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Population growth, nauplii production and post-embryonic development of *Pseudodiaptomus annandalei* (Sewell, 1919) in response to temperature, light intensity, pH, salinity and diets

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The present attempt revealed influence of salinity, temperature, pH, light intensity and diet on survival, fecundity, population density and embryonic development of the marine calanoid copepod, *Pseudodiaptomus annandalei*. Various levels of salinity *viz*., 15, 20, 25, 30, 35, and 40 ppt; temperature (21, 24, 27, 30, 33, and 36 °C); pH (6.5, 7, 7.5, 8.0, and 8.5); light intensity (500, 1500, 3000, and 4500 lux); and different microalgal feed *viz*., *Chlorella marina* (CHL), *Isochrysis galbana* (ISO), *Tetraselmis suecica* (TET), *Nannochloropsis occulata* (NAN), *Dunaliella salina* (DUN), *Picochlorum maculatum* (PICO) and mixed microalgae (MIX) at equal ratio were employed to determine the impact on biology of *P. annandalei*. The better survival and reproduction was achieved under the salinity 25 ppt, temperature 27 °C, pH 8, light intensity 500 lux and with ISO diet. The developmental time was recorded to be short at 25 ppt, 30 ºC, pH 8 and light intensity 500 lux with ISO diet. Although, the highest yield was obtained under those parameter regimes, *P. annandalei* seems to be optimistic with wide range of environmental conditions. This study has confirmed that *P. annandalei* can be cultured at commercial scale as aqua feed and as model organism in toxicity experiments.

[**Keywords**: Developmental rate, Fecundity, Population density, *Pseudodiaptomus annandalei*]

Introduction

Copepods are found abundantly in earth ecosystems and represents to about 80 % of the zooplankton in the ocean[1]. Marine copepods are considered as ecologically and economically important organisms as they help in maintaining the fish reserves by serving as key feed for most of the brackish water and marine fishes. Copepods form momentous trophic link between phytoplankton, microzooplankton and higher tropic levels such as fish and shrimps[2]. The major advantages of copepods over the traditional live feeds (Rotifers and *Artemia*) are their superlative nutritional profile and numerous metamorphosis stages which provide broad spectrum of food size for the farmed young one. Many researchers have attempted the culturing of copepods over the year, but still there is no standard technology that improvises the production up to the need. Conversely, intensive production of copepods in comparable kinds of environmental and water quality conditions are the basic approaches to adjudicate production, growing, and propagative factors. Such facts are vital for the sustainable production of copepods and for their use in aquaculture[3]. Though, not all the copepod species are preferable for large scale production due to their evolutionary behaviours; evaluating the growth parameters of certain copepod species such as survival, development biology and egg or nauplii production will provide the required information for large scale culture of copepods.

In the context of increasing prominence for copepods as feed in aquaculture, there is need of understanding the rudimentary information on their biology. The water quality parameters especially temperature and salinity besides quality and quantity of the diets adjudicate the copepod production in captive condition. The influences of diverse algal regimes on egg producing capacity[4-7], egg hatching success, mortality and development[8,9] in numerous copepods were reported earlier. Certain experiments have investigated the response of paracalanoid copepods to diverse nutrition bases, salinity regimes and temperature[10,11]. Culture of copepods in intensive system is directly dependent on fecundity of female copepods, and also on a measure of the net production rate of adult females[12]. Though, successive hatching, survival and development of nauplii and copepodite can influence the yield of copepod cultures[13,14].

There are ten orders of copepods described so far, of which the culture data exist for only three orders such as Calanoids, Harpacticoids and Cyclopoids. Among these, the calanoid group gets considerable devotion due to their richness in surface waters and easiness of culture in organised circumstances. Copepod, *P. annandalei* is found throughout the year in coastal, estuarine[15] and in brackish waters[16,17] along the tropical and subtropical region[18,19]. This copepod is considered as key diet for most of the brackish water fish larvae in the wild; besides being used as live feed in the commercial aquaculture[20,21]. The objective of this study is to examine the temperature, salinity, pH, light intensity and dietary impacts on endurance, fecundity and reproduction of *P. annandalei* and to ascertain the finest water quality and diet intensities for enhanced production in controlled conditions.

Materials and Methods

Copepod stock culture maintenance

The experimental copepod species *P. annandalei* was collected from the Nagore, coastal waters (Lat. 10º49'37.8048" N; Long. 79º51'49.0608" E), Southeast coast of India, using a zooplankton net with a mesh size of 158 µm. The collected copepods were taken to the laboratory with mild aeration using battery aerators. Then the copepod samples were coarsely filtered through the superimposed sieves to eliminate the nauplii and other larval forms. From the diluted samples, males and females of *P. annandalei* were isolated and initially stocked at 250 mL glass beakers for culture. Then the copepods mass culture was done in 100 L fiber glass tank filled with filtered seawater. The salinity and temperature of culture medium were adjusted to 26 ppt and 28 ºC. *P. annandalei* was fed with mixed algae at a concentration of 30,000 cells/mL for once in two days. The fecal pellets and debris were siphoned out daily and the expelled culture water was replaced with filtered fresh seawater.

Microalgae culture

The microalgae species such as *Isochrysis galbana* (ISO), *Chlorella marina* (CHL), *Picochlorum maculatum* (PICO), *Dunaliella salina* (DUN), *Nannochloropsis occulata* (NAN) and *Tetraselmis suecica* (TET) were cultured separately in glass containers filled with filtered (1µm) and sterilized seawater using Walne medium to feed copepods. The water quality and environmental parameters such as temperature, salinity and light intensity were maintained at 23-25 ºC, 30 ppt and 45-60 mmol photons/m^2/sec, respectively. The microalgae were harvested during exponential phase and fed to copepods.

Experiments

Survival (SUR)

All the copepods used in the survival experiments were starved for 24 hours before the start of experiment. From the stock culture, 10 healthy adults of *P. annandalei* were handpicked and inoculated in a 100 mL glass beaker filled with 50 mL filtered seawater. Parameters such as salinity, temperature, pH, light intensity etc., were adjusted accordingly to the corresponding experiments. The copepods were examined daily and counted for survival. The experiments were done in triplicate and continued for a period of 14 days. The debris and faecal materials were removed daily by siphoning. The culture medium was replaced once in two days to avoid the supremacy of poor water quality over survival of the copepods. The number of live copepods remained on the final day were counted for survival corresponding to each parameter.

Population density (PD)

For the estimation of population density, 5 pairs of adult of *P. annandalei* (1 Male: 1 Female ratio) were stocked in 250 mL glass beaker. The copepods were fed once in every two days. No copepods were removed from the containers during the experimental period of 15 days. On the final day, all the copepods including nauplii and copepodites were filtered through 48 µm sieve. The total copepods added through reproduction over the period of experiment were counted using Sedgewick Rafter counting chamber under microscope. The density of nauplii, copepodites and adult copepods were counted separately.

Nauplii production rate (NPR)

For determining the nauplii production capacity of *P. annandalei*, a gravid female was stocked in a glass test tube containing 20 mL of filtered seawater. The copepod was examined at regular intervals (every 2 hour) for the hatching of nauplii. Once the nauplii released, the adult female was carefully removed from the test tube and the nauplii were counted under the microscope.

Developmental time (DT)

To assess the developmental period, a gravid female was stocked in a clean test tube. The copepod was checked for hatching at regular intervals. After hatching of the nauplii, the adult female was removed from the plate and the nauplii were observed for further development. In this experiment, the time taken for eggs to hatch from spawning (embryonic development), the development of naupliar stages (NI – NVI) and copepodite stages (CI – CVI) were noted and generation time (egg to egg) was also recorded. Few nauplii from each treatment were cultured separately until they spawn to determine the generation time.

Results

Effect of temperature on SUR, PD, NPR and DT

All the examined temperature tests resulted above 50 % of survival except for 36 ºC, which recorded the lowest survival rate (43 %) compared to other tests (Fig. 1a). The highest survival rate was observed in 27 ºC with a staggering 86 % of survival rate. Similarly, the survival percentages tend to decrease with the lower and higher temperature ranges. But there was a gradual decline in the survival of copepods in 36 ºC trials from the very start of the experiment compared to other tests.

The highest number of total copepod population was obtained in 27 ºC with 514 ind L^{-1} (P < 0.05) followed by 30 ºC and 24 ºC with 408 ind L^{-1} and 312 ind L^{-1}, respectively (Fig. 1b). The lowest mean population density was obtained in 36 ºC, which produced only 124 ind L^{-1}. The reason behind the lowest population density at higher temperature may be the fact that many nauplii did not took them to the adult stages and only a portion of the nauplii carried themselves to the copepodite stages.

The nauplii hatching rate of copepods was affected by temperature likewise in the survival too. At higher temperature, the production of nauplii seemed to be decreased (P < 0.001; 16.6 to 7.66 naupli/female). The highest number of nauplii was recorded in 27 ºC with an average of 16.6 nauplii per female followed by 14.3 nauplii in 24 ºC. The lowest number of nauplli was recorded in 36 ºC with only 7.6 nauplii per female (Fig. 1c).

The embryonic development time was found to be shorter in 27 ºC, which hatched at 1.4 ± 0.5 days, followed by 1.7 ± 0.4 and 1.9 ± 0.18 days in 30 and 24 ºC, respectively (Table 1a). The longest embryonic development time was observed in 36 ºC with 2.4 ± 0.27 days. The shortest duration taken for nauplius development (N1-N6) was observed at 5.4 ± 0.4 days in 27 ºC, followed by 30 ºC with 5.8 ± 0.4 days. The copepodite to adult development

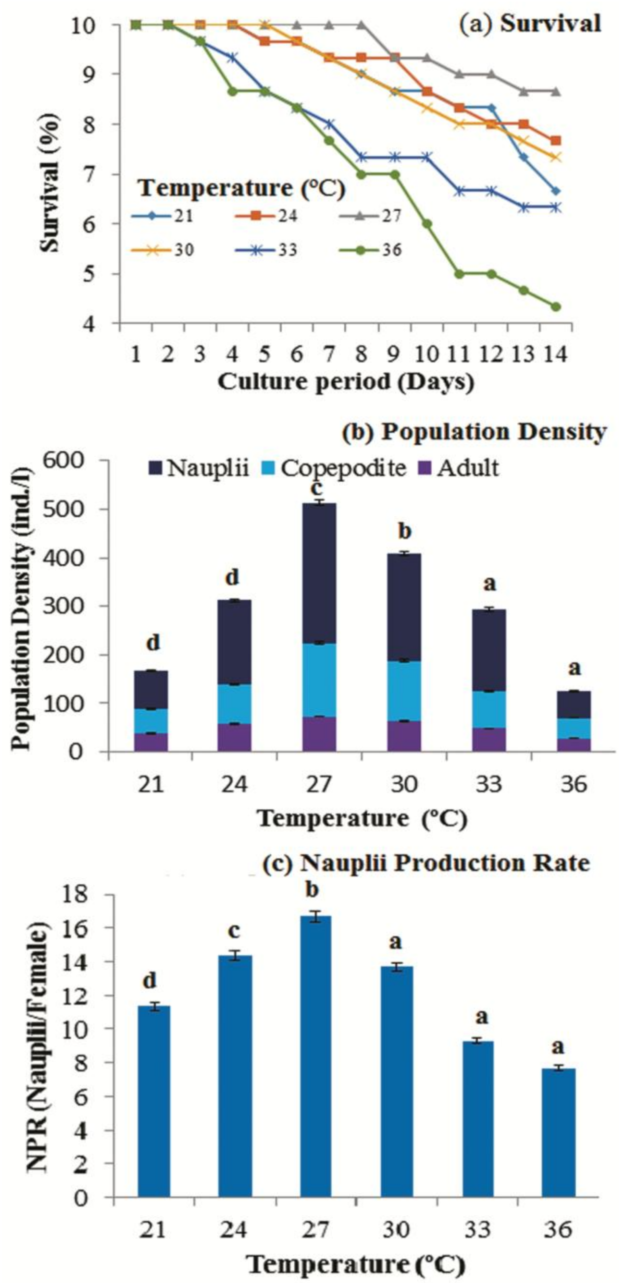

Fig. 1 — Effect of temperature on: (a) survival, (b) population density, and (c) nauplii production rate; of marine copepod *P. annandalei*

Table 1 — Effect of culture environmental conditions and feed on development and generation time of marine copepod *P. annandalei*

Culture conditions	Egg (Days)	N1 - N6	C1 - C6	Generation Time
(a) Effect of temperature (ºC)				
21	2.1±0.2	6.7±0.3	7.8±0.6	16.1±0.4
24	1.9±0.18	6.1±0.5	7.2±0.4	15.8±0.4
27	1.4±0.5	5.4±0.4	6.5±0.5	14.6±0.7
30	1.7± 0.34	5.8±0.4	6.9±0.6	15.1±0.3
33	2.2±0.41	5.9±0.4	7.2±0.3	15.7±0.5
36	2.4±0.27	6.2±0.24	7.4±0.3	16.4±0.5
(b) Effect of light intensity (lux)				
500	1.6±0.24	6.1±0.16	6.6±0.21	15.4±0.3
1500	1.9±0.24	6.3±0.2	6.7±0.31	15.8±0.4
3000	2.1±0.46	6.9±0.32	7.4±0.16	16.2±0.23
4500	2.3± 0.32	7.1±0.6	7.1±0.23	16.7±0.41
(c) Effect of salinity (ppt)				
15	2.3±0.4	7.1±0.5	7.8±0.4	16.7±1.2
20	2.1±0.37	6.9±0.4	7.4±0.4	16.4±0.5
25	1.6±0.41	5.8±0.3	6.2±0.2	15.2±0.2
30	1.8±0.26	5.9±0.3	6.4±0.1	15.8±0.3
35	1.8±0.58	6.8±0.3	7.4±0.3	15.8±0.2
40	2.8±0.73	7.4±0.4	8.2±0.28	17.4±0.3
(d) Effect of pH				
6.5	2.6±0.4	6.9±1.3	7.6±0.8	16.8±0.5
7	2.3±0.32	6.4±0.32	7.2±0.8	16.1±0.3
7.5	2.1±0.3	6.5±0.43	6.8±0.6	15.8±0.4
8	1.9± 0.24	5.8±0.32	6.7±0.7	15.4±0.9
8.5	1.6±0.27	5.9±0.46	6.5±0.3	15.8±0.7
9	1.9±0.34	6.7±0.6	7.2±0.3	16.3±0.4
(e) Effect of feed				
CHL	1.6±0.42	5.6±0.3	7.6±0.2	15.2±0.4
ISO	1.4±0.3	5.2±0.6	6.8±0.7	13.8±0.4
TET	2.1±0.4	6.5±0.3	8.2±0.4	16.3±0.5
NAN	2.3±0.56	6.1±0.7	7.8±0.2	15.6±0.3
DUN	2.1±0.28	6.7±0.4	7.2±0.5	16.1±0.3
PICO	1.9±0.41	5.8±0.5	7.1±0.1	14.8±0.3
MIXED	1.8±0.26	5.9±0.3	7.3±0.2	15.1±0.4

(C1-C6) was shorter in 27 ºC with 6.5 ± 0.5 days and longer in 21 ºC with 7.8 ± 0.6 days. The shortest generation time of 14.6 ± 0.7 was observed in 27 ºC whereas it was longer in 36 ºC with 16.4 ± 0.5 days.

Influence of light intensity on SUR, PD, NPR and DT

During the experiment of effect of light intensity on copepods, all the tested trials showed above 50 % of survival (Fig. 2a). Among the light intensity experiments, 500 lux showed highest survival (83 %), while the lowest survival rate was observed in the light intensity of 4,500 lux with only 53 %. It was clearly observed that the increase in the light intensity in copepod culture eventually reduced the rate of survival.

The maximum population density in copepods was achieved in the light intensity trial of 500 lux which produced about 696 ind L^{-1} ($P < 0.01$), including 98 adults, 138 copepodites and 460 nauplii; followed by 1,500 lux which produced a total of 508 ind L^{-1} comprising of 78 adults, 110 copepodites and 320 nauplii. The minimum population density was recorded in 4,500 lux i.e. 326 ind L^{-1} including

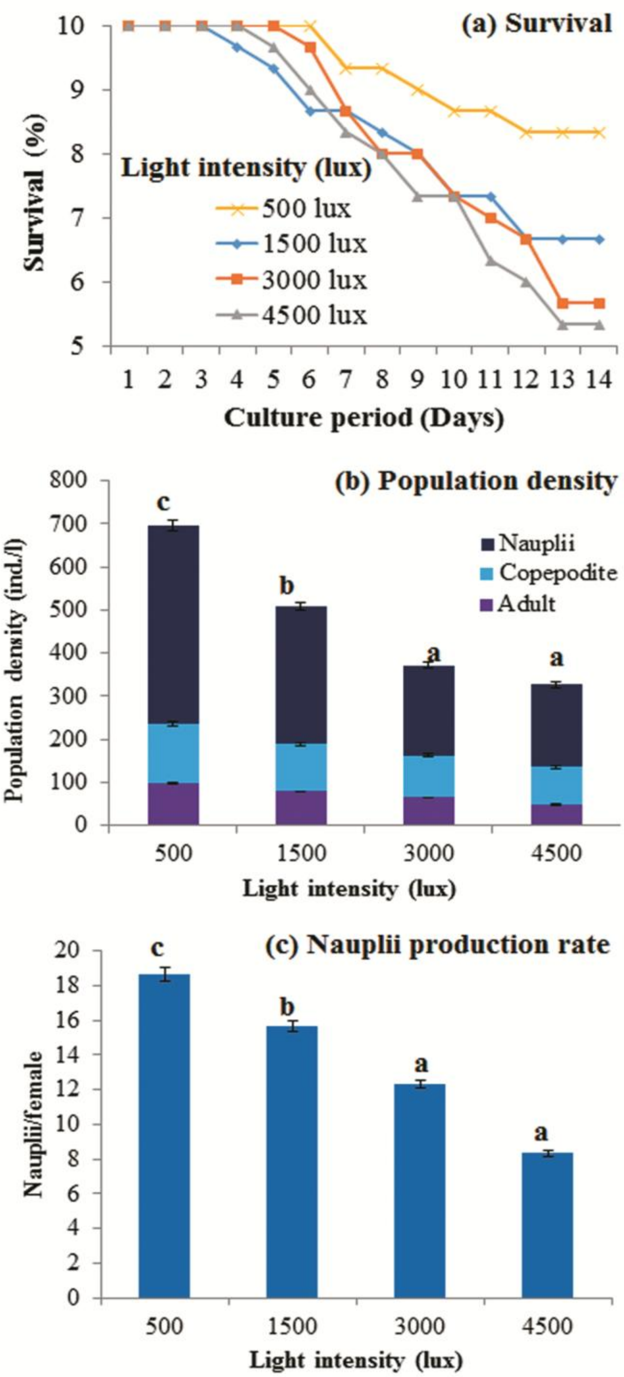

Fig. 2 — Effect of light intensity on: (a) survival, (b) population density, and (c) nauplii production rate; of marine copepod *P. annandalei*

48 adults, 88 copepodites and 190 nauplii ($P < 0.05$; Fig. 2b).

The highest number of nauplii was produced by the copepods which were exposed to the minimum range of light intensity and the production rate was observed to be decreasing with higher illumination. An average of 18.6 nauplii female^{-1} ($P < 0.001$) was recorded in copepod exposed to 500 lux, whereas the lowest value was obtained in copepod exposed to 4,500 lux which produced only 8.3 nauplii female^{-1} ($P < 0.05$; Fig. 2c).

The lowest embryonic development period was recorded in 500 lux (1.6 ± 0.4 days) and the longest duration was recorded in 4,500 lux (2.6 ± 0.3 days; Table 1b). The minimum duration of naupliar development was recorded in 500 lux with 6.1 ± 0.16 days and the longest duration was recorded in 4,500 lux with 7.1 ± 0.6 days. The copepodite stages were developed in a short period when they exposed to 500 lux (6.6 ± 0.2 days) and the longest duration took place in 4,500 lux with 7.9 ± 0.2 days. The generation time was also shorter in copepods exposed to 500 lux (15.4 ± 0.3 days), whereas the copepods exposed to 4,500 lux took 16.7 ± 0.1 days.

Effect of salinity on SUR, PD, NPR and DT

All the tests showed above 50 % of survival rate and total mortality was not observed in any test. However lack of liveliness was observed in animals cultured at higher salinity. Among the different variants of salinity tested, the highest percentage of survival rate was obtained at 25 ppt with 86 % of survival rate followed by 83 % at 30 ppt (Fig. 3a); whereas, 40 ppt salinity showed the lowest survival rate (53 %). The survival rate of copepods at 40 ppt had started a rapid decline from the 4th day itself, but few copepods managed to withstand the elevated salinity in the final few days of the experimental period.

The average total number of copepods (inclusion of nauplii and copepodites) at the end of an experimental period of 15 days is shown in Figure 3(b). The highest number of copepod density was attained at 25 ppt with a total of 542 ind/L ($P < 0.01$) whereas the lowest density was counted at 40 ppt, with an average of 244 ind/L. The recorded lowest value of copepod density at 40 ppt is might be due to the mortality of newly hatched nauplii which could not withstand the higher salinity and only few nauplii were developed into a copepodite stage.

Likewise in survival test, the nauplii producing capacity of *P. annandalei* was considerably affected by the salinity. An average of 20 nauplli ($P < 0.001$)

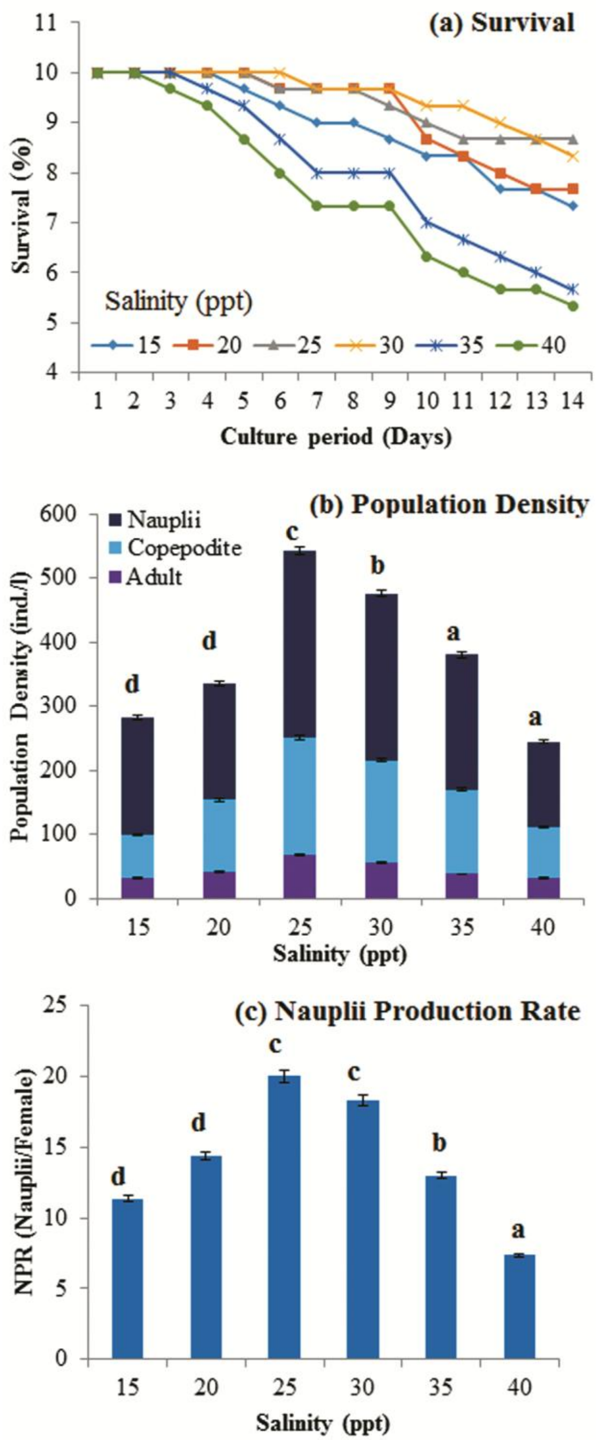

Fig. 3 — Effect of salinity on: (a) survival, (b) population density, and (c) nauplii production rate; of marine copepod *P. annandalei*

were produced by a female in a single brooding at 25 ppt, which is highest among the trials followed by 30 ppt which produced 18.3 naulii per female (Fig. 3c). The lowest number of nauplii was produced at 40 ppt salinity at an average of 7.3 nauplii ($P <$

0.05) per female. The nauplius production rate seemed to be decreasing with increased salinity.

The lowest embryonic development period of 1.6 ± 0.26 days was recorded at 25 ppt, whereas the longest embryonic development period was recorded at 40 ppt in 2.8 ± 0.73 days (Table 1c). The development period for naupliar stages (N1-N6) took 5.8 ± 0.3 days in 25 ppt, which is the shortest duration among all the trials. The longest time taken for the development of naupliar stages (N1-N6) was 7.4 ± 0.4 days in 40 ppt. The development time taken for copepodite stages (C1-C6) was shortest in 25 ppt with 6.2 ± 0.1 days and the longest development period occurred in 40 ppt with 8.2 ± 0.28 days. Likewise the shortest generation time was recorded in 25 ppt with 15.2 ± 0.3 days and the longest duration was recorded in 40 ppt with 17.4 ± 0.3 days.

Effect of pH on SUR, PD, NPR and DT

All the tested pH variants resulted above 50 % of survival rate except for pH 6.5 which showed only 43 % of survival rate, the lowest among all the pH trials (Fig. 4a). The pH 8.5 showed the highest survival rate with 83 % followed by pH 8 (80 % of survival). In pH 6.5, the survival of copepods seemed to be decreasing daily at a constant rate.

The mean population density of copepods in different pH trials were calculated after 15 days in which the pH 8 produced the highest number of copepods with 512 ind L^{-1} ($P < 0.001$) comprising of 86 adults, 162 copepodites and 264 nauplii (Fig. 4b). The pH 8.5 also produced an adorable number of nauplii when compared to other trials. The lowest population density was obtained in pH 6.5 with only 113 ind L^{-1} ($P < 0.01$) comprising of 14 adults, 38 copepodites and 61 nauplii.

From the obtained results, it is clear that pH play a substantial role in the nauplii production of copepod. Among the tested trials, the pH 8 produced the highest number of nauplii with 19.3 nauplii female^{-1} ($P < 0.001$), while pH 6.5 produced the lowest number of nauplii with 6.3 nauplii female^{-1} ($P < 0.05$; Fig. 4c). The naupliar production rate was evidently lower in the acidic pH.

Likewise all other parameters examined, pH also showed an inevitable impact in the developmental duration of copepods. In the present study, the shortest embryonic developmental period was recorded in pH 8.5 in which nauplii hatched in 1.6 ± 0.27 days followed by pH 8 with 1.9 ± 0.24 days (Table 1d). The longest embryonic developmental

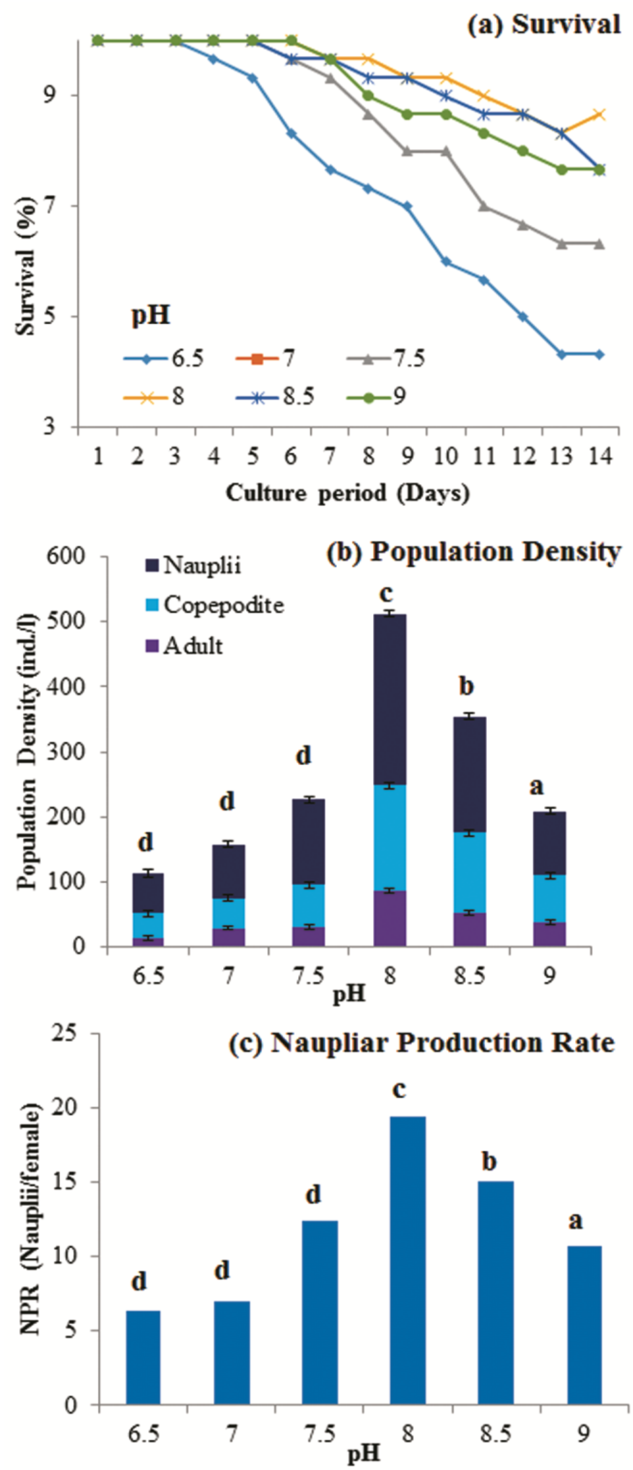

Fig. 4 — Effect of pH on: (a) survival, (b) population density, and (c) nauplii production rate; of marine copepod *P. annandalei*

duration was observed in pH 6.5 with 2.6 ± 0.42 days. The development time for naupliar stages (N1-N6) was found shorter in pH 8 which took 5.8 ± 0.32 days and the longest duration was recorded in pH 6.5

with 6.9 ± 1.3 days. The duration for copepodite to adult development was shorter in pH 8.5 with 6.5 ± 0.3 days and longer in pH 6.5 with 7.6 ± 0.8 days. The generation time was recorded shorter in pH 8 with 15.4 ± 0.9 days and longer in pH 6.5 which took 16.8 ± 0.5 days.

Effect of diet on SUR, PD, NPR and DT

Among the seven different microalga feeds tested, ISO supported highest survival percentage i.e. 83 % followed by CHL with 76 % (Fig. 5a). The MIX algal feed also showed better survival rate when compared to other test feeds such as TET, NAN and DUN in which TET showed the lowest survival rate of 53 % followed by NAN and DUN showing 56 % of survival each.

The mean population density of copepods after the experimental period of 15 days with different algal feeds showed that the highest number of copepods were produced in ISO diet (557 ind L^{-1}; $P < 0.001$) followed by PICO which produced 460 ind L^{-1} ($P < 0.01$; Fig. 5b). The lowest number of population density was recorded in TET with only 220 ind L^{-1} followed by DUN with 300 ind L^{-1} ($P < 0.05$).

The observed results suggest that the maternal feeding impacts the production of nauplii in copepods. The highest naupliar production rate was observed in ISO with an average of 21 nauplii $female^{-1}$ ($P < 0.001$) followed by 19.3 nauplii $female^{-1}$ in PICO ($P < 0.01$; Fig. 5c). The minimum number of nauplii production was recorded in DUN which produced only 13 nauplii $female^{-1}$ ($P < 0.05$). The MIX algal feed produced 17.6 nauplii $female^{-1}$.

The minimum duration taken for the embryonic development was recorded in ISO diet which hatched within 1.4 ±0.3 days followed by CHL and MIX algal diets which hatched within 1.6 ± 0.42 and 1.8 ± 0.26 days respectively (Table 1e). The longest embryonic development time was recorded in NAN which took 2.3 ± 0.56 days to hatch. The development time for naupliar stages (N1-N6) was found shorter in ISO diet which took 5.2 ± 0.2 days and the longest duration was recorded in DUN with 6.7 ± 0.7 days. The duration for copepodite to adult development was shorter with ISO feed i.e. 6.8 ± 0.7 days and longer in TET with 8.2 ± 0.4 days. The generation time was recorded shorter in ISO with 13.8 ± 0.4 days and longer in TET which took 16.3 ± 0.5 days. In general, ISO diet aided the shortest development period of both nauplii and copepodite stages when compared to all other diets tested.

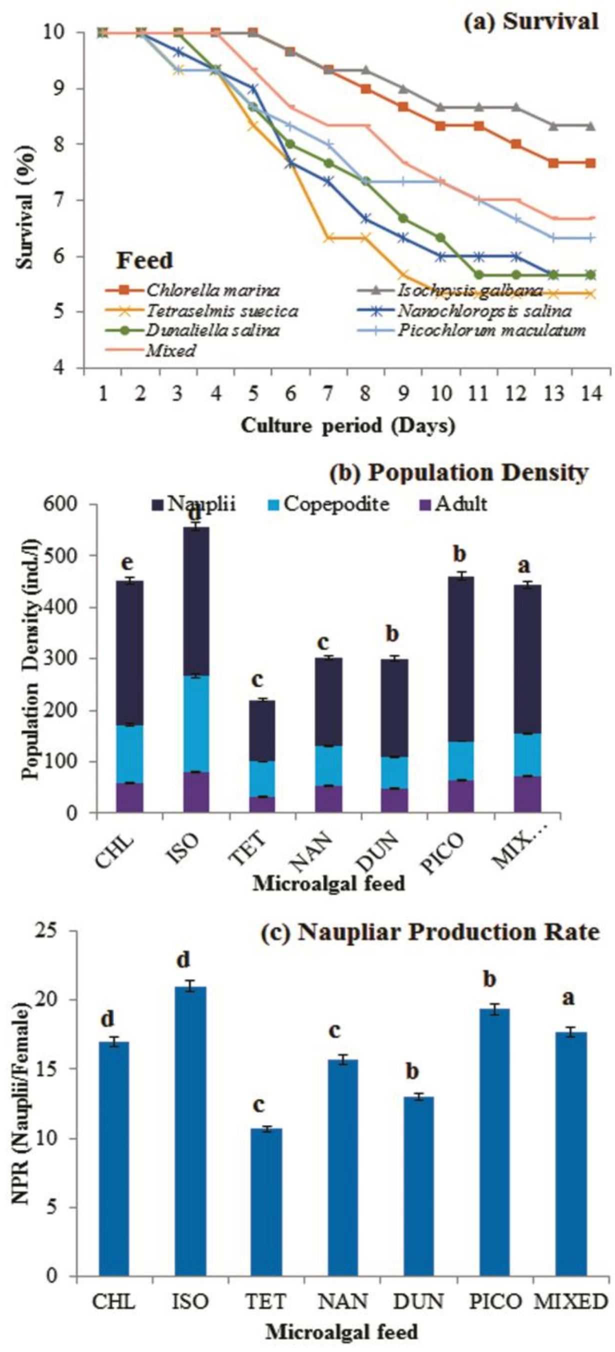

Fig. 5 — Effect of different diet on: (a) survival, (b) population density, and (c) nauplii production rate; of marine copepod *P. annandalei*

Discussion

The attainment of any culture system largely depend upon survival and egg production rates of the cultured animals, which are limited by food availability, water temperature, pH, light and salinity[22]. The water temperature could severely affect the respiration, reproduction, growth, development and gut evacuation of marine copepods[23]. The present findings revealed that the environmental parameters have strong and substantial effects on the overall survival, nauplii production, population density and developmental time of *P. annandalei*. This experimental study concluded that the calanoid copepod *P. annandalei* can survive and reproduce in a wide range of salinity (15 to 40 ppt) and at different temperatures (21 to 36 ºC). Many authors have clarified that *P. annandalei* is a well suited copepod species for culturing in a large scale as it can tolerate a wide salinity range of 3-30 ppt[15,18,24]. The present results showed that *P. annandalei* was able to survive and reproduce better and develop over a temperature range from 21 to 33 ºC. Peters and Downing[25] stated that filtering and grazing rate has been increased with increase in temperature from the low to high up to certain level. The *Pseudodiaptomus* species cannot withstand higher temperature as they follow a diel vertical migration pattern. In which, they stay on the bottom during daytime and drift to the surface water during night, and remain in pelagic realm until dawn[26]. The temperature above 30 °C resulted in lengthened development from nauplius to adult stage. An elevated temperature could also impact on reproductive parameters by controlling the metabolic activity of copepods[27]. Apart from water quality parameters, algal prey also contributed significantly in reproduction of the copepod. Among the various diets tested, ISO was found to be very influential in *P. annandalei* culture and it may be due to the presence of DHA and EPA in ISO. This result was also supported by Lee *et al*.[28] in brackish water cyclopoid copepod *Paracyclopina nana* culture experiments.

The artificial illumination normally inhibits the growth, development and reproduction rate of aquatic invertebrates[29]. In this present study, it was clearly found that increased light intensity negatively impact the production and development rate of copepod *P. annandalei*. The changing illumination affects the endocrine activity in marine copepods and thereby directly affects reproduction, maturation and also impact spawning, hatching, and survival[30]. The light sensitivity and vision in copepods depend on the compound lateral eyes and on a dermal light sense[29]. Increased artificial illumination impels the copepod to invest most of their energy in metabolic activities than under normal lighting. In this study, *P. annandalei* cultured under low light intensity (500 lux) exhibited maximum production compared with high light intensity levels (4,500 lux). The important reason to infer these results are related to stress and energy consumption by copepods for living under intense light conditions.

In general estuarine copepods are known to have a wide range of tolerance to salinity changes as they are inhabiting in different natural habitats with periodic exposure to salinity fluctuations. But the rate of survival and reproduction were limited and controlled accordingly with increased and decreased values of each environmental parameter. The maximum population and higher reproductive activity were observed at 25 ppt salinity and at 27 ºC temperature. The salinity above 35 ppt was not found suitable for reproduction and population growth of *P. annandalei*, perhaps due to the physiological stresses at high salinity.

The fluctuations in pH might be adversely affecting the survival and reproduction of copepods. While in the ocean, the copepods likely to move some other places in terms of depth with sudden changes in pH[31]. In this study, it was found that pH considerably affected the survival and reproduction of copepod *P. annandalei*. The maximum survival was recorded in pH 8.5 and the minimum was recorded in pH 6.5. Similar results were found in *Oithona similis* where the survival rate was decreased with high pCO_2 than the control[32]. The nauplii production rate of *P. annadalei* was significantly higher in pH 8. The acidic pH 6.5 may adversely affect the copepod nauplii production than the alkaline pH 9. The development period for nauplii and copepodite was found to be shorter in pH 8 and 8.5 and the development time was longer in acidic pH (6.5).

Our study reveals the influence of microalgae diet on the survival and nauplii production of copepod *P. annandalei*. In case of diet, ISO proved to be a prominent feed for increasing the survival of copepods. Many have suggested mixed algal feed as a better option for copepod culture. But this study confirmed that the *Isochrysis galbana* is well suited for *P. annandalei* for its feeding style and also due to its smaller cell size (~3–6 μm), it is suitable to feed all the developmental stages. Minimum survival and reproduction was observed in DUN and NAN could be ascribed to the poor nutritional quality of the microalgae. Many workers have stated that the *I. galbana* is suitable feed for most of the filter feeding copepods, because of the high DHA content which can help better survival and reproduction[33]. This study also inferred that the *P. annandalei* could produce maximum nauplii when fed on *I. galbana* at an optimum concentration of 30,000 cells/ml.

These results attributed to the fact that the presently maintained ranges of environmental parameters are very similar to that of the natural habitat of copepods where it was collected. It is inferred that the water temperature affects respiration, reproduction, growth, development, and gut evacuation of marine copepods[34,35]. In natural environment, most of the copepods are personalized to deal with seasonal changes in temperature, salinity and feed[13]. Particularly, estuarine copepods have broad tolerance to a range of salinities. Therefore, in the present study temperature, light intensity, pH, salinity and diet conditions were optimized to assess population, nauplii production and developmental biology of *P. annandalei*. This is a useful attribute for aquaculture, as copepods are used as live feed for fish larvae and proved to remain alive and available in the water column under various water quality and environmental conditions that are suitable for the culture of fish larvae[6].

Conclusions

The environmental parameters are very important for the well-being of live feed production. This study have established that *P. annandalei* cultured under different levels of salinity, temperature, pH, light and feed can assist the animal for better survival and also reproduce very well. The culture environmental parameters like 25 ppt of salinity, 27 ºC of temperature, 8 to 8.5 pH, 500 lux of light intensity and *Isochrysis galbana* feed provide better survival, total population and high nauplii hatching which could make *P. annandalei* as good live feed candidate for aquaculture industry.

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Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

MK: Conceptualization, methodology, sample analyses, and writing - original draft; PS: Designing of work, resources, review & editing; SA: Sample analyses, and writing; SDK: Editing the draft, statistical analyses, plotting graph; PR: Sample analyses and data analyses; and SK: Editing draft and review.

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