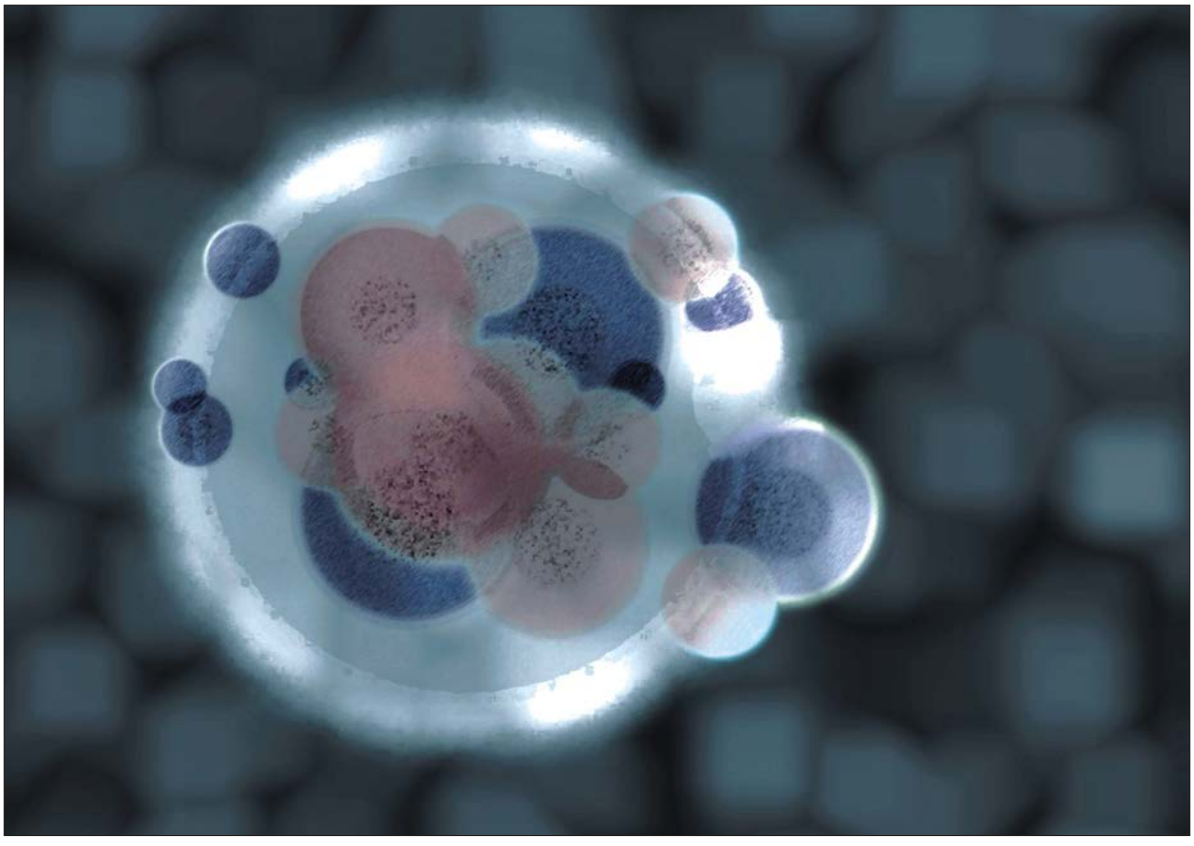

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Generation Time of Some Marine Harpacticoid Species in Laboratory Condition

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Abstract: Recent investigations on harpacticoid copepods have demonstrated their higher nutritional values compared to *Artemia* and rotifer. Nevertheless, studies on the potential use of tropical harpacticoid copepods as live feed in aquaculture are still limited. The present study was carried out to compare the generation time between selected harpacticoid species cultured in laboratory condition as an early step to choose a potential live feed for aquaculture practices. Some estuarine species of harpacticoids were isolated from Merchang river mouth, an estuary of the South China Sea in Terengganu and undergone trial culture procedure. Three species (*Paradactylopodia oculata*, *Schizopera knabeni* and *Robertsonia knoxi*) were successfully adapted to the laboratory condition thus their generation time were recorded. Copepod samples were cultured under controlled laboratory condition at temperature 25±1°C and salinity 27±1 ppt for 40 days and fed with 0.1 mL of baker's yeast (0.02 g/L/day). The mean generation time (day) was different for each species where *P. oculata* showed the long generation time (17.19±4.74 days) followed by *S. knabeni* (10.19±3.51 days) and *R. knoxi* (8.93±1.00 days). The species with short generation times could be a better choice for fish larval rearing and hatchery activity due to the early time of nauplii production and hence we suggest the *R. knoxi* could be used as a potential live feed (on the basis of their generation time) in aquaculture practices.

Key words: Estuarine harpacticoid, generation time, laboratory condition, south China sea

INTRODUCTION

Fish larvae directly depends on the live feed organism for their survival both in the wild and hatchery system. At present, various live feed organisms are used in aquaculture practices as a larval feed to enhance the production and reducing feed cost. Studies have shown that for a number of marine fish species larvae fed with copepod nauplii as a diet resulted in faster growth, enhanced nutritional content and better survival rate of first feeding larvae compared to diets consisting solely of rotifers and Artemia (Watanabe *et al*., 1983; Stottrup *et al*., 1986; Kraul *et al*., 1992; Stottrup and Norsker, 1997; Schipp *et al*., 1999; Shields *et al*., 1999; Stottrup, 2000; Payne and Rippingale, 2000). Interestingly, high natural omega-3 profile in copepods compared to other commercially available live feed organism together with better preference of copepod nauplii by the fish larvae has driven researchers to show greater attention on copepod culture in recent years (Evjemo *et al*., 2003). Copepods are among the most abundant and important components of aquatic invertebrates in many marine and freshwater ecosystem. They become the major biomass in zooplankton community in the water column and at many occasions they form significant entity of benthic community structure on bottom sediment. They are a major food source for organisms in higher tropic level such as juvenile fishes and shrimps (Vincx, 1996; Penchenik, 2004). They constitutes large percentage of larval diet and proven to be the best alternative source to rotifer and *Artemia nauplii* containing high level of DHA and other PUFA that are necessary for the growing marine fish larvae (McKinnon *et al*., 2003; Stottrup, 2000). They also contain high level of exogenous digestive enzymes that are believed to be playing notable role in fish larval digestion.

Among the copepods, harpacticoids becomes an important live feed source for aquaculture industry since they have combination of an appropriate size for larval fish, adaptability to culture condition and high nutritional values. Copepod diets were proved to increase the growth of fish and crustacean larvae compared to *Artemia* or

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rotifer *Brachionus plicatilis* (Kuhlmann *et al*., 1981; Watanabe and Kiron, 1994) because of the low in HUFA's content in the later group (Barclay and Zeller, 1996; Rainuzzo *et al*., 1997; Shields *et al*., 1999).

Harpacticoid copepods have been found to be a good candidate in aquaculture industry since they have a high reproductive potential, short generation time, high population growth, flexible in diet and tolerate a wide range of environmental factors such as temperature and salinity (Sun and Fleeger, 1995; Stottrup and Norsker, 1997). There are reports that indicate the availability of enzyme in harpacticoid copepods which enable the organisms to convert any type of their organic food into lipids stored in their body (Nanton and Castell, 1998). They have direct benthic development and pass through six naupliar stages followed by six copepodite stages, then copepodite VI being the adult (Nybakken, 2001). Sun and Fleeger (1995) indicated that the average turn-over time (generation time from egg to egg) of *Amphiascoides atopus* was between 21 and 26 days at 30 ppt and 24°C. A study by McKinnon *et al*. (2003) showed nauplii development will be completed by 2.46 days in *Parvocalanus crassirostris* and *Acartia sinjiensis* and by 3.2 days in *Bestiolina similis*. Due to high commercial values of copepod, present study was aimed to compare the generation time of three harpacticoid species (*Paradactylopodia oculata*, *Schizopera knabeni* and *Robertsonia knoxi*) collected from estuarine habitat and cultured in a laboratory condition as to evaluate their potential as a live feed source for fish larval rearing practice.

MATERIALS AND METHODS

Sample collection: Stocks of harpacticoid copepods used in this study were collected from the sea grass patch at estuaries of Merchang, Terengganu (5°2.260'N, 103°17.821'E) in the South China Sea on 16 January 2007. Meiobenthic samples were collected using the 62 μm plankton net (Somerfield *et al*., 2005), where the net was towed on the surface of sediment at seagrass patch. The samples were then placed in aquarium containing aerated seawater and immediately transported to the Biodiversity Laboratory, Universiti Malaysia Terengganu (UMT), Malaysia.

Culture of harpacticoid copepod: Harpacticoid copepods were reared in static (batch) cultures in 20 mL culture medium since 2007 (Rippingale and Payne, 2001). For the present study, seawater was filtered with GFC membrane filter and then autoclaved for 15 min. Culture was initiated by the single gravid female of harpacticoid copepod. The copepods were fed with 1 mL of baker's yeast (0.02 g L^{-1}) daily (Nanton and Castell, 1998).Temperature was maintained at 25±1°C and salinity was at 27±1 ppt. Once the copepods produced the eggs, the hatched nauplii were counted. Then the female copepod was transferred into a new culture medium. Total number of copepods were counted under dissection microscope. The observations were carried out each day for 40 days.

Data analysis: Accidentally two culture vessels were found to support the same species of *R. knoxi* after identification. Thus data for both cultures were included in this report. Life table parameters were calculated using standard life table methods following Ricklefs (1990). Fertility at age x (m_x) was the average number of births per female of age x, during the time interval x to x+1. Net reproductive rate, Ro, represented the total expected number of offspring produced by a female during her lifetime. The calculation for Ro as follows:

$$Ro = \Sigma l_x m_x$$

Mean generation time (T) is the time required for a population to increase by the factor Ro. It is derived from the equation:

$$T = \frac{\Sigma x l_x m_x}{Ro}$$

RESULTS

The life-history parameters including generation time, T (days) for three species of harpacticoids cultured under laboratory condition (temperature 25±1°C and salinity 27±1 ppt) for 40 days are given in Table 1. *P. oculata* showed the longer mean generation time (17.19±4.74 days) compared to other two species. The mean generation time of *S. knabeni* and *R. knoxi* were 10.19±3.51 days and 8.93±1.00 days respectively. The net reproductive rate (Ro) was higher in *P. oculata* (100.00±28.28) followed by *R. knoxi* (22.50±20.51) and *S. knabeni* (13.00±9.90). The Intrinsic growth rate (r) was higher in *R. knoxi* (0.58±0.18) followed by *S. knabeni* (0.50±0.22) *and P. oculata* (0.45±0.13).

Naupliar stages for all three species were completed within two days in the second generation though it took

Table 1: Life-history parameters of *P. oculata, S. knabeni* and *R. knoxi* under laboratory condition

Parameters	*P. oculata*	*S. knabeni*	*R. knoxi*
Net reproductive rate (Ro)	100.00±28.28	13.00±9.90	22.50±20.51
Generation time (T) (day)	17.19±4.74	10.19±3.51	8.93±1.00
Intrinsic growth rate (r) (day)	0.45±0.13	0.50±0.22	0.58±0.18

Values are Mean±SD, Ro: $\Sigma l_x m_x$, T: $\Sigma x l_x m_x/Ro$, r: lnRo/T

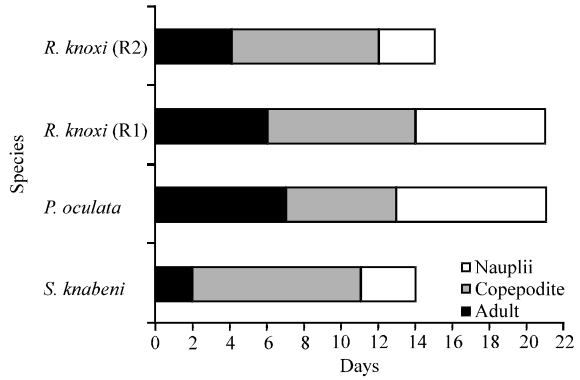

Fig. 1: Population of the first generation of *P. oculata*, *S. knabeni* and *R. knoxi*, under laboratory condition

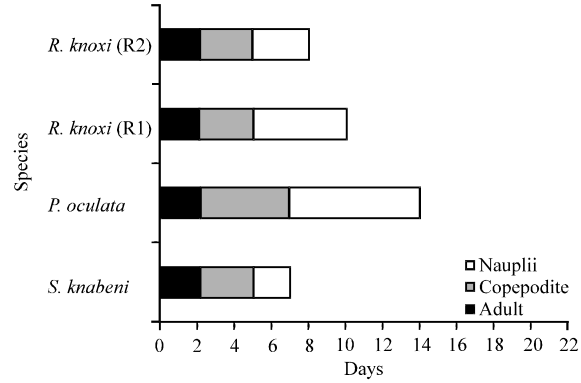

Fig. 2: Population of the second generation of *P. oculata*, *S. knabeni* and *R. knoxi*, under laboratory condition

between two and seven days in the first generation. Copepodites also showed the same trend and took only the maximum of four days to complete the stages in the second compared to between six and 10 days in the first generation. Despite that, the duration for female to become adult and gravid was more or less the same between the first and second generation. Overall the time required for all the developmental stages to grow up to adult stage has notably reduced from first generation to the second (Fig. 1, 2).

DISCUSSION

Copepod production and their aquaculture nutritional values were extensively discussed by Stottrup (2000) and their suitability as a larval feed was studied (McKinnon *et al*., 2003; Gopakumar and Santhosi, 2009). In this study, three harpacticoid species (*P. oculata*, *S. knabeni* and *R. knoxi*) were observed to have a short generation time and the population growth seemed to be quickly adapted to laboratory culture in the second generation. Recent studies on the effect of food, environmental stability and temperature suggested that they could play an important role in affecting the generation time of copepod (Zaleha and Busra, 2012). Generation time of *R. knoxi* was considered short compared to the other two species in this study and others species in previous study. Similar results were noted by Rhodes (2004) who observed a generation time of 10-12 days for *Nitokra lacustris*. On the other hand *Amphiascus tenuiremis* has a generation time of 16 to 17 days (Chandler *et al*., 2004) when cultured under laboratory condition. Harpacticoids with short generation time proved their suitability for mass culture. This was found in *Tisbe biminiensis* and *Tigriopus japonicus* (Pinto *et al*., 2001). The longer generation time in most of the life stage period in the first generation might be caused by the physiological adjustment to the culture condition. The past moving water and tidal change from the South China Sea influenced the harpacticoids in the area (Zaleha *et al*., 2006) and naturally they need to adjust their physiology in response to the calm environment in the culture bottle. Some species collected from the same vegetative area including *Paralaophonte octavia* and *Longipedia* sp. (Nurul Huda and Zaleha, 2005) failed to survive the same laboratory condition.

Nauplii stage is an important first day feed for some fish larvae (Stottrup and Norsker, 1997). Larval stages of shrimp such as *Litopenaeus vannamei* was also reported to prefer the naupliar over the copepodite stage of harpacticoid (De Lima and Souza-Santos, 2007). The short life stage for the species found in this study (2 days) needs an efficient collecting or harvesting technique if they are planned to be used as live feed in aquaculture. Culture technique by Sun and Fleeger (1995) for negative phototaxis species might be a good example. Nevertheless, further study of phototaxism of the present species need to be carried out to facilitate the culture technique design.

CONCLUSION

Of the three cultured species, *R. knoxi* showed its potential as a good candidate for mass culture due to their short generation time (8.93±1.00 days). On the other hand, *P. oculata* showed more stable and high population starting from the first generation thus also indicates its commercial potential. The Naupliar and copepodite stage took longer time during the first generation in all studied species. The finding on the longer time taken by the harpacticoids to stay in their juvenile stages as shown in

this study could be further investigated as to be manipulated for hatchery industry. Further research is also important to design a technique to sample the different life stage of the population.

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REFERENCES

Barclay, W. and S. Zeller, 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia* by feeding spray-dried *Schizochytrium* sp. J. World Aquacult. Soc., 27: 314-322.

Chandler, G.T., T.M. Cary, D.C. Volz, S.S. Walse, J.L. Ferry and S.L. Klosterhaus, 2004. Fipronil effects on estuarine copepod (*Amphiascus tenuiremis*) development, fertility and reproduction: A rapid life-cycle assay in 96-well microplate format. Environ. Toxicol. Chem., 23: 117-124.

De Lima, L.C.M. and L.P. Souza-Santos, 2007. The ingestion rate of *Litopenaeus vannamei* larvae as a function of *Tisbe biminiensis* copepod concentration. Aquacult., 271: 411-419.

Evjemo, J.O., K.I. Reitan and Y. Olsen, 2003. Copepods as live food organisms in the rearing of Atlantic halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on nutritional value. Aquacult., 227: 191-210.

Gopakumar, G. and I. Santhosi, 2009. Use of copepods as live feed for larviculture of damselfishes. Asian Fish. Sci., 22: 1-6.

Kraul, S., H. Ako, A. Nelson, K. Brittain and A. Ogasawara, 1992. Evaluation of live feeds for larval and postlarval mahimahi, *Coryphaena hippurus*. J. World Aquacult. Soc., 23: 299-306.

Kuhlmann, D., G. Quantz and U. Witt, 1981. Rearing of turbot larvae (*Scophthalmus maximus* L.) on cultured food organisms and postmetamorphosis growth on natural and artificial food. Aquacult., 23: 183-196.

McKinnon, A.D., S. Duggan, P.D. Nichols, M.A. Rimmer, G. Semmens and B. Robino, 2003. The potential of tropical paracalanid copepods as live feeds in aquaculture. Aquacult., 223: 89-106.

Nanton, D.A. and J.D. Castell, 1998. The effects of dietary fatty acids on the fatty acid composition of harpacticoid copepods, *Tisbe* sp., for use as a live food for marine fish larvae. Aquacult., 163: 251-261.

Nurul Huda, A.I. and K. Zaleha, 2005. Report on some phytal harpacticoid copepod from Terengganu Coast. Proceedings of the KUSTEM 4th Annual Seminar, May 2-5, 2005, Primula Beach Resort, Kuala Terengganu, Terengganu, pp: 281-285.

Nybakken, J.W., 2001. Marine Biology: An Ecological Approach. 5th Edn., Benjamin Cummings, New York, USA., ISBN-13: 9780321030764, pp: 43-44.

Payne, M.F. and R.J. Rippingale, 2000. Rearing West Australian seahorse, *Hippocampus subelongatus*, juveniles on copepod nauplii and enriched *Artemia*. Aquacult., 188: 353-361.

Penchenik, J.A., 2004. Biology of the Invertebrates. 5th Edn., McGraw-Hill, New York, USA., ISBN-13: 978-0071111751, Pages: 608.

Pinto, C.S.C., L.P. Souza-Santos and P.J.P. Santos, 2001. Development and population dynamics of *Tisbe biminiensis* (Copepoda: Harpacticoida) reared on different diets. Aquacult., 198: 253-267.

Rainuzzo, J.R., K.I. Reitan and Y. Olsen, 1997. The significance of lipids at early stages of marine fish: A review. Aquacult., 155: 103-115.

Rhodes, A.C.E., 2004. Marine harpacticoid copepod culture for the production of long chain highly unsaturated fatty acids and carotenoid pigments. Ph.D. Thesis, North Carolina State University, Raleigh, NC, USA.

Ricklefs, R.E., 1990. Birds as flying machines. [C. J. Pennycuick, Bird flight performance. A practical calculation manual. Oxford University Press, New York (1989)]. Sci., 248: 1562-1563.

Rippingale, R.J. and M.F. Payne, 2001. Intensive Cultivation of a Calanoid Copepod Gladioferens Imparipes. A Guide to Procedures. Curtin University of Technology, Perth, Australia.

Schipp, G.R., J.M. P. Bosmans and A. Marshall, 1999. A method for hatchery culture of tropical calanoid copepods, *Acartia* spp. Aquacult., 174: 81-88.

Shields, R.J., J.G. Bell, F.S. Luizi, B. Gara, N.R. Bromage and J.R. Sargent, 1999. Natural copepods are superior to enriched *Artemia* nauplii as feed for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. J. Nutr., 129: 1186-1194.

Somerfield, P.J., R.M. Warwick and T. Moens, 2005. Meiofauna Techniques. In: Methods for the Study of Marine Benthos, Elefteriou, A. and A.D. McIntyre (Eds.). 3rd Edn., Chapter 6. Blackwell Science Ltd., Oxford, UK., ISBN-13: 978-0632054886, pp: 229-272.

Stottrup, J., K. Richardson, E. Kirkegaard and N.J. Pihl, 1986. The cultivation of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. Aquacult., 52: 87-96.

Stottrup, J.G. and N.H. Norsker, 1997. Production and use of copepods in marine fish larviculture. Aquacult., 155: 231-247.

Stottrup, J.G., 2000. The elusive copepods: Their production and suitability in marine quaculture. Aquac. Res., 31: 703-711.

Sun, B. and J.W. Fleeger, 1995. Sustained mass culture of *Amphiascoides atopus* a marine harpacticoid copepod in a recirculating system. Aquacult., 136: 313-321.

Vincx, M., 1996. Meiofauna in Marine and Freshwater Sediment. In: Methods for the Examination of Organism Diversity in Soils and Sediments, Hall, G.S. (Ed.). CAB International, Wallingford, UK., pp: 187-195.

Watanabe, T. and V. Kiron, 1994. Prospects in larval fish dietetics. Aquacult., 124: 223-251.

Watanabe, T., C. Kitajima and S. Fujita, 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: A review. Aquacult., 34: 115-143.

Zaleha, K. and I. Busra, 2012. Culture of Harpacticoid Copepods: Understanding the Reproduction and Effect of environmental Factors. In: Aquaculture, Muchlisin, Z. (Ed.). InTech Open Access Publisher, Rijeka Croatia, ISBN: 978-953-307-974-5, pp: 343-360.

Zaleha, K., M.N. Roswati and N. Iwasaki, 2006. Distribution of some species of Harpacticoid Copepods in East Coast of Peninsular Malaysia. Coastal Mar. Sci., 30: 140-145.