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Original Article

Cultivation of harpacticoid copepods (families harpacticidae and laophontidae) under selected environmental conditions

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ABSTRACT

The objective of this study was to find the optimal conditions for harpacticoid copepod cultivation at the laboratory scale. Harpacticoid copepods were collected from the macroalgae, *Padina* sp. and *Amphiroa* sp., and used to study the effects on their survival rates of temperature, salinity, diet, substratum and initial density. Mixed cultures of the dominant harpacticoid copepods (Families Harpacticidae and Laophontidae), were used for subsequent experiments. After 7 d, copepods reared at 30 °C showed the highest mean (±SE) survival rate (46.67 ± 5.85%; $p < 0.05$), followed by the copepods cultivated at 25 °C and 35 °C, respectively. Harpacticoid copepods could survive in brackish water to hyper-saline water, between 10 practical salinity units (psu) and 40 psu, but not in freshwater ($p < 0.05$). In addition, they also showed favorable results with salinity at 27 psu and 30 psu with mean survival rates of 44.72 ± 6.35% and 42.78 ± 4.94%, respectively, which were higher than the mean survival rate of 26.67 ± 13.33% recorded in salinity at 10 psu ($p < 0.05$). The feeding experiment was inconclusive since there were no significant differences between the survival rates of copepods on the different algal diets. On the other hand, copepods fed with commercial shrimp feed had the mean lowest survival rate ($p < 0.05$). No significant differences among the different sediment types were observed. However, harpacticoid copepods showed a preference for smaller-sized particles as higher survival rates were obtained for copepods reared in sand-silt sediment rather than in fine sand, coarse sand, vermiculite (artificial sediment) and no substrate. Culture at low densities such as 100 individuals/L and 500 individuals/L is suggested for initial cultivation based on this experiment.

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Introduction

At present, copepod cultivation for use as live feed has increased, with success reported with approximately 60 species (Drillet et al., 2011). Among the copepod groups, the harpacticoid copepods are favored over the calanoids and cyclopoids, since the former have a short generation period, high reproduction, high population growth, flexible diet and tolerance to environmental fluctuation (Støttrup and Norsker, 1997). In addition, harpacticoid copepods are better food for fish larvae than the other common live feed types used in larviculture such as *Artemia* and rotifers, due to their ability to convert any type of organic food into lipid storage such as excess folic acids (EFAs) (Nanton and Castell, 1998). To achieve this, optimal conditions for culturing harpacticoid copepods, including temperature, salinity, diet, substratum and rearing density, must be considered. It has been reported that lower temperatures could increase the total body length of *Tisbe holothuliae* and result in longer development and maturation of copepods, but a reduced number of offspring per egg sac (Miliou and Moraitou-Apostolopoulou, 1991). In addition, temperature also influences the nutritional quality of copepods; for example, the percentage of polyunsaturated fatty acid (PUFA) in two harpacticoid copepod species, *Amonardia* sp. and *Tisbe* sp., was increased at lower temperatures (Nanton and Castell, 1999). Similarly, salinity affects osmoregulation in copepods and this is important for the fecundity and longevity of harpacticoid copepods, while this condition is species- and strain-dependent (Cutts, 2001). The results from laboratory studies by Bergmans and Janssens (1988) showed that a higher growth rate of *Tisbe holothuriae* occurred at 32 practical salinity units (psu), while the higher growth rate of *T. battagliai*

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was at 20 psu. The euryhaline harpacticoid species, *Nitokra lacustris*, was able to grow in salinity ranging from 10 psu to 40 psu (Rhodes, 2003a), while the optimum salinity for the development of a tropical harpacticoid, *Pararobertsonia* sp., during laboratory cultivation was at 35 psu (Zaleha and Jamaludin, 2010). Moreover, it is likely that the estuarine copepods are more sensitive to a change of temperature rather than salinity because they can adapt to the salinity fluctuations due to the daily tidal cycle (Zaleha and Jamaludin, 2010).

Diet can be the most vital condition for the survival of harpacticoid copepods, and a variety of food sizes may increase their survival (Cutts, 2001). In particular, the protein and lipid contents of the diet seem to be important for harpacticoid copepods since the algal diet with the highest protein and lipid contents gave the best productivity in *Tisbe carolinensis* (Lee et al., 1985). In culture, various diets such as *Chaetoceros calcitrans*, *Dunaliella tertiolecta*, *Isochrysis galbana* and baker's yeast, have been fed to *Tisbe* sp. and resulted in a consistently high docosahexaenoic acid (DHA): eicosapentaenoic acid (EPA) ratio in harpacticoid copepods (Nanton and Castell, 1998). Since harpacticoid copepods are benthic, choosing a suitable substratum and available area are also concerns for mass culture. The impact of inorganic sediments on rocky shore meiofauna has been documented by Gibbons (1988) who concluded that the habitat complexity and sediment contents have a positive effect on the diversity and abundance of harpacticoid copepods of both the adult and nauplius stages. Moreover, it has already been reported that the density can affect the growth, survival, development and fecundity of harpacticoid copepods (Cutts, 2001). In particular, since harpacticoid copepods are benthos, the available of substratum area would be more important than the available water volume. Complex chemical compounds may be produced by copepods reared in high density and result in different response levels (Zhang and Uhlig, 1993).

There have been few reports on harpacticoid cultivation in Thailand. Chullasorn (2010a) investigated the post-embryonic development of five harpacticoid copepod species—*Tigriopus thailandensis*, *T. japonicus*, *Paramphiascella choi*, *Nitokra karanovici* and *Tigriopus* sp.—collected from algal bases along the eastern coast of the Upper Gulf of Thailand. That author concluded that the optimal conditions for culturing these copepods were: temperature, 25–30 °C; salinity, 25–33 psu; and three algal diets (*Isochrysis galbana*, *Chaetoceros* sp. and *Tetraselmis* sp.) However, mass cultures of harpacticoid copepods to date have not yet proven successful due to the lack of information on the effects of environmental factors on survival and biology of harpacticoid copepods. Thus, the objective of this study was to determine the optimal environmental parameters (temperature, salinity, diet, substrate and density) for the paramount survival of harpacticoid copepods at the laboratory scale. The findings could then be keys for the future development of copepod cultivation.

Materials and methods

Sampling of harpacticoid copepods

Harpacticoid copepods were collected during July 2013 to April 2014 from the rocky intertidal zone of the eastern coastal area near the Sichang Marine Research and Training Station (SMaRT), Sichang Island, Chonburi Province, Thailand. The sampling area (about 10 m wide and 30 m long) was a rocky shore lined with a narrow sandy beach of mostly fine sediments. *Amphiroa* sp. and *Padina* sp. were the two dominant algae in the tidal pools (Fig. 1). The copepods were collected after flushing thallus of algae, *Amphiroa* sp. and *Padina* sp., using filtrated seawater. Then, they were sieved through a 100 μm mesh net and identified under a stereo microscope (model SZ30; Olympus Corp.; Tokyo, Japan) at an objective lens maximum magnifying power of 4× after being fixed in neutral formalin and dyed with Bengal's Rose with a final concentration of 4–6% for 24 h. Live mixed species of the co-occurring harpacticoid copepods in the Families Harpacticidae and Laophontidae were finally sorted using a capillary tube under a stereo microscope after which they were acclimated in beakers for 12–24 h before conducting the experiments. Each experiment was repeated three times.

Effect of temperatures on survival rate of harpacticoid copepods

The experiment was conducted during November to December 2013. Ten of the selected harpacticoid copepods (Families Harpacticidae and Laophontidae) were reared in 50 mL beakers containing 20 mL filtered seawater (salinity 27–30 psu to mimic natural seawater). Then, they were exposed to three temperature levels, 25 °C, 30 °C or 35 °C, by placing each beaker in water baths with three replicates for each temperature. The experiments on other parameters were conducted following the culture method used by Chullasorn (2010b). In detail, copepods were fed every other day with a combination of microalgae—*Isochrysis galbana*, *Chaetoceros calcitrans* and *Tetraselmis gracilis* (ratio 1:1:1 by volume). The photoperiod was controlled at 12 h light:12 h dark. Copepod mortality was monitored at 1, 2, 3, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h using a stereo microscope.

Effect of salinity level on survival rate of harpacticoid copepods

The experiment was conducted during July to August 2013. Ten of the harpacticoid copepods were placed in multi-well plates containing 3 mL filtrated seawater with triplicate salinity treatments of 0, 10, 20, 30 and 40 psu, with natural seawater (salinity of 27 psu) as a control. The temperature of the culture environment was in the range 28–32 °C. The samples were fed with a combination of the three microalgae every other day and the photoperiod was the same in as the previous experiment. Copepod mortality was monitored after 1, 2, 3, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h using a stereo microscope.

Effect of selected diets on survival rate of harpacticoid copepods

The experiment was conducted in April 2014. Ten of the harpactiocoid copepods were reared in plastic cups containing 20 mL of filtrated natural seawater (salinity 27–30 psu). Triplicate cups of the copepods were fed daily with one of six different feed types: 1) *I. galbana* (cell size: 4–7 μm in diameter), 2) *C. calcitrans* (cell size: 3–4 μm in diameter), 3) *T. gracilis* (cell size: 10–25 μm long and 7–20 μm wide), 4) a combination of these three algae, 5) commercial shrimp feed pellets (the general feed used for sandworm, *Perinereis nuntia*), and 6) non-feed (as the control), respectively. The temperature and photoperiod were the same as in the previous experiments. Copepod mortality was monitored every 24 h for 7 d (168 h) under a stereo microscope.

Effect of substrata on survival rate of harpacticoid copepods

The experiment was conducted during January to February 2014. Thirty of the harpacticoid copepods were reared in plastic cups containing 20 mL filtrated seawater (salinity 27–30 psu). Triplicates of the five different substrate conditions were assigned: 1) no substrate, 2) coarse sand (particle size > 1 mm), 3) fine sand (particle size ≤ 1 mm), 4) vermiculite (an artificial sediment used for sandworm culture), and 5) sand-silt sediment (mixture of fine sand and silt particles collected from the study sites), respectively.

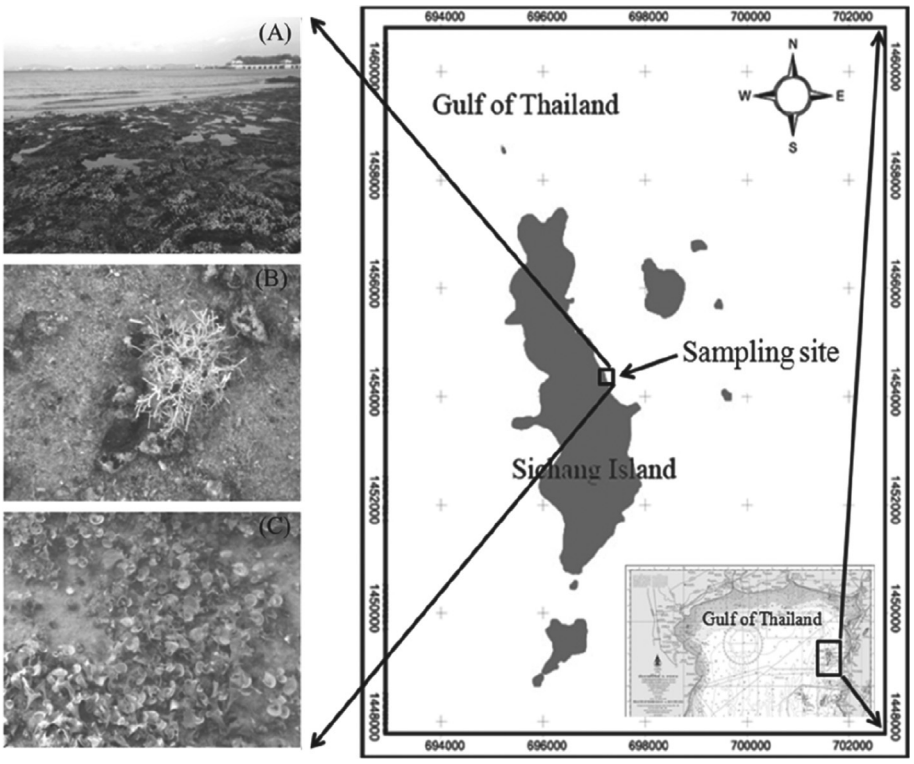


Fig. 1. Sampling site of harpacticoid copepods from the rocky shore area of Sichang Island, Chonburi Province, Thailand (A), covered by the two dominant macroalgae, *Amphiroa* sp. (B) and *Padina* sp. (C) in tidal pools.

Then, they were fed with a combination of the three microalgae every other day. The water temperature and photoperiod conditions for the experiment were the same as in the previous experiments. Copepod mortality was monitored under a stereo microscope at the end of the experiment (7 d), when the samples were preserved in a 4–6% final concentration of neutral formalin and dyed with Bengal's Rose for 24 h prior to the investigation. The copepods were differentiated from the sediment by their reddish pink color.

Effect of initial rearing density on survival rate of harpacticoid copepods

The experiment was conducted during September to October 2013. The harpacticoid copepods were reared at different densities with triplicate sets of initial densities of 100, 500, 1,000, 3000 and 8000 individuals/L. The culture conditions including temperature, salinity, diets and photoperiod were the same as in the previous experiments. Copepod mortality was monitored after 6, 12, 24, 48, 72, 96, 120, 144 and 168 h using a stereo microscope.

Statistical analysis

Analysis of variance was used to analyze differences among treatments followed by a *post-hoc* test (Tukey's comparison test), with a significance level of 95% (p-value ≤ 0.05) using the SPPS statistical package (version 22; SPSS Inc.; Chicago, IL, USA).

Ethical statement

This study was approved by the Ethics Committee of Chulalongkorn University, Bangkok, Thailand (Approval no. U1-02205-2558).

Results

Diversity of harpacticoid copepods

Four genera from four families of harpacticoid copepods were found in the study area: *Nitokra* (Family Ameiridae), *Tigriopus* (Family Harpacticidae), *Tisbe* (Family Tisbidae) and an unidentified genus from the Family Laophontidae. The variation in the harpacticoid copepods corresponded to the alternation of algae, *Amphiroa* sp. and *Padina* sp., with *Amphiroa* sp. dominating during March to September and *Padina* sp. during October to February. The genus *Tigriopus* was the dominant genus during the study period (approx. 60–70%). A harpacticoid copepod in the Family Laophontidae was found in higher density (approx. 25–30%) related to the domination of *Padina* sp. while the genus *Tisbe* was found only during the domination of *Amphiroa* sp. (approx. 5–10%). In addition, *Nitokra* sp. was found only in small numbers during the study period (less than 5%). Therefore, the co-occurrence of the dominant harpacticoid copepod Families of Harpacticidae and Laophontidae was selected for the subsequent experiments. The ratios of Harpacticidae: Laophontidae used in each experiment were 7:3 or 6:4 depending on the abundance of the Family Laophontidae.

Effect of temperature on survival rate of harpacticoid copepods

Throughout the experiment, the survival rate of harpacticoid copepods reared at 30 °C was significantly higher than in the group cultured at 35 °C, but was not significantly different from the group reared at 25 °C. Likewise, the group reared at 25 °C was not significantly different from the 35°C-group throughout the rearing period. After 7 d rearing, the group at 30 °C had the highest mean (±SE) survival rate (46.7 ± 5.85%), with the first mortality being observed at 12 h followed by the group at 25 °C (21.67 ± 10.93%,

with the first mortality noted after 6 h cultivation), and the group at 35 °C (100% mortality after 144 h (6 d) rearing, with the first mortality occurring after rearing for 2 h) as shown in Fig. 2.

Effect of salinity on survival rate of harpacticoid copepods

The survival rates of harpacticoid copepods reared at various salinity levels of seawater (0 psu, 10 psu, 20 psu, 30 psu and 40 psu) are shown in Fig. 3. The harpacticoid copepods survived in brackish water to hyper-saline water between 10 and 40 psu but they could not survive in freshwater, with more than 60% of the harpacticoid copepods dying after 1 h and all of them had died within 72 h when exposed to freshwater. Although the survival rates of the groups reared in a salinity of 20–40 psu were not significantly different and were similar to the survival rate of the control group, the statistical testing revealed that the survival rates of the 20 psu and 40 psu groups were also similar to the 10 psu group ($p > 0.05$). As such, the best salinity for survival would be at 30 psu. After 7 d, the highest mean (±SE) survival rates of 44.72 ± 6.35% and 42.78 ± 4.94% were recorded for copepods cultured in 27 psu (control) and 30 psu seawater, respectively (Fig. 3).

Effect of diet on survival rate of harpacticoid copepods

Among the six diets, the combination of three algae (*I. galbana*, *C. calcitrans* and *T. gracilis*) and the mono-algal (*C. calcitran*) diet gave relatively high mean (±SE) survival rates (44.44 ± 6.76% and 43.33 ± 5.09%, respectively) which surprisingly were not different from the group without feeding (31.11 ± 7.78% survival rate). The diets that produced low survival rates comprised the other two mono-algal diets (*I. galbana* or *T. gracilis* with survival rates of 23.33 ± 0.00% and 22.22 ± 5.56%, respectively) and the commercial shrimp feed (2.22 ± 2.22%). Noteworthy was that the survival rate of the group fed commercial shrimp feed was very low but not significantly different from the two mono-algal fed groups (Fig. 4).

Effect of substrata on survival rate of harpacticoid copepods

The harpacticoid copepods reared with coarse sand, fine sand, vermiculite, sand-silt sediment and without substrate, showed similar survival rates ($p > 0.05$) of more than 50%. The relatively high mean (±SE) survival rate of 76.11 ± 1.67% ($p > 0.05$) was found in the sand-silt sediment followed by 68.33 ± 5.00%, 65.56 ± 7.78%, 63.89 ± 9.44 and 56.67 ± 1.11%, for the fine sand, no substrate, coarse sand and vermiculite, respectively (Fig. 5).

Effect of initial rearing density on survival rate of harpacticoid copepods

The harpacticoid copepods cultured at various initial densities showed significantly different mean survival rates between the lowest density (100 individuals/L) and the density of 1000 individuals/L but no significant differences was observed between the density of 100 individuals/L and other densities (500, individuals/L 3000 individuals/L and 8000 individuals/L). After 7 d, a mean survival rate of over 50% was recorded for harpacticoid copepods reared at low densities such as 100 individuals/L and 500 individuals/L, which had mean (±SE) survival rates of 61.11 ± 5.56% and 53.33 ± 10.18%, respectively. In contrast, lower mean survival rates of 35.56 ± 12.37%, 34.81 ± 8.35% and 43.89 ± 11.81% occurred when the densities were 1000 individuals/L, 3000 individuals/L and 8000 individuals/L, respectively (Fig. 6).

Discussion

The impact of habitat complexity and the relationship between algal species and harpacticoid copepod species was demonstrated more than 30 yrs ago by Hicks (1980). The harpacticoid copepods preferentially live with a seaweed base such as *Enteromorpha clathrata*, *Caulerpa* spp., *Sagassum* sp., *Padina* sp., *Neomeris vanbosseae* and *Gracilaria* spp. (Chullasorn, 2010b). In the present study, four families of harpacticoid copepod—the Families Ameiridae (genus *Nitokra*), Harpacticidae (three species in the genus *Tigriopus*), Laophontidae (an unidentified genus) and Tisbidae (the genus *Tisbe*)—were found in the two seaweed bases of *Amphiroa* sp. and *Padina* sp., near the Sichang Marine Research and Training Station on Sichang Island, Chonburi province, Thailand. The co-occurrence of the copepod Families Harpacticidae and Laophontidae, which were the dominant harpacticoid copepods in the study area, were then selected for the further experiments.

The effects of selected environmental conditions (temperature, salinity, diet, substrata and initial rearing density) on the survival rate of harpacticoid copepods were studied under laboratory conditions. After 7 d, a relatively high survival rate (over 45%) was

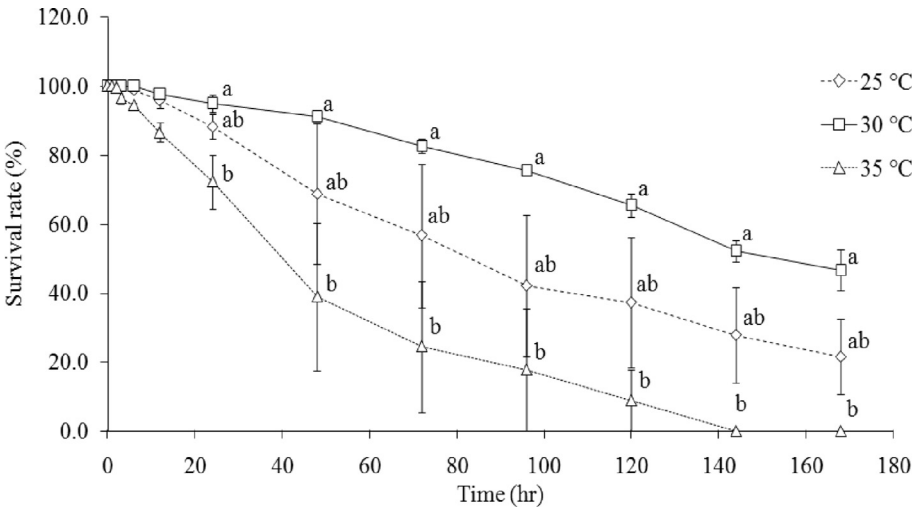


Fig. 2. Survival rate (mean ± SE) of harpacticoid copepods reared at different temperatures (different lowercase letters at each time point indicate significant differences at $p < 0.05$.).

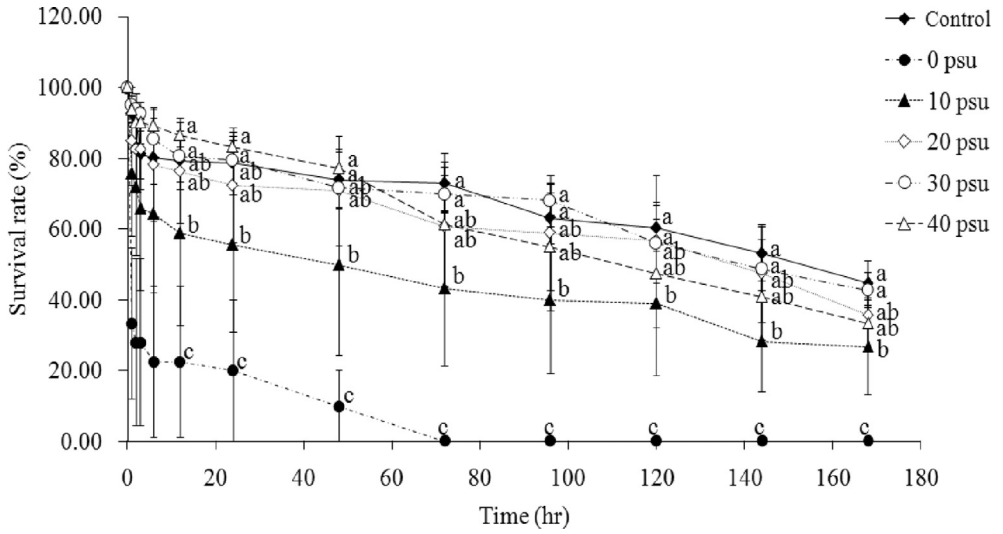


Fig. 3. Survival rate (mean ± SE) of harpacticoid copepods cultured at various salinity levels (practical salinity units; psu) and different letters at each time point indicate significant differences at $p < 0.05$.

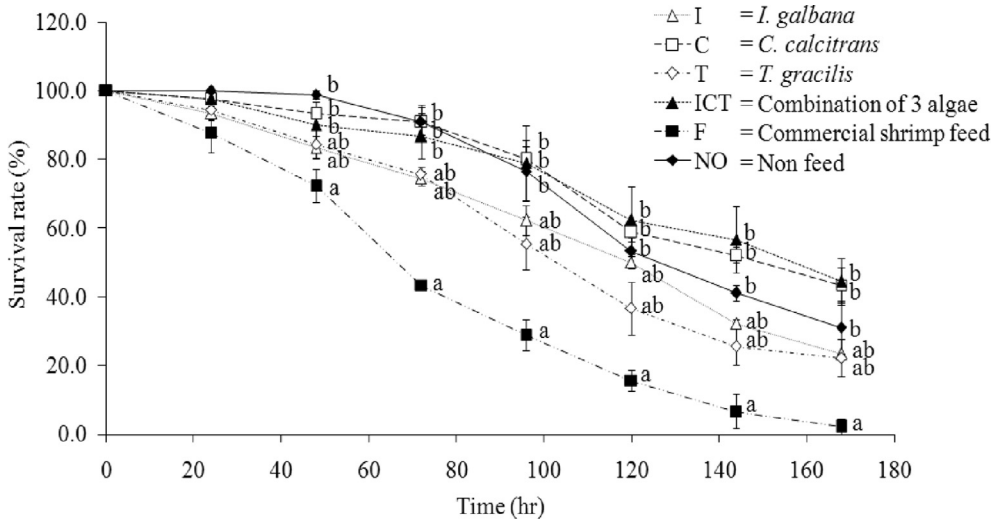


Fig. 4. Survival rate (mean ± SE) of harpacticoid copepods fed with six different diets and different lowercase letters at each time point indicate significant differences at $p < 0.05$.

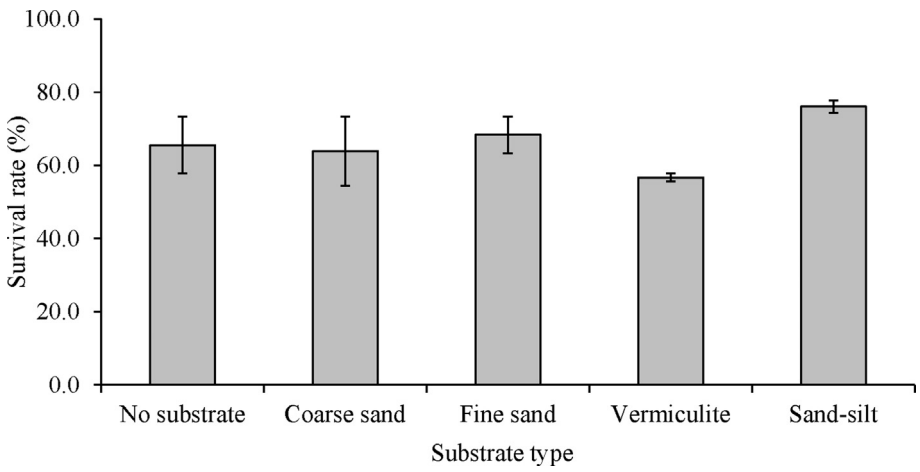


Fig. 5. Survival rate (mean ± SE) of harpacticoid copepods cultivated in designated substrata (there were no significant differences in the survival rates in different substrate conditions at $p > 0.05$.).

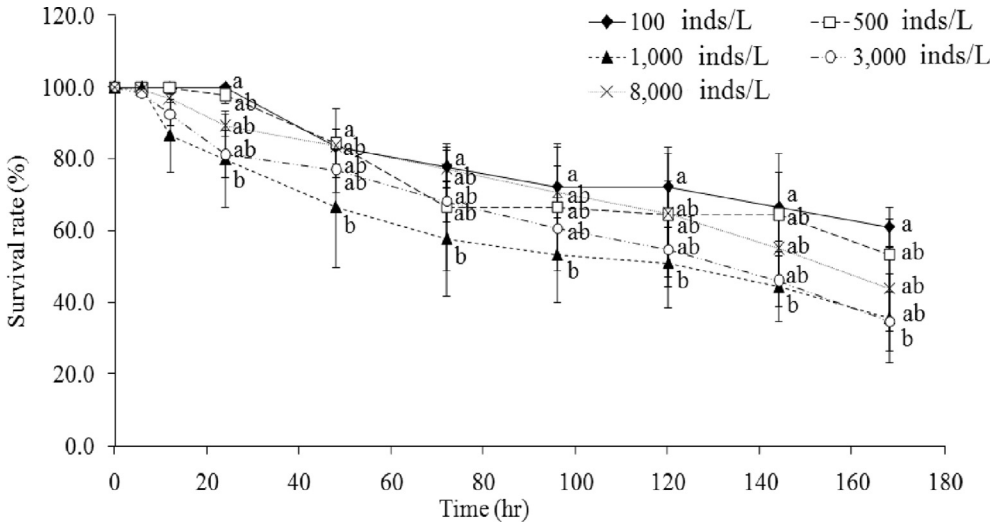


Fig. 6. Survival rate (mean ± SE) of harpacticoid copepods reared at different initial densities measured in individuals (inds)/liter; (different lowercase letters at each time point indicate significant differences at $p < 0.05$.).

recorded for harpacticoid copepods reared at 30 °C and salinity of 27 psu (natural seawater as control) and 30 psu compared to other the temperatures and salinities. This was similar to the cultivation of *Tigriopus thailandensis*, *T. japonicus*, *Paramphiascella choi*, *Nitokra karanovici* and *Tigriopus* sp. under laboratory conditions conducted by Chullasorn (2010a) with the temperature ranging from 25 °C to 30 °C and salinity varying from 25 psu to 33 psu. Harpacticoid copepods are tolerant to environmental variations, but they do show positive effects when cultured at favorable temperature and salinity, though this is species- and strain-dependent (Cutts, 2001). A tropical harpacticoid in the Family Miraciidae (*Pararobersonia* sp.), obtained from the Merchang estuary, South China Sea, was grown at 25 ± 1 °C (Zaleha and Jamaludin, 2010) and the Family Tisbidae, *Tisbe biminiensis*, in Brazil was cultured at 28–32 °C (Ribeiro and Souza-Santos, 2011), while a widely distributed copepod in the Family Harpacticidae, *Tigriopus* sp., found in Korea had its highest production at 28 °C (Lee and Park, 2005). It was known that temperature affects the metabolisms of marine copepods (Ikeda et al., 2001). Lower temperatures caused longer development and maturation of copepods, while also reducing the number of offspring per egg sac (Miliou and Moraitou-Apostolopoulou, 1991). Temperature affects not only development and production, but also the nutritional quality of copepods. Nanton and Castell (1999) reported that a lower temperature (6 °C) increased the concentration of n-3 HUFA in the two marine harpacticoid copepods, *Amonardia* sp. and *Tisbe* sp., compared to higher temperatures (15 °C and 20 °C). Moreover, storage fatty acids such as DHA, in starved copepods were more depleted when cultured at higher temperature, indicating that the metabolic cost of living increases with temperature (Werbrouck et al., 2016). Likewise, temperature and salinity are important factors that affect the survival of crustaceans, including copepods (Lance, 1963). In the present study, the harpacticoid copepods tolerated salinity that varied from 10 psu to 40 psu, similar to the study by Matias-Peralta et al. (2005) which reported that a tropical harpacticoid copepod, *Nitokra affinis*, can survive in salinity ranging from 10 psu to 35 psu. However, the optimal salinity range for harpacticoid copepods in the present study (27 psu to 30 psu) falls within that observed in the vicinity of the sampling site (Sichang Island, 22 psu to 33 psu, Rungsupa, 2012), and was similar to the salinity of 25–33 psu in the laboratory conditions employed by Chullasorn (2010a). In particular, Raisuddin et al. (2007) concluded that the most suitable salinity for the copepod *Tigriopus* spp. in the Family Harpacticidae was 27.1–34.3 psu.

Foods are also an essential issue for copepod survival. In general, harpacticoid copepods distribute in coastal waters and usually live as benthic organisms with their food being algae or settled organic particles (Coull and Wells, 1983). In particular, copepods in the Family Hapacticidae, such as the genus *Tigriopus*, are generalist feeders which utilize algae such as diatoms to various particles sizes in the range 0.5–50 μm in diameter and including bacteria (Powlik et al., 1997), while the Family Laophontidae prefers sediments rather than benthic algae (Hicks, 1977). Various food sizes may enhance copepod production by affecting both the feeding activity and survival rate of copepods (Lee et al., 1985; Cutts, 2001). The present study aimed at the cultivation of harpacticoid copepods for use as live feed for sandworm, *Perinereis nuntia*. This was a reason for including commercial shrimp diets in the present study as these are regularly fed to sandworm (Techaprempreecha et al., 2011). However, the results showed that the commercial shrimp diet was not suitable for feeding to the selective harpacticoid copepods as shown by the lowest survival rate relative to other algal diets ($p < 0.05$). It is likely that shrimp feed pellets contain excessive oil contents which when released into the water resulted in an oil film attaching to copepod appendages, thus leading to mortality of copepods.

The results from the present study showed a tendency, although with non-significant difference with other treatments, that the combination of the three algae, and *C. calcitrans* had the highest survival rate of the copepods. Nevertheless, the survival rates obtained from these two diets were not different from the treatment without feeding. This probably indicates that copepods can tolerate starvation during environmental stress such as under the laboratory conditions, and this led to them surviving longer. Starvation can induce changes in the feeding behavior and digestive enzyme activity (Hassett and Landry, 1990) and there has been a report that copepods can tolerated starvation for 1–3 wk both in the nauplius and copepodite stages (Tsuda, 1994). In addition, there are several conditions that affect the feeding rate of copepods, such as the food concentration which has led to functional responses of copepods to prey species and prey size (which resulted in feeding selection and changes to the feeding mechanisms of copepods) and turbulence which enhances the chance of encounters between copepods and their prey (Miller and Wheeler, 2012). In general, the mixed algal

diets were always better than the mono algal diets and this supported the use of a multi-algal diet. In general, the copepods fed a combination of various algae always performed better than when fed on a mono-species algal, presumably because the mixed diet generally provided more balanced nutrition, including vitamins, minerals and trace elements that are vital for the survival, growth and reproduction of copepods (Lee et al., 1985). Miles et al. (2001) recommended that *Tisbe holothuriae* be fed a mixture of *Chaetoceros mulleri*, *Rhinomonas reticulata* and *Isochrysis galbana* to produce a high survival rate and nauplii production, and that this diet also provided an adequate content of DHA and a good DHA:EPA ratio. Moreover, different sizes of the mixed algae suited the requirement for different particle sizes due to the changing size of copepod mouthparts during development (Cutts, 2001; De Troch et al., 2006a).

The habitat complexity and sediments influence the micro-environmental conditions and in turn have an important impact on meiofaunal communities (Hicks, 1980; Gibbons, 1988). Different grain sizes also affect the grazing rate of the harpacticoid copepods (De Troch et al., 2006b). Therefore, having an appropriate substratum is also necessary for good productivity in a copepod culture (Cutts, 2001). In the present study, the survivals rates of harpacticoid copepods showed no significant differences among substrates, with survival rates of over 50% observed for all sediment types, including no substrate. This was in line with the results reported for *Tisbe biminiensis*, which showed low sensitivity to different particle sizes, while a higher number of nauplii and fecundity were obtained with the smallest grain size (less than 63 μm) rather than in larger grain sizes (from greater than 63 μm to less than 2 mm) and no substrate (Araújo-Castro et al., 2009). The importance of substrate types may be more related to the organic content which has been considered a driving force in harpacticoid copepod—*Robertsonia propinqua*—distribution (Stringer et al., 2012), rather than grain sizes alone. This has been supported by previous investigations where high values of organic matter, bacterial abundance and chlorophyll *a* content were obtained using smaller particle sizes (Hargrave, 1972; Cammen, 1982). Despite the non-significantly different survival rates among different substrate particle sizes, the present study showed a tendency for the harpacticoid copepods to favor a substrate with small particle size as was shown by the relatively high survival rate (76%) and the number of females with egg sac production (10%) in sediment with smaller particles (sand-silt), rather than larger particles (fine sand, and coarse sand), and no substrate (data not shown). Less ovigerous females (2–3%) were also observed in the groups reared in the substrate with large particles (data not shown). Regarding harvesting ease, Chandler (1986) suggested that a small particle size (less than 0.125 mm) such as cleaned and sorted mud was more suitable than a larger particle size because adults and late-stage copepodites are usually larger than 0.125 mm allowing them to be easily separated in a sieve and make harvesting easy.

The density of a population can affect the growth, survival, development and fecundity of copepods, particularly in mass culture operations (Cutts, 2001). In the present study, a higher survival rate was observed in harpacticoid copepods cultured at low density (100 and 500 individuals/L). However, it should be noted that the harpacticoid copepods used in this study were collected from natural habitats; therefore they possibly developed stress during the sampling or sorting process. Subsequently, rearing in abundant numbers, copepods could feel more stress and be subjected to more excretion, which could cause high mortality in higher density cultivation, since there it been stated that complex chemical compounds may be produced by the animals as a result of crowding, allowing them to perceive and respond to different crowding levels (Fava and Crotti, 1979). Therefore, the density might be increased after generations of culture (Rhodes, 2003b; Ribeiro and Souza-Santos, 2011). However, in some harpacticoid copepods species, such as *Tisbe* spp., the density was related to the available substratum area, rather than the available water volume (Cutts, 2001). Likewise, the female fecundity was influenced by the density of breeding females and nauplii (Zhang and Uhlig, 1993).

Based on the current results, it can be concluded that the optimal conditions for harpacticoid copepod cultivation are a temperature of 25–30 °C, salinity of 27–30 psu, small particles or no substrate culture and rearing at a low initial density. Unfortunately, in the present work, the diet result was inconclusive because the results of feeding experiment were unclear and the experimental time was short. However, culture methods have to be developed further to approach industrial scale cultivation based on this research. An emphasis on mono-species culture requires further study. In particular, harpacticoid copepods in the genus *Tigriopus*, which was the dominant genus in the present study, can be improved for intensive mono-species cultivation in the future as recommended by Chullasorn (2010a) because of their high tolerance and adaptation to a wide range of salinity and other environmental fluctuations.

Conflict of interest

There is no conflict of interest.

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