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Effect of temperature on population growth of copepod, *Euterpina acutifrons*

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Abstract

This study was aimed at determining the optimum temperature for culturing the copepod, *Euterpina acutifrons*. The trial was conducted for 10 days in chambers at temperatures of 25^{0}C, 27^{0}C, 29^{0}C and 31^{0}C. Ten adult individuals of the copepod were randomly collected and placed into three replicate experimental flasks for each treatment. Throughout the trial, the salinity, light intensity, and photoperiod were maintained at 30 ±2psu, 100μmolm^{-2}s^{-1} and 12:12 light-dark cycle, respectively. The copepods were fed with 80,000cell/ml *Isochrysis* sp. daily. At the end of the trial, the total numbers of *E. acutifrons* nauplii, copepodites and adults were determined and counted using Sedgwick-Rafter. The highest population was found at 27^{0}C with mean total population of 800±100 individuals from an initial of 10 individuals. This was followed by those reared at 25^{0}C and 29^{0}C where the population counts were 700±100 individuals and 367±115 individuals, respectively. At the 31^{0}C, all the copepod specimens were found dead on day 5th. Statistical analysis showed that the temperature had a significant effect ($P<0.05$) on the population growth of the copepod. The population of nauplii was higher in lower temperature (25^{0}C) set compared to the one at higher temperature (29^{0}C). However, the copepodite number was greater at 27^{0}C. Growth of the copepod was highest at 27^{0}C (0.438K) followed by sets at 25^{0}C (0.425K) and 29^{0}C (0.361K). Based on the results of this analysis, it is suggested to culture copepod at temperature 25^{0}C for nauplii production and 27^{0}C for producing more copepodites.

Keywords: Aquaculture, Live Feed, Copepod, Temperature, Population growth

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Introduction

A major concern in successful larval rearing of commercial fish is about the appropriate type of live feed at the first feeding phase (Ohs et al., 2009). It is the most critical period in the larviculture that determines the survival of the fish and success of the culture system (Agh and Sorgeloos, 2005). During this phase the marine fish larvae require a live feed of suitable nutritional value and size range (Ohs et al., 2009).

In South East Asia, aquaculture of most species involves capturing young or immature wild stocks and culturing them to marketable sizes. This is an unsustainable practice that contributes to depletion of wild stocks. The viable alternative is establishing the full cycle culture in the hatchery and producing seeds to support aquaculture development. However, the larviculture of most of the species remains a major bottleneck in this effort. Larval mortality is high due to gap in knowledge of nutritional requirement of the larvae and difficulty in producing the required feed suitable for the first feeding (Sorgeloos and Leger, 1992).

At first feeding, the mouth gap of most larvae is small which limits the size of food the larva can capture to survive (Chesney, 2005). Formulated feed, though nutritionally adequate, have been able to replace live feed due to problems pertaining to their digestibility and palatability (Kolkovsky, 2001). The use of rotifer as live feed has revolutionized the development of full cycle culture of many species. The brine shrimp, *Artemia*, is also used as food for many marine fish larvae due to its commercial availability. However, it has been reported that some species of marine fish do not survive on rotifers and *Artemia* (McKinnon et al., 2003; Chesney, 2005; O'Bryen and Lee, 2005). These species include *Epinephelus* sp. (Knuckey et al., 2000; McKinnon et al., 2003; Toledo et al., 2005), *Pagrus* sp. (Payne, 2000) and *Lutjanus* sp. (Ogle et al., 2005; Phelps et al., 2005; Su et al., 2005). Comparatively, the copepod provides good results (Stottrup, 2000; Lee, 2003). The advantages of copepods over rotifers and *Artemia* include size range at the naupliar, copepodite or adult stages. Any of these can be chosen according to the mouth size of the larvae (Chen et al., 2006). Their nutritional content should match the requirements of marine fish larvae (Stottrup, 2000; Evjemo et al., 2003; McKinnon et al., 2003) especially in the amounts of DHA and EPA (Bell et al., 2003). Moreover, their swimming behaviour can stimulate a stronger foraging response in fish larvae (Stottrup, 2000).

Several species of copepods have shown potential in aquaculture particularly in rearing marine fish larvae. The preferred species belong to the genera *Acartia*, *Centropages*, *Eurytemora*, *Euterpina*, *Tigriopus*, *Tisbe*, *Oithona* and *Apocyclops* (Stottrup, 2003). The benefits of using copepods as live feed are well known, but reliable supply of copepods remains as a challenge for aquaculture due to technical constraints and it still remains a work in progress (Stottrup, 2000, Hagiwara et al., 2001). The major drawback of copepods as live feeds for larviculture compared to *Artemia*

and rotifers is their low productivity in mass culture (Milione and Zeng, 2007; Camus and Zeng, 2008).

In copepod propagation, temperature is one of the main parameters affecting survival and growth. Knowing the optimum temperature will maximize the production of this live feed (Milione and Zeng, 2008). This experiment was conducted to determine the optimum temperature for the population growth of the copepods. *Euterpina acutifrons* was chosen due to its high potential as live feed for aquaculture.

Materials and Methods

Experimental trials on the effect of temperature on the growth of *E. acutifrons* were conducted over a period of 10 days by keeping the cultures in temperature-controlled rooms at salinity of 30±2psu. Four different temperatures of 25^0C, 27^0C, 29^0C and 31^0C were selected for this experiment. The fluctuations in the temperature were about ±2°C between day and night. At the beginning of the experiment, adult copepods were separated from stock culture using 200µm sieve. The copepods caught with the help of a mesh and placed in a Petri dish with a small amount of seawater. The specimens were randomly chosen and following the usual isolation procedure transferred to the experimental flasks. This experiment was performed using Erlenmeyer flasks (250ml) filled with 150ml of filtered seawater with three replicates for each treatment. Each experimental flask was stocked with 10 adult individuals per 150ml. Adults were used in this experiment due to unavailability of gravid females. Experimental flasks were covered during the experiment to prevent evaporation.

Except the temperature, other experimental conditions, including the salinity (30±2psu), light intensity (100µmol$m^{-2}s^{-1}$) and photoperiod (12:12 LDC) were similar for the sets. Approximately, 80% culture water was changed daily. The number of copepods was counted at the first and final day of the experiment. The copepods were fed with 80,000cell/ml *Isochrysis* sp. daily based on the results of the previous dietary experiments conducted in laboratory (unpublished work). The amount of feed left was checked daily and the amount of algae fed was adjusted accordingly. Haemocytometer was used to count the algal density. At the end of the experiment, the contents of each replicate bottle were drained through a 20µm sieve and fixed in 10% formalin solution. The total number of *E. acutifrons*, nauplii, copepodites and adults were counted using a Sedgwick-Rafter counting slide under optical microscope at 400 times magnification. Results of cell densities were reported as the mean ±SD of 3 replicate cell counts.

At the end of the experiment, maximum specific growth rate (K) of copepod was calculated using the method used by Levasseur *et al.* (1993). Two points of X_1 and X_2 at the extremes of the linear phase were taken and substituted into the equation below:

Growth rate, $K = \ln(X_2)-\ln(X_1)/t_2-t_1$

X_1 = initial number of copepods at the start of selected time interval

X_2 = final number of copepods at the completion of selected time interval

t_2-t_1 = selected time (in days) for the determination of the number of copepods

Where X_1 and X_2 = biomass at time 1 (t_1) and time 2 (t_2) respectively.

Results

Figure 1 shows the mean total population of *E. acutifrons* treated at different temperatures after 10 days of culture. The highest population was recorded at 27^0C reaching a mean total population of 800±100 individuals from an initial 10 individuals of adults per 150ml. This was followed by 25^0C and 29^0C with mean total population of 700±100 individuals and 367±115 individuals, respectively. At 31^0C, all copepods were found dead on day 5. Statistical analysis showed that the temperature had a significant effect ($P<0.05$) on the population growth of *E. acutifrons*. Final population at 27^0C was significantly higher than that 29^0C, however, it shows that there was no significant difference ($P>0.05$) between 27^0C and 25^0C.

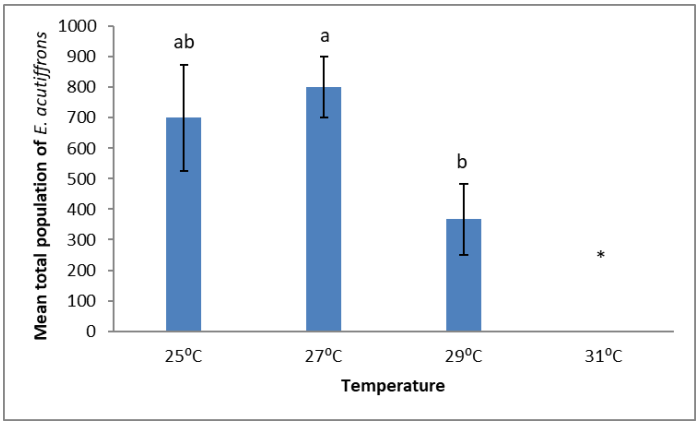

Figure 1. Mean total population of *E. acutifrons* cultured for 10 days at 4 different temperatures maintained at salinity 30±2psu and photoperiod 12:12 light-dark.

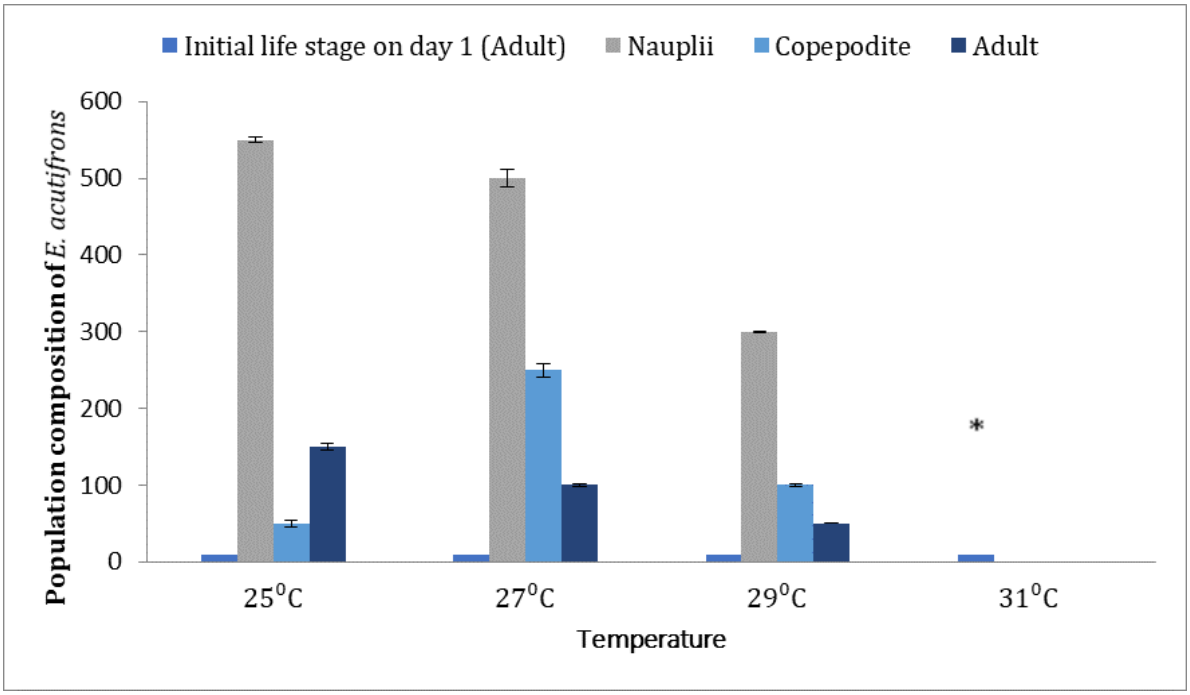

Figure 2. Mean number of individuals at different developmental stages of *E. acutifrons* cultured in salinity of 30±2psu for 10 days under four different temperatures. * denotes no data on day 10.

Figure 2 shows the counts of different life stages of *E. acutifrons* at various temperatures at the end of 10 days of culture. The distribution of developmental stages within the population was affected by temperature. In general, the size of the nauplii was between 50 - 200 micron (N1 to N6). At the lower temperatures, the population had a larger number of nauplii compared to the higher temperature. Nauplii were abundant at 25^{0}C which were 20% and 75% higher than at 27^{0}C and 29^{0}C, respectively. On the other hand, the copepodite (C1 to C6) resembling the adult with simple unsegmented urosome and a size range of 200 – 500 micron were more abundant at 27^{0}C. The results have shown that the copepodite production at 27°C was 83.33% higher than at 25°C and 71.43% higher than at 29°C. In addition, the total number of adults in the experiment from highest to lowest was 50% at 25°C, 33.33% at 27°C and 16.67% at 29°C. The maximum growth was seen at 27^{0}C with 0.438K as shown in Table 1. This was followed by 25^{0}C and 29^{0}C with 0.425K and 0.361K, respectively.

Table 1. The maximum specific growth rate of *E. acutifrons* grown in 30±2 psu salinity for 10 days under different temperature conditions.

Temperature (^{0}C)	Maximum specific growth rate (K)
25	0.425
27	0.438
29	0.361

Discussion

It was observed that *E. acutifrons* population growth at 30psu was optimum at 27^{0}C, with maximum specific growth 0.438K although there was no significant difference between 27^{0}C and 25^{0}C. Therefore, it can be suggested that the best range of temperature for culturing of *E. acutifrons* is between 25^{0}C to 27^{0}C. Previous study on *A. tsuensis* carried out by Takahashi and Ohno (1996) also showed optimal growth and minimum mortality at temperature of around 25^{0}C. In general, population growth in harpacticoid copepods is highly temperature dependent (Bergmans, 1984), with most rapid growth for tropical species at ≥25^{0}C. The tropical species are better adapted to higher temperature than species in temperate or polar zones (Moreira et al., 1982). Malaysia is a tropical country with a mean annual temperature range of 25°C-28°C (Yusoff et al., 1997). Therefore, culturing copepod at these temperatures without using a cooling incubator or air conditioner is possible. This reduces the cost of the live feed production.

Population growth was significantly lower when the temperature increased to 29^{0}C, with specific growth rate 0.361K, and all copepods were died at 31^{0}C. Mortality occurred at 31^{0}C is because the temperature increase exceeded the bearing capacity of *E. acutifrons*. This is apparent by the absence of *E. acutifrons* at the end of the experiment at 31^{0}C. In general, the rate of metabolism of organisms increases with increase in temperature (McNab, 2002; Clarke, 2004). The increase is because the reactants in cell gain greater thermal energy and many cellular enzymes become more active as temperature increases. However, when the temperature rises above 30^{0}C, enzymes become denatured and lose their functional properties (Rhyne et al.,

2009). Similar findings have been observed in *Diaptomus pallidus* (Geiling and Campbell, 1978), *Acrocalanus gibber* (McKinnon, 1996), *A. sinjiensis* (Milione and Zeng, 2008) and *Pseudodiaptomus pelagicus* (Rhyne et al., 2009).

In this study, the distribution of developmental stages in the population was also affected by temperature. At temperature 25^{0}C, the population consisted of a greater amount of nauplii. While at 27^{0}C and 29^{0}C, the populations comprised more copepodite stages. For aquaculture, the small size of nauplii can be used for first feeding to a newly hatched fish larvae, while the copepodites are more suitable for the older larvae. Similar trend was observed in an earlier study done by Milione and Zeng (2008) on *A. sinjiensis* cultured at 30±1psu. According to these authors, the population consisted of a greater number of nauplii at lower temperatures (15^{0}C to 24^{0}C) but consisted of advanced stage individuals at higher temperatures (25^{0}C to 34^{0}C).

Conclusion

Marine fish hatcheries should consider propagating copepod especially this species *E. acutifrons* as a live feed, considering the size range (50-500 micron) that suits the fish larvae with a smaller mouth gap size (80-150 um) at fist feeding. Based on the finding of this study, the optimum temperature for nauplii production is 25^{0}C but for copepodites, the temperature should be 27^{0}C. For maintenance of stock culture of pure species of *E. acutifrons*, the room temperature should be controlled at 29^{0}C as the growth rate is relatively slow and lower rearing efforts will be required.

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