Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Effect of temperature and algal food on egg production and hatching of copepod, *Paracalanus parvus*

P. Santhanam*, N. Jeyaraj and K. Jothiraj

Department of Marine Science, Bharathidasan University, Tiruchirappalli-620 024, India

**Corresponding Author email : sanplankton@yahoo.co.in*

Publication Info

Paper received:
23 September 2011

Revised received:
11 February 2012

Accepted:
26 May 2012

Abstract

There are relatively few reports on the influence of environmental factors on the development and reproduction of calanoid copepods. In this study, we establish over the 12 days culture experiment at different temperature (18, 20, 25, 30 ^{0}C) and different algal cell densities 1000, 5000, 10,000, 20,000 cells ml^{-1}. The results revealed that the high egg production (42.33 eggs female^{-1} day^{-1}) and hatching rate (92.65%) was reported in the temperature of 25 ^{0}C. It is understood that the egg production of *P.parvus* clearly affected by temperature was supported by ANOVA value (F= 4.792909) while the egg production and hatching rate was found to be high as 53.5±4.94 eggs female^{-1} day^{-1}, 96.26% respectively with 20,000 cells ml^{-1}, The temperature of 25 ^{0}C and algal concentration was positively supported by ANOVA . The present study concluded that the temperature of 25 ^{0}C and food concentration of 20,000 cells ml^{-1} was found to be favorable water temperature and algal food concentration to obtain high fecundity and hatching success in copepod, *P. parvus.*

Key words

Copepod, *Paracalanus parvus*, Temperature, Food concentration

Introduction

An adequate production of quality of live food microorganisms at a lowest possible cost is essential for the successful development of a commercial fish or shrimp hatchery (Stottrup, 2003). Marine copepods are a critical component of marine trophic food webs and are the main prey item for a vast majority of marine fish larvae (Farhadian *et al*., 2009). Rotifers, *Brachionus* spp., and brine shrimp, *Artemia* spp. are live feed organisms commonly fed to cultured larval fish, primarily due to their availability and convenience of culture (Olivotto *et al*., 2008). Natural deficit in the nutritional composition of *Artemia* nauplii and rotifers require an "enrichment" process to occur before being fed to larvae. In this circumstance, marine copepods are considered to be one of the most important grazers in pelagic ecosystems. A key species in marine ecosystems is the calanoid copepod. *P.parvus* is one of the dominating copepods in tropical waters due to their continuous breeding nature, high reproductive capacity besides its general adaptability to the prevailing environmental conditions. Several earlier workers reported that the copepod, *P.parvus* as common one in Indian estuaries and mangrove ecosystem (Karuppasamy and Perumal, 2000; Santhanam and Perumal., 2003; Vengadeshperumal *et al*., 2009; Santhanam *et al*., 2012). The copepods present in the Indian waters have been studied well taxonomically, but biologically has not been well studied. However, inadequate knowledge of the influence of environmental factors on fecundity or egg production of *P.parvus* is not reported previously.

Several investigations were made earlier in experimental study in various copepod species (Ku Kang *et al*., 2000; Ara, 2001; Longoria, 2003; Chinnery and Williams, 2004; Milione and Zeng, 2008; Farhadian *et al*., 2008; Rhyne *et al*., 2009; Devreker, 2009). But very limited reports are available on the biology of our Indian copepods including effect of temperature, food concentration on varies species of copepod reproductive biology (Shrivastava *et al*., 1999; Perumal *et al*., 2000; Santhanam and Perumal, 2012). Hence, an attempt was made on the effect of temperature and algal food concentration on the reproduction of

P.parvus. Such information is most valuable for the mass culture of this species and also adds to understanding of the influence of environmental factors on calanoid reproduction.

Materials and Methods

Algal feed : Pure strains of the algae viz: *Chlorella marina*, *Dunaliella* sp., *Isochrysis galbana, Nannochloropsis* sp., *Coscinodiscus centralis, Chaetoceros affinis* and *Skeletonema costatum* were obtained from the Central Institute of Brackishwater Aquaculture (ICAR), Chennai and Central Marine Fisheries Research Institute (ICAR), Cochin. All the species of algae were grown at 25°C, 30‰ salinity, and 14 l: 10 D light regimes and fertilized with f/2 medium (Guillard and Ryther, 1962). The algae were harvested during the log phase (approximately 35,000 cells ml^{-1}) for feeding to the copepods.

Copepod culture: Zooplankton samples were collected using 158 µm mesh size plankton nets (0.35 m diameter opening) from the Muthupet mangrove waters (10º 20'N latitude and 79º 35'E longitude) early in the morning during the full moon phase. The samples were immediately transported to the laboratory. Samples were screened through a 500 µm mesh to eliminate the fish and prawn larvae. Then the samples were rinsed for 2 hr in a zooplankton washer (190 µm mesh) to remove rotifers and nauplii of copepods and barnacles. After rinsing, the remaining adult copepods and larger copepodids were used to start the culture in a rearing container (fiberglass tank 550 mm diameter, 850 mm height) filled with 200 l UV filtered seawater which was daily changed and vigorously aerated. Rearing containers were covered with nylon cloth to prevent excessive evaporation. Seawater was filtered through a membrane filter (pore size 500 micron). Temperature, salinity, pH and dissolved oxygen were maintained at between 28 and 32°C, 30 and 34‰, 7 and 8.5 and 5 and 6.8 ml^{-l}, respectively during the rearing period. The copepods were cultured and fed equal quantities of 7 algal species each at 30,000 cells ml^{-1}.

Temperature experiments : The adult male and females copepod, *P.parvus* maintained under food saturated conditions were placed in pyrex test tubes (2.5 cm mouth diameter; 15 cm deep) containing 20 ml of the culture water. The temperature below 25ºC was maintained by using air conditioning and above 25ºC was using a immersion heater in the water bath. The water was stirred using an air stone to avoid overheating. Eggs produced in each temperature effect were placed on a watch glass and counted under binocular microscope. The egg production rate was determined by incubating eggs in a test tube placed in air-conditioned room by maintaining temperatures of 18, 20, 25 and 30ºC. The experiments were conducted in triplicate.

Algal food concentration experiments : The male and female copepods were isolated and kept in beakers containing filtered seawater and starved for 24 hours. Food concentrations mixtures of seven algal species were diluted from stock cultures of known concentrations determined by microscopic count. The food was kept constant at 1000, 5000, 10,000 and 20,000 cells ml^{-1}, with one control containing filtered water without algae. During the experiment, a stirrer agitated the water in the beakers at 2-6 hr intervals. Cell counts were measured before and at the end of the experiment. Temperature was kept constant at 25±3ºC. The beakers were covered with black cloth for reduced illumination. After the experiment, survival of the male and females were checked and the eggs were counted.

Hatching succession : To estimate the hatching success, laid eggs were incubated in test tubes along with filtered seawater for 96 hrs. The hatching rate was determined by incubating eggs in a test tube placed in air-conditioned room by maintaining temperatures of 18, 20, 25 and 30 ºC. The hatched out nauplii from individual test tube were placed in petriplates and counted under binocular microscope. For the experiment on algal concentration effect on copepod hatching, the eggs were placed in test tubes with filtered seawater fed with following algal concentrations: 1000, 5000, 10,000 and 20,000 cells ml^{-1} with one control containing eggs without algae. The nauplii released were counted under binocular microscope. The experiments were conducted in triplicate.

Results and Discussion

The minimum egg production (8 eggs female^{-1}) was observed at temperature 18ºC while the maximum number of eggs (45.33 eggs female^{-1}) was noticed at the temperature of 25 ºC. At 30 ºC, the mean egg productions slightly decreased to 32 eggs female^{-1} (Table 1).

The egg production was found to increase with increasing algal food concentration (Table 2). The highest mean egg production (53.5 eggs female^{-1} day^{-1}) was achieved with the highest food concentration of 20,000cells ml^{-1}, while the lowest mean egg production (9) was noticed in the lowest food concentration of 1000 cells ml^{-1}. The egg production and hatching of *P. parvus* were highly significant with temperature, food concentration (r=0.99541, r=0.99629, respectively).

The lowest percentage of hatching (58.25%) was observed at 18±2ºC whereas the highest hatching of 92.65% was reported at 25±3ºC (Table 1). At lowest food concentration, the hatching percentage was recorded as 44.44% and maximum of 96.26% was noticed at maximum

Table 1 : Mean egg production and hatching rate of *P. parvus* at different temperatures

Temperature (°C)	Egg production rate (Eggs female^{-1} day^{-1})	Hatching rate	Hatching (%)
18±2°C	8.0±2.82	4.66±2.12	58.25
20±3°C	16.0±3.53	12.50±0.00	78.12
25±3°C	45.3±1.41	42.00±2.12	92.65
30±1°C	32.0±3.05	26.00±2.30	81.25

Values are mean of replicates ±SD

Table 2 : Mean Egg production and hatching rate of *P.parvus* in different algal food concentration

Algal concentration (cells ml^{-1})	Fecundity rate (Eggs female^{-1} day^{-1})	Hatching rate	Hatching (%)
1000	9.0±2.08	4.0±0.57	44.44
5000	14.5±2.82	10.0±2.82	68.96
10000	30.5±2.12	26.0±1.41	85.24
20000	53.5±4.94	51.5±5.65	96.26

Values are mean of replicates ±SD

food concentration. Correlation between food concentration and hatching was highly significant (Table 2).

Present result showed that the temperature and algal food concentration had significant effects on egg production and egg hatching of *P.parvus*. It is known that the temperature has a major effect on metabolic rate and at suboptimal temperature, higher maintenance costs may reduce the level of investment in reproduction (Hirche and Halsband, 2001; Chinnery and Williams, 2004; Milione and Zeng, 2008). In this study, the optimum temperature for egg production was found to be 25°C. The egg production of *P.parvus* were clearly affected by temperature, which is supported by ANOVA value (F=4.792909). The more number of eggs were produced at the optimum temperature of 25 °C while low eggs were noticed at low temperature of 18 °C. In contrast, the egg production decreased at unstable temperature of 30 °C as reported earlier by Milione and Zeng (2008) in calanoid copepod *Acartia sinjiensis*.

The egg production and hatching rate are normally lower at low temperature, and generally increase with increasing temperature up to a thermal threshold, after which decline begins. Such a trend has been reported for the egg production of some other copepods, *A. bifilosa* (Uriarte *et al*., 1998), *Oithona rigida* (Santhanam and Perumal, 2012), *A. lilljeborgi* (Ara, 2001) for both egg production and hatching rate of *A.tsuensis* (Takahashi and Ohno, 1996), *A.discaudata, A.clausi, A.tonsa, A.margalefi* (Longoria, 2003) and the Baltic *A.tonsa* (Holste and Peck, 2006). Recently, Rhyne *et al*. (2009) have reported the increased egg production with increasing temperature in *A.sinjiensis*. Further, the egg hatching rate was significantly higher at warmer temperatures (34°C) than lower temperatures in calanoid copepod, *Pseudodiaptomus pelagicus*. Furthermore, the high survival and the greatest nauplii production were also reported in the temperature between 26-30°C.

The egg production and hatching succession of copepod was also influenced by the quantity of algal food. The egg production values of *P.parvus* were relatively higher in comparison with those in the literature for *P. parvus* and *Pseudodiaptomus pelagicus* (Sun *et al*., 2008; Rhyne *et al*., 2009). In the present experiment, the temperature increase of up to 25±3°C resulted in high egg production indicating its adaptation to temperature extremes. The present study was similar to Hirche and Halsband (2001) who recorded the peak egg production in *Centropages typicus* and *Acartia clausi* in the North Sea of Germany during summer season when the temperature was high. Likewise, Kiorboe and Nielsen (1994) reported low egg production in *Temora* sp. and *Pseudocalanus* sp. during winter season in Kattegat when the temperature was low. The present observation varied from Koski and Kuosa (1999) who stated that the increase in temperature above 13±1°C did not significantly increase the egg production in *A. bifilosa*.

The egg production was found positively correlated with algal concentration : (r=0.99541). Presently, the highest mean egg production was achieved with the highest cell concentrations. These results strongly support the hypothesis that the rate of egg production of *P.parvus* is limited immediately by the availability of phytoplankton (Santhanam and Perumal, 2012). Hirche *et al.* (1997) described the feeding history had a strong influence on egg production rate, which was much higher in females of *C. finmarchicus* exposed to different feeding concentrations. The present study inferred that the egg production increased with increasing food concentration while the increasing temperature did not significantly increase the egg production. However, *P. parvus* seemed to have given good results in egg production with high food concentrations. Similar observation was reported earlier by Koski and Kuosa (1999) in calanoid copepod, *A. bifilosa* and Abu Rezq *et al*. (1997) in harpacticoid copepod, *Tisbe furcata.* Santhanam and Perumal (2012) observed larger clutch sizes with increased algal concentration in cyclopoid copepod, *Oithona rigida*.

The reproductive success of the copepods can also depend on the egg hatching succession. It is widely reported that, the hatching rate also increased with increased temperature and food concentration. In the present study, the low hatching rate was observed in low temperature and low food concentration whereas high hatching rate was obtained in the optimum temperature and high food concentration is in conformation with the study of Koski and Kuosa (1999) and Milione and Zeng (2008) who reported lowest egg production and hatching rate in calanoid copepods. From these results, it is understood that calanoid copepod, *P.parvus* was most suitable candidate species to culture and other physiological monitoring studies if its reared at optimum temperature and maximum algal cell concentration.

Acknowledgments

The authors are thankful to the Head, Department of Marine Science and authorities of Bharathidasan University for facilities provided. The authors are indebted to the Department of Biotechnology, Govt. of India for providing financial support for this work.

References

Abu Rezq, S., A.B. Yule and S.K. Teng: Ingestion, fecundity, growth rates and culture of the harpacticoid copepod, *Tisbe furcata* in the laboratory. *Hydrobiologia,* **347**, 109-118 (1997).

Ara, K.: Daily egg production rate of the planktonic calanoid copepod *Acartia lilljeborgi* Giesbrecht in the Cananeia Lagoon estuarine system, Sao Paulo, Brazil. *Hydrobiologia,* **445**, 205-215 (2001).

Burkhart, C.A. and G.S. Kleppel: A new incubation system for the measurement of copepod egg production and egg hatching success in the field. *J. Exp. Mar. Biol. Ecol.,* **221**, 89-97 (1998).

Chinnery, F.E. and J.A. Williams: The influence of temperature and salinity on *Acartia* (Copepoda:Calanoida) nauplii survival. *Mar. Biol*., **145**, 733-738 (2004).

Devreker, D.: Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: A laboratory study. *J. Exp. Mar. Biol. Ecol.,* **368**, 113-123 (2009).

Farhadian, O., F.M. Yusoff and A. Arshad: Population growth and production of *Apocyclops dengizicus* (Copepoda: Cyclopoida) fed on different diets. *J. World Aquacult. Soc*., **39**, 384-396 (2008).

Farhadian, O., F. Yusoff, S. Mohamed and C. Saad: Use of cyclopoid copepod *Apocyclops dengizicus* as live feed for *Penaeus monodon* postlarvae. *J. World Aquacult. Soc.,* **40**, 22-32 (2009).

Guillard, R.R.L. and J.H. Ryther: Studies on marine planktonic diatoms: I. *Cyclotella nana* Hustedt and Detonula Conference (Cleve) Gran. Can. *J. Microbiol.,* **8**, 229–239 (1962).

Hirche, H.J., V. Meyer and B. Niechoff: Egg production of *Calanus finmarchicus*: Effect of temperature, food and season. *Mar. Biol.,* **127**, 609-620 (1997).

Hirche, H.J. and C. Halsband: Reproductive cycles of dominant calanoid copepods in the North Sea., *Mar. Ecol. Prog. Ser*., **209**, 219-229 (2001).

Hirche, H.J. and C. Halsband: Reproductive cycles of dominant calanoid copepods in the North Sea. *Mar. Ecol., Prog. Ser*., **209**, 219-229 (2001).

Holste, L. and M.A. Peck: The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): A laboratory investigation. *Marine Biology,* **148**, 1061-1070 (2006).

Karuppasamy, P.K. and P. Perumal: Biodiversity of zooplankton at Pichavaram mangroves, South India. *Ad. Bios.,* **19,** 23-32 (2000).

Kiorboe, T. and T.G. Nielsen: Regulation of zooplankton biomass and production in a temperate coastal ecosystem. Copepods. *Limnol. Oceanogr.,* **39**, 493-507 (1994).

Koski, M. and H. Kuosa: The effect of temperature, food concentration and female size on the egg production of the planktonic copepod *Acartia bifilosa*. *J. Plankton. Res.,* **21**, 1779- 1789 (1999).

Ku Kang, H., S. Poulet, A. Lacoste and Y.J. Kang: A laboratory study of the effect of non-phytoplankton diets on the reproduction of *Calanus helgolandicus*. *J. Plankton Res*., **22**, 2171-2179 (2000).

Longoria, E.: Egg production and hatching success of four *Acartia* species under different temperature and salinity regimes. *J. Crustacean Biol.,* **23**, 289-299 (2003).

Milione, N. and C. Zeng: The effects of temperature and salinity on population growth and egg hatching success of the tropical calanoid copepod *Acartia sinjiensis*. *Aquaculture,* **275**, 116-123 (2008).

Olivotto, I., I. Buttino, M. Borroni, C.C. Piccinetti, M.G. Malzone and O. Carnevali: The use of the Mediterranean calanoid copepod *Centropages typicus* in Yellowtail clownfish (*Amphiprion clarkii*) larviculture. *Aquaculture*, **284**, 211-216 (2008).

Perumal, P., V. Ashokprabu, T. Nedumaran and P. Santhanam: Studies on behaviour and survival rate of *Oithona rigida* Giesbrecht (Copepoda: Cyclopoida) fed with *Coscinodiscus centralis* Ehrenberg and *Skeletonema costatum* (Grev) Cleve. *Seaweed Res. Utiln.,* **22**, 135- 137 (2000).

Rhyne, A.L., C.L. Ohs and E. Stenn: Effects of temperature on reproduction and survival of the calanoid copepod *Pseudodiaptomus pelagicus*. *Aquaculture*, **292**, 53-59 (2009).

Runge, J.A.: Egg production of the marine planktonic copepod *Calanus pacificus* Brodsky: Laboratory observations. *J. Exp. Mar. Biol. Ecol.,* **74**, 53-6 (1984).

Santhanam, P. and P. Perumal: Diversity of zooplankton in Parangipettai coastal waters, southeast coast of India. *J. Mar. Biol. Assoc. India*, **45**, 144-151 (2003).

Santhanam, P., P. Perumal, S. Ananth and A. Shenbaga Devi: Copepod population in Velar estuary, Parangipettai coast in relation to environmental conditions. *J. Environ. Biol*., **33**, (2012).

Santhanam, P. and P. Perumal: Feeding, survival, egg production and hatching rate of the marine copepod *Oithona rigida* Giesbrecht (Copepoda: Cyclopoida) under experimental conditions. *J. Mar. Biol. Ass. India*, **54**, 38-44 (2012).

Shrivastava, Y., B. Fernandas, S.C. Goswami, U. Goswamy and C.T. Achuthankutty: Observation on feeding behaviour and survival rates in the estuarine calanoid copepods *Acartia spinicauda* and *Heliodiaptomus cinctus* (Crustacea: Copepoda: Calanoida). *Indian J. Mar. Sci.,* **28**, 222-224 (1999).

Stottrup, J.G.: Production and nutrition value of copepods. In: Live feeds in marine aquaculture (Eds.: J.G. Støttrup and L.A. McEvoy). Blackwell Publishing, Oxford. pp. 145-205 (2003).

Sun, X.H., S. Sun, C.L. Li and G.T. Zhang: The seasonal and spatial variation in abundance and egg production of *Paracalanusparvus* (Copepoda: Calanoida) in/out Jiaozhou Bay, China. *Estuar. Coast. Shelf Sci*., **79**, 637-643 (2008).

Takahashi, T. and A. Ohno: The temperature effect on the development of calanoid copepod, *Acartia tsuensis*, with some comments to morphogenesis. *J. Oceanography,* **52**, 125-137 (1996).

Uriarte, I., U. Cotano and F. Villate: Egg production of *Acartia bifilosa* in the small temperate estuary of Mundaka, Spain, in relation to environmental variables and population development. *Marine Ecology Progress Series*, **166**, 197-205 (1998).

Vengadesh Perumal, N., M. Rajkumar, P. Perumal and K. Thillai Rajasekar: Seasonal variations of plankton diversity in the Kaduviyar estuary,Nagapattinam, southeast coast of India. *J. Environ. Biol*., **30**, 1035-1046 (2009).

Whitehouse, J.W. and B.G. Lewis: The effect of diet and density on development size and egg production in *Cyclops abyssorum* Sars, 1863 (Copepoda:Cyclopoida). *Crustaceana,* **25**, 225-236 (1973).