Sains Malaysiana 39(1)(2010): 135–140

Culture and Growth of a Marine Harpacticoid, *Pararobertsonia* sp. in Different Salinity and Temperature

(Kultur dan Pertumbuhan Harpacticoida Marin, *Pararobertsonia* sp. dalam Kemasinan dan Suhu Berbeza)

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ABSTRACT

Copepods play a major role as food link for larger animals and it is also important as a live food source for the aquaculture industry. There are only few reports on the influence of temperature and salinity towards the growth and development of tropical copepods. In this study, the effect of temperature (5 and 25°C) and salinity (5, 10, 25 and 30 psu) on population growth and density of a marine harpacticoid, Pararobertsonia *sp. in a laboratory condition is investigated. The species was first obtained from seagrass samples from Merchang estuary, Terengganu, which receives seawater from the South China Sea. It has been cultured in the laboratory condition through generations. The findings show that there is a significant difference (P<0.05) detected between the salinity treatment and it shows that difference in salinities give different effects on the population number of the harpacticoids cultured in the same temperature (25 ± 1°C). On the other hand, harpacticoids reared in cold temperature (5 ± 1°C) did not survive the condition. Harpacticoids reared in 25 psu salinity show the highest population density (mean of 3.7 ind./ml), but the most stable population growth is shown in 35 psu treatment as shown by its high value of maximum specific growth rate (K). From this experiment, it can be concluded that 35 psu and temperature of 25 ± 1°C is the optimum condition for the maximum production of a tropical* Pararobertsonia *sp. in the laboratory condition.*

Keywords: Harpacticoida; laboratory culture; tropical species

ABSTRAK

Kopepoda memainkan peranan utama sebagai penghubung makanan bagi haiwan yang lebih besar dan ia juga penting sebagai sumber makanan hidup di dalam industri akuakultur. Tidak banyak laporan yang telah diterbitkan mengenai pengaruh suhu dan kemasinan terhadap perkembangan Kopepoda di kawasan tropika. Kajian kesan suhu (5 and 25°C) dan kemasinan (5, 10, 25 dan 30 psu) ke atas pertumbuhan populasi dan kepadatan Harpaktikoid marin, Pararobertsonia *sp. telah dijalankan di dalam makmal. Spesies ini telah pertama kali diperolehi daripada sampel rumput laut dari kawasan muara sungai di Merchang Terengganu yang menerima air masin Laut China Selatan. Ia telah dikultur di dalam keadaan makmal sejak beberapa generasi. Kajian ini menunjukkan terdapat perbezaan yang bererti (P<0.05) di antara rawatan-rawatan kemasinan, dan ini menggambarkan kemasinan yang berbeza memberi kesan yang berbeza ke atas bilangan populasi haiwan tersebut yang dikultur di dalam suhu yang sama (25 ± 1°C). Sebaliknya, haiwan yang dikultur di dalam suhu rendah (5 ± 1°C) tidak dapat bertahan. Harpacticoida yang dipelihara di dalam kemasinan 25 psu menunjukkan kepadatan populasi tertinggi (min 3.7 ind./ml), tetapi, pertumbuhan populasi paling stabil ditunjukkan oleh rawatan kemasinan 35 psu di mana ia menunjukkan nilai Kadar Pertumbuhan Khusus (K) maksimum. Daripada eksperimen ini, dapat dirumuskan bahwa 35 psu dan suhu 25 ± 1°C merupakan keadaan optimum persekitaran makmal bagi penghasilan maksimum spesies tropika,* Pararobertsonia *sp.*

Kata kunci: Harpacticoida; kultur makmal; spesies tropika

Introduction

Copepods have been cultured using various techniques with different type of diets. Schipp et al. (1999) used a 5000 L fiberglass tank to produce 1000 L adult of a calanoid, *Acartia* spp. per culture cycle. Sun and Fleeger (1995) managed to produce a total of one million individuals and a total over 5 g of dry weight biomass per day using a 4 m^2 basal surface area culture system for a harpacticoid, *Amphiascoides atopus*. The copepods were fed with either cultured algae *Chaetocerous muelleri*, or commercial fish-flake, and they seems to grow well on each diet or a mixture. Harpacticoids are generally tolerant to environment fluctuations but they do have temperature and salinity optima, and these will be species- and strain-dependent (Cutts 2003).

Marine harpacticoids in tropical water are exposed to high temperature and salinity throughout the year. An experiment showed that for *Nitokra lacustris* Shmankevich,

the most suitable and best salinity for the harpacticoids to grow is in a range of 10 to 40 psu (Rhodes 2003). In a study done by Matias-Peralta et al. (2005), the maximum growth rate of *Nitocra affinis* f. *californica* Lang was reported at 30-35psu and decreasing at lower salinities (10-25 psu). The species survived more than 80% of its population in all tested salinity (10-30 psu).

Reproduction and development in harpacticoid is strongly temperature dependent (Hicks & Coull 1983) where development time decreases with increasing temperature. Williams & Jones (1999) reported that *Tisbe battagliai* reproduced at the optimum temperature of 20°C. They found that females reared at 15°C lived twice as long as the one in 25°C but increase in temperature will decrease the number of offspring per day.

Studies on culture of tropical marine harpacticoids fewer if compared to the temperate species. Basic effect of temperature and salinity on the growth and population is rarely reported although this organism is proven as better starter feed than *artemia* or rotifers in larviculture industry. A tropical harpacticoid *Pararobertsonia* sp. was first collected along with other species from wild and tested for laboratory culture in 2006. The species showed acceptable growth performance on yeast diet and easy to adapt to small culture vessel in laboratory (Busra et al. 2008). Thus, this species is selected in the study as to determine the effect of different salinity and temperature on the population density and production of a tropical harpacticoid. The finding would be used as a guideline to maintain harpacticoid culture for other field of study.

Materials and Methods

Seawater was collected from the area where the copepods were firstly obtained in Merchang estuary (5^0 02.260' N, 103^0 17.821' E) facing the South China Sea. It was filtered through a GFC membrane filter and autoclaved at 120°C for 15 minutes (Carli et al. 1995). Experiments were carried out in glass petri dishes filled with 15 ml treated seawater with salinity of 5, 10, 25 and 35 psu (5 replicates each). Salinity in glass petri dishes was measured using a refractometer (model ATAGO).

Pararobertsonia sp. has been cultured in the laboratory for about six months and the species has already adapted to its new environment. One gravid female was placed in each petri dish. No aeration was given to the culture as aeration will only increase evaporation (Nanton & Castell 1998). Two different sets of culture were maintained in two different temperature, the room temperature of 25 ± 1°C and a refrigerator temperature (5 ± 1°C) for 45 days. The temperature was measured everyday using a mercury thermometer.

Everyday each individual of copepods at all stages were counted under a Leica stereo microscope before being fed with Baker's yeast at 0.1 ml (0.02 g/L). Dead animals, molted exoskeleton and any debris were taken out from the culture using a wire loop.

Data collected were all analyzed using one way analysis of variance (ANOVA, treatment vs population). These data were tested at 0.05 level of probability. Turkey's comparison test was used to test for significant differences within the different salinity levels. The statistical analysis was performed using a SPSS Version 11.5 programme.

At the end of the experiment, the maximum specific growth rate (*K*) of copepod was calculated using the method used by Omori and Ikeda (1984):,

$$K = \frac{\ln (X_2) - \ln (X_1)}{t_2 - t_1}$$

where X_1 is the number of copepods at the initial of selected time interval, $X_{2\ is}$ the number of copepods at the final of selected time interval and t_2-$t_{1\ is}$ the selected time (in days) for the determination of number of copepods.

Results and Discussion

There was no development of harpacticoids observed at 5 ± 1°C. Copepod does not grow and no production of nauplii occurred. Adult female with egg sac still attached to the body as the first day they were introduced to the culture medium. Movement was only observed in the first few days and there was no moving action after 5 days.

The population density was determined by measuring the number of individuals produced in 1 ml of seawater for every treatment. The maximum specific growth rate (K) was then calculated (Table 1). The growth rate increased with the increase in salinity.

TABLE 1. The maximum specific growth rate of *Pararobertsonia* sp. grown under different salinities (ambient temperature, 25 ± 1° C)

Salinity (psu)	Maximum specific growth rate (K)
5	0.0308
10	na
25	0.0462
35	0.0602

na = not available

At 25°C, the harpacticoids seemed to grow well and developed during the first week. They reached their maximum density at different time for different salinity. Harpacticoid cultured in 25 psu salinity showed the highest population density with 3 ind./ml during the 4th week of culture (Figure 3). As for 5 psu salinity, the population density reached up to 3.5 ind./ml at 4th week of culture (Figure 1), 10 psu salinity with 2.9 ind./ml in 2nd week (Figure 2) and 2.3 ind./ml for 35 psu at the 6th week (Figure 4).

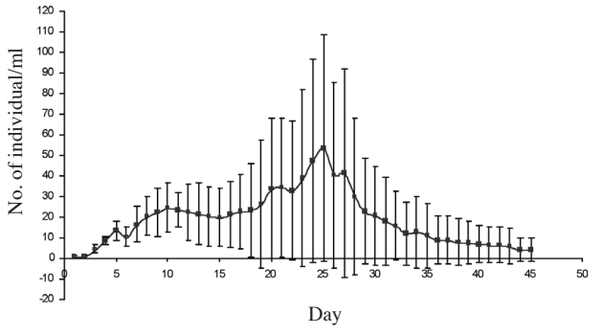

FIGURE 1. The population density (number of individual in 15 ml) with a standard deviation of *Pararobertsonia* sp. at 25°C for salinity of 5 psu

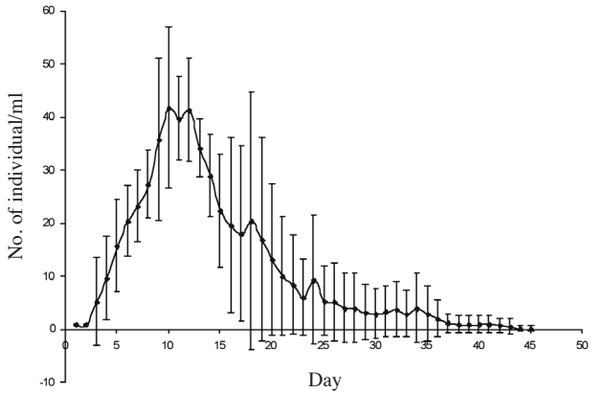

FIGURE 2. The population density (number of individual in 15 ml) with a standard deviation of *Pararobertsonia* sp. at 25°C for salinity of 10 psu

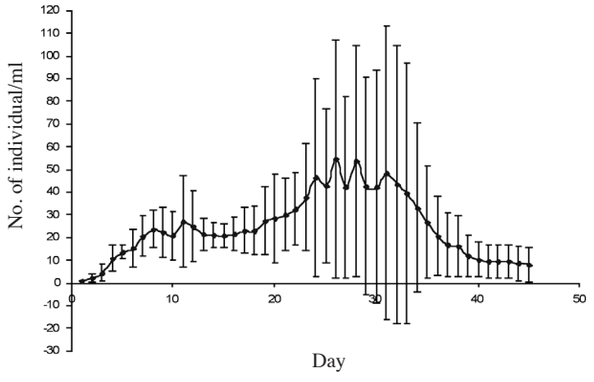

FIGURE 3. The population density (number of individual in 15 ml) with a standard deviation of *Pararobertsonia* sp. at 25°C for salinity of 25 psu

The production of nauplii and gravid females along with other stages, cultured in different salinities at 25°C is summarized in Figure 5-8. Peak of nauplii production for 5 psu, 10 psu, 25 psu and 35 psu was shown at day 5 and 27, 6 and 19, 7 and 27 and 9 and 37, respectively. Nauplii existed in each treatment until at day 33, 28, 38

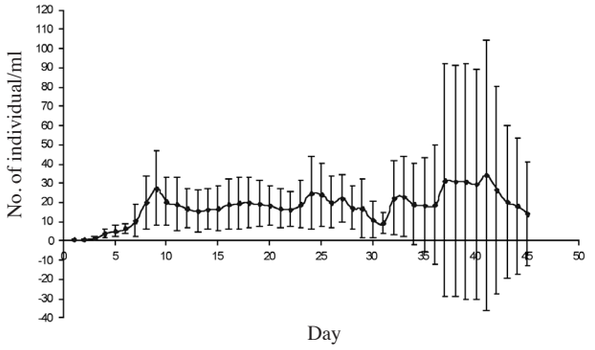

FIGURE 4. The population density (number of individual in 15 ml) with a standard deviation of *Pararobertsonia* sp. at 25°C for salinity of 35 psu

and 45 respectively. It is interesting to note that in 35 psu the nauplii survived until the 45th day.

Gravid females occurred several times in the culture indicating the occurrence of several new generations throughout the experiment period. Gravid females found more frequent in 35 psu than other treatments although higher number was in 25 psu. They also occurred in shorter duration during the overall 10 first day of the experiment suggesting the more favorable culture condition in the early period of the study.

One-way ANOVA analysis shows that there was a significant difference ($P<0.05$) of population density between salinity treatments. The replicate treatments for all salinity (5, 10, 25 and 35 psu) also shows significant difference ($P<0.05$) between the replicates as indicated by the large values of standard deviation. Different salinities have different effects on the population number of the copepod cultured in the same temperature (25 ± 1°C). Each nauplii and adult stages also show significant different ($P<0.05$) between the treatment indicating that there were different effects on the number of nauplii and adults produced in the different salinity.

For a female adult that has mating before, the probability of getting gravid again two to three times without mating is very high. Females are fertilized soon after the entry into adulthood and can produce several brood sacs from one fertilization event (Rhodes 2003). According to Hicks and Coull (1983), harpacticoid copepods need to mate once to produce a multiple of egg sacs. Therefore, for every female individual, the tendency of producing eggs is different from one another and could resulting in a significant difference of population density between replicate samples.

This significant difference could potentially due to the cannibalism activity perform by certain species of copepods. When the food source is limited, cannibalism of nauplii by adults and later stage copepodids could occur and thus contribute to the declining of nauplii. This finding was reported by Schipp et al. (1999), where a calanoid copepod, *Acartia* spp. was found to perform cannibalism during the 8th day of culture cycle due to shortage of food.

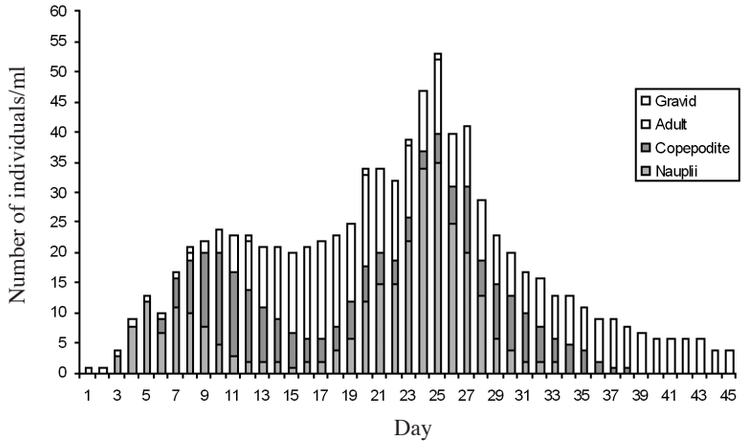

FIGURE 5. The mean number of nauplii, copepodite and adult of *Pararobertsonia* sp. produced at 25°C for salinity of 5 psu

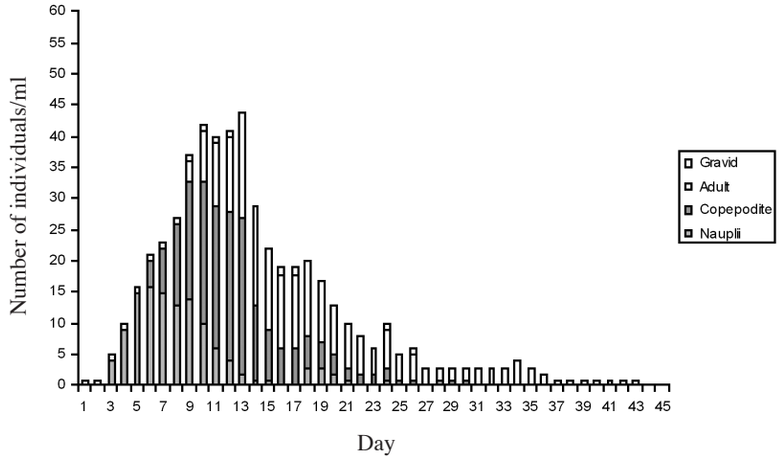

FIGURE 6. The mean number of nauplii, copepodite and adult of *Pararobertsonia* sp. produced at 25°C for salinity of 10 psu

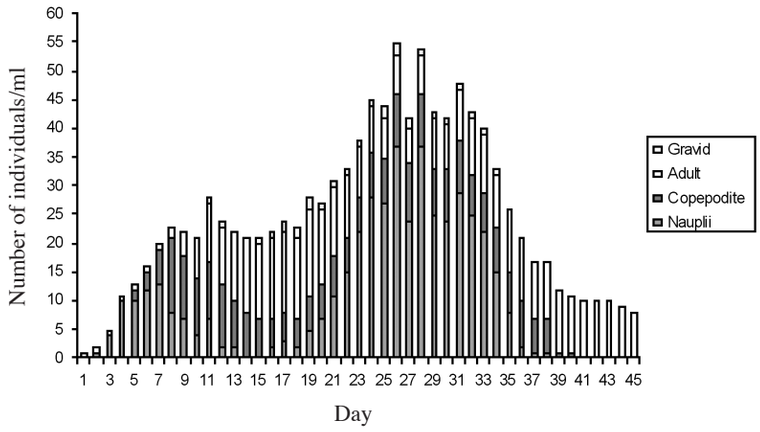

FIGURE 7. The mean number of nauplii, copepodite and adult of *Pararobertsonia* sp. produced at 25°C for salinity of 25 psu

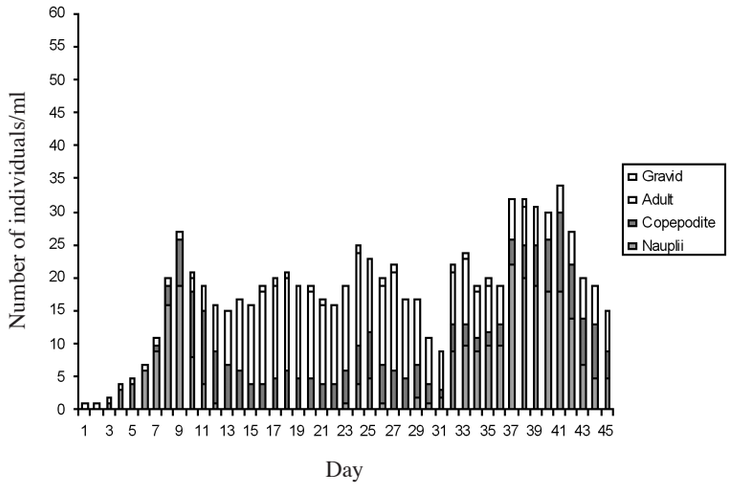

FIGURE 8. The mean number of nauplii, copepodite and adult of *Pararobertsonia* sp. produced at 25°C for salinity of 35 psu

Camus and Zeng (2009) found that cannibalism rate on naupliar stage increases with increasing adult stocking density of *Acartia sinjiensis*.

The sharp decline of the number of nauplii could also be due to the number of nauplii that decreased drastically during the culture time. It could be influenced by the food concentration in the culture medium since the food supplied was maintained at 0.1 ml everyday regardless that the population of the copepods has increased. Williams and Jones (1999) noted that in laboratory studies, offspring production generally declines with reduction in food supply below the optimal levels.

Salinity level of 35 psu was found to provide the best condition for the development of *Pararobertsonia* sp. from nauplii to adult, and it shows that this culture condition is the most stable among the others. This result is comparable to a study done by Matias-Peralta et al. (2005) where a tropical harpacticoid species, *Nitocra affinis* showed the maximum growth when cultured in 35 psu salinity compared to lower salinities. Sun and Fleeger (1995) found that a harpacticoid *Amphiascoides atopus* from the same family (Diosaccidae) grew best in the salinity regime of 25-35 psu while it can continue to survive in the salinity range of 10 to 60 psu.

A sudden decrease in salinity and temperature do affect the survival. The results of this study show that *Pararobertsonia* sp. is more sensitive to the sudden change of temperature rather than salinity. Its physiological response and tolerance towards temperature and salinity might be the reason for the different sensitivity to the two parameters. Being an estuarine species, its more exposed to the salinity fluctuation due to daily tidal cycle, thus developed an osmo-regulatory adaptation to survive. As a result, they are ready to adapt to the sudden change of salinity in laboratory experiment (Bollmohr et al. 2009). The different tolerant level towards temperature and salinity in culture vessel was also reported for a calanoid species *Eurytemora affinis* (Devreker et al. 2009). They also found that the calanoid was more sensitive to temperature change than salinity.

The different species might have different thermal limit in term of reproductive response. Rhyne et al. (2009) confirmed the important role of temperature in copepod culture. They found that 26-30°C was the best range for nauplii production while 28-32°C was the best for fast maturation rate of nauplii. Earlier, Miliou and Moraitou-Apostolopoulou (1991) reported that a reduction in the number of egg sacs and the total number of offspring produced by the Greek strain of *Tisbe holothuriae* was observed when the temperature was lower or higher than the optimum (19°C). A study by Williams and Jones (1999) also noted that a benthic harpacticoid, *Tisbe battagliai* has their best temperature at 20°C and increasing of temperature towards 25°C decreased the production rate. In the case of salinity effect, Staton et al. (2002) found that there is a non-linear survival response of *Microathridion littorale* (estuarine harpacticoid copepod) to short term immersion of 24 hours in 3, 12 and 35 psu. Copepods that were transferred in the 12 psu showed the lowest survival rate. They also noted that exposure of low salinity in more than 24 hours for this species will only cause death, as what happened to *Euterpina acutifrons* which survived only for 24 hours when transferred directly from 35 to 5 psu seawater.

CONCLUSION

A tropical harpacticoid, *Pararobertsonia* sp. shows its ability to grow well in 15 ml seawater culture dish with the maximum population of about 4 ind./ml when maintained in 25°C in laboratory condition. Increase in salinity gives positive effect to the species as it can increase the growth rate and the population becomes more stable in high salinity (25-35 ppt). The effect of low temperature might be more crucial than salinity as shown in this study particularly when the stress is sudden. Further study should

be carried out to understand the reproductive biology of the species in response to the environmental stress from salinity and temperature change.

ACKNOWLEDGEMENT

The authors are grateful to the National Oceanographic Directorate Ministry of Science and Innovation Malaysia for the research grant (NOD/R&D/03/005).

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Received: 14 January 2009
Accepted: 16 July 2009