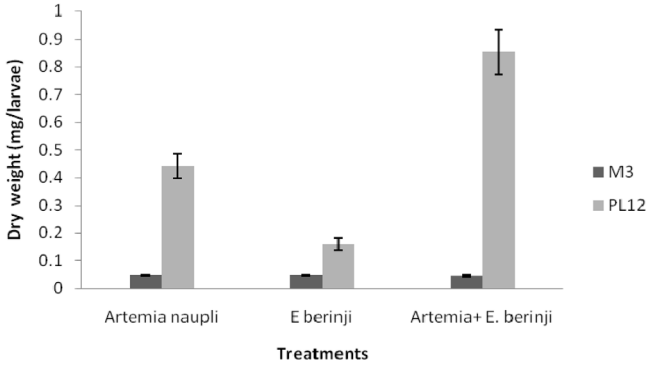

Fig. 10. Dry weight of post larvae of *P. indicus* fed on different diets.

of 5887 ± 109 nos/L followed by 4586 ± 167 nos/L and then 4003 ± 116 nos/L fed with combination of NAN + CHL, RB + BY, NAN + BY respectively (Fig. 8). Microscopic observation of *E. beringi* sp. nov. under 4X magnification (Plate 1) found yellowish orange resembles with artemia nauplii. There is no significant difference ($p < 0.05$) among treatments fed with monospecific and mixed feed diets although highest density was found in the treatment fed with mixed diets.

The role of *E. beringi* sp. nov. as a live feed using *P. indicus* early post larval stages was investigated by replacing *Artemia* naupli with *E. beringi* sp. nov. as monospecific and mixed diets (1:1). Highest survival was found in the treatment fed with combination of *Artemia* naupli and *E. beringi* sp. nov. followed by *Artemia* naupli and treatment fed with *E. beringi* sp. nov. alone (Fig. 9). There was no significant difference among treatment fed with combination of live feeds and treatment with *Artemia* naupli alone. Post larvae fed with *E. beringi* sp. nov. and *Artemia* showed highest IGR (22.5% day^{-1}) followed by *Artemia* naupli (17.3% day^{-1}) and *E. beringi* sp. nov. (9.5% day^{-1}). Dry weight of *P. indicus* larvae higher in the treatment fed with mixed diet than fed with monospecific diet (Fig. 10). The result of experiment demonstrated that during early post larval rearing of *P. indicus* larvae, the period of dependence of costly artemia can be replaced considerably using *E. beringi* sp. nov. at ration of 1:1 combination without any loss in terms of larval development and production.

4. Discussion

The life history of tropical plankton showed fast maturation leads to fast growth and proliferation (Murugan, 2006). *E. beringi* sp. nov. matured within a short duration of 3 to 6 days depending on the quality of feed diets. Selection of suitable feed diets in

Plate 1. Microscopic view of *E beringi* sp. nov. (4X) on 3rd day of culture.

terms of quality and quantity directly affect population growth and survival rate (Zadereev and Lopatina, 2012). *E. beringi* sp. nov. fed with microalgal species showed highest survival rate compare to rice bran and baker's yeast. Selection of microalgae for feeding cladoceran based on the ease of culture and availability, light and temperature tolerance. Cladoceran culture can be maintained at monospecific diets (Sipauba-Tavares and Bachion, 2002; Nandini and Sarma, 2003; Han et al., 2011) and combination of two or more microalgae (Pagano et al., 2000; Pagano, 2008). Uses of mixed diets meet the nutritional requirements of target species compare to monospecific diet (Cai et al., 2007; Puello-Cruz et al., 2009). High price of Artemia cysts has increased shrimp seed production cost, therefore cheaper alternative diets with comparable nutritional quality needed to maintain competitiveness of ornamental fish on global market (Altaff and Mehraj-Ud-Din, 2010; Kumar et al., 2005)). Moina comes under cladocera group are reported as an ideal food organism for larval rearing of penaeid, non penaeid shrimp and catfishes (Masters, 1975; Huisman, 1976; Styczynska-Jurewicz et al., 1979). Nutritional profile of cladoceran is superior than other live food sources for many fish larvae because of their broad size range, ease of digestibility, better growth and development (Sipauba-Tavares and Bachion, 2002; Lemke and Benke, 2003; Sarma et al., 2005; Ismail et al., 2011). Nutritional profile of live food organism will vary with species and also depending on the type of feed diet taken by the organism. The development time, size and reproductive performance of adult cladoceran varies according to type of food preferred for culture (Matias Peralta et al., 2012).

E. *beringi* sp. nov. reproduce parthenogenetic young ones with a minimum size of 300 µm and getting matured within a size range of 866 to 1098 µm within a short duration of 3 days. Cladocerans include *Moina* and *Daphnia* reproduce parthenogenetically under favourable condition and males and ephippia appears under unfavourable conditions (Dodson and Frey, 2001). The present study reveals that *E. beringi* sp. nov. can be cultured within a salinity range of 3 to 35‰ under controlled condition and highest survival was observed at higher salinities. Laboratory culture of *D. australis*, euryhaline cladoceran species survive over salinities ranges from 5 to 33‰ at a temperature of 22.8 °C (Ismail et al., 2011). Brackishwater copepod, *Eurytemora velox* showed less sensitive to low salinity at high temperature and more sensitive to high salinity at lower temperature (Nagaraj, 1988). *E. beringi* sp. nov. fed with *Chlorella* sp and other microalgal species at a density of 1×10^5 cells/ml generated a production of 3–6 neonates/ broodstock. *Moina* species fed with *Chlorella* species at a density of 1×10^6 cells/ml generated a production of 12–14 nos/broodstock (Malla and Banik, 2015). Fecundity and growth of *M. macrocopa* decreased when quantity and quality of feed decreased and population density increased (Loh et al., 2016; Zadereev and Lopatina, 2012). Fecundity of cladocera is adversely affected by concentration of protein, fat and aminoacids such as arginine and histidine (Koch et al., 2011) in diets. Some species of cladocera have an ability to convert acid α linoleic acid into EPA and DHA with varying abilities (Masclaux et al., 2012). Growth rate is a critical parameter for most of the live animals which affect age at first reproduction, body size and other factor of their life history (Mcfeeters and Frozt, 2011). Somatic growth rate of *E. beringi* sp. nov. was higher on 2nd day in all the treatment fed with microalgae monospecifically and as mixed diets and on 3rd day in rest of the treatments. Linear size of cladoceran continues to increase throughout their life cycle, although the somatic growth rate of body length slows down according to size, with maximum somatic growth rates in terms of body length observed on first day. This matches with the earlier reports where specific somatic growth rate of body length of juvenile *M. brachiata* reported to increase with the food concentration (Manuilova, 1964).

Treatment fed with NAN + CHL [5887 ± 109 individual (Ind)/L] has highest density followed by RB + BY(4586 ± 167 Ind/L) within one week culture period at 30 ‰ salinity in outdoor culture system with an initial inoculants of 100 nos/L indicate potential of *E. beringi* sp. nov. to use as live feed for aquaculture practices. Highest production of *Moina micrura* was obtained with a density of 1050–2600 Ind/L (Punia, 1988) and 2500–3500 Ind/L (Majumdar and Nandy, 1989). *M. macrocopa* fed with *Chlorella* sp (1×10^6 cells/ml) produced highest density of about 15 000 to 20 000 Ind/L with an initial inoculants of 40–50 Ind/L when compared to species fed with animal waste such as fowl and cow manure (1301 Ind/L) with an initial stocking density of 45 Ind/L (Ventura et al., 2012; Siddque et al., 2004). Rice bran can be used as feed diet for the mass culture of *Daphnia* and *Artemia* sp (Sorgeloos et al., 1980; Depauw et al., 1981) as it contains various nutrients such as protein (12%–13%), lipid (16%–20%), linoleic acid (6.35–6.85%), acids α linolenate (0.2–0.27%), vitamin B and minerals (6%–9%) dominated by calcium and iron content (Faria et al., 2012; Murtaza et al., 2011). Dried algae are also recorded as an excellent food for live feed culture but they are too expensive to be used at large scale (Altaff and Mehraj-Ud-Din, 2010). Proximate composition of *E. beringi* sp. nov. vary depending of the environmental conditions and type of feed provided during the culture period. Enrichment of zooplankton with microalgae or artificial feed with high in essential fatty acid is very essential prior to feeding to fish and shell fish larvae (Sorgeloos and Lavens, 1996). The enrichment ability of *E. beringi* sp. nov. with different microalgae and enrichment medium is a matter of further study.

The study shown that *E. beringi* sp. nov. release 2–8 number of offspring depending the feed diets and can survive in low dissolved oxygen concentration (2–3 mg/L). Water temperature of 25 to 33 °C and pH of 7–8.2 requires during mass culture with least disturbances. *Moina macrocopa* comes under cladoceran can produce a highest fecundity of 37 eggs/broodstock (Rietzler et al., 2014) and optimum population growth was attained at water temperature of 25–31 °C, pH of 7–8 and dissolved oxygen of greater than 4 mg/L with water hardness of 50 mg/L (Tan and Wang, 2010). Ephippia hatching time of *M. macrocopa* is temperature dependent and takes 8 days at 14 °C and 2.5 days at 28–29 °C (Ivleva, 1973). Highest survival of early post larvae of *P. indicus* observed in the treatment fed with combination of *Artemia* naupli and *E. beringi* sp. nov. (1:1) reveals its potential to use as live feed during larval rearing. For the larval rearing of finfish and shell fish species, mass culture of zooplankton carried out using BY, fish meal and ground trash fish (Rottmann et al., 1992). The statistical

evaluation of rate of dry matter accretion showed that post larvae fed with *Artemia* + *E beringi* sp. nov. or *Artemia* nauplii grew at significantly higher rate than post larvae fed only *E. beringi* sp. nov.. *Artemia* are reported to be replaced by *M. macrocopa* during the larval rearing of *Macrobrachium rosenbergii* (Aniello and Singh, 1982). *M. micrura* mixed with *Artemia* sp at 50:50 ratio given highest survival rate compare to monospecific diet (Alam et al., 1993) apart from its role in toxicological bioassays (Hatakeyama and Yasuno, 1981).

5. Conclusion

The present study standardizes the optimum salinity and varying diet combination to attain highest population growth and reproductive phase under laboratory and mass scale culture of cladoceran, *E. beringi* sp. nov. The incorporation of this brackishwater cladoceran in early larval rearing of *P. indicus* explore its potential to partially replace *Artemia* in light of the spiralling cost of Artemia cyst during shrimp seed production. Proximate compositions of cladoceran warrant the scope for nutrient enrichment of the species with varying diet and enrichment medium. The highest survival, ability to grow on different diet, minimum maturation period, wide salinity tolerance, minimum aeration requirement and, ease of mass culture within short duration observed in this study indicates potential of *E. beringi* sp. nov. to use as live feed diet during the larval rearing of both finfish and shell fish species.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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