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A Tropical Harpacticoid Copepod, *Nitocra affinis californica* Lang As an Effective Live Feed for Black Tiger Shrimp Larvae *Penaeus monodon* Fabricius

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ABSTRACT

Survival and specific growth rates of *Penaeus monodon* larvae (post-larval stages 1-15), fed with different live feeds and artificial diets, were evaluated using three different treatments, namely: i) *Nitocra affinis*, ii) combination of *Artemia* nauplii + *N. affinis* and iii) artificial diet. The experiment was carried out in 10-L aquaria with 30% daily water exchange for a period of 16 days. The survival rate (61%) and specific growth rate (16.7 %day^{-1}) were highest ($p<0.05$) in the treatment with shrimp larvae fed with *N. affinis*. Likewise, the protein contents of *N. affinis* was found to be the highest ($p<0.05$) among all the diets used. The fatty acids of *N. affinis* was dominated by polyunsaturated fatty acids (PUFA) (22:6n3) forming 19.5% of the total PUFA identified. In fact, *N. affinis* contained the highest ($p<0.05$) amount of PUFA and the highest ($p<0.05$) n-3/n-6 ratio amongst the three diets. Analysis of the copepod fed shrimp showed significantly higher ($p<0.05$) amount of long chain PUFA, both of the n-3 and n-6 series fatty acids, when compared to the artificial diet fed larvae. The results of this study showed that *N. affinis* has the potential to be used as an effective live feed for *P. monodon* due to their high PUFA contents and broad size range (nauplii to adults) to cater for different shrimp post-larval stages.

Keywords: Harpacticoid copepod, Live feed, *Nitocra affinis californica, Penaeus monodon*, Shrimp larvae

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INTRODUCTION

Today, the larviculture of marine decapod crustaceans is still regarded as one of the major bottlenecks impairing the production of several commercially important species (Calado *et al*., 2008). Meanwhile, live food has been considered to be a limiting factor in the commercial larval production of many fish and crustacean species (Kovalenko *et al*., 2002), and it forms an important factor in overall production cost. For penaeid larvae, animal protein is the most important component of the diet (Anderson & De Silva, 2003; Perera *et al*., 2005), and this is generally supplied by live feeds such as rotifers and brine shrimp, *Artemia*. Although live feeds have been proven successful as animal protein source in raising the larvae of many species, inherent problems still remain. The problems include variable nutrient composition and availability, potential introduction of pathogens into the culture system, and in the case of rotifers, the cultures are prone to crashes (Jones *et al*., 1993; Kovalenko *et al*., 2002). Adequate production of quality live food microorganisms, at a lowest possible cost, is essential for the successful development of commercial fish or shrimp hatchery since artificial diets developed have so far not been fully adequate, and generally, the use of live food has yielded better results (Tseng & Hsu, 1984; Watanabe & Kiron, 1994; Calado *et al*., 2008).

The increased global demand for seed-stock experienced by shrimp farming industry, specifically *P. monodon*, during the last two decades has prompted extensive improvement and refinement of the basic larval rearing techniques (Jory, 1997; Martin *et al*., 2006). The objective of this study was to obtain predictable production of high quality hatchery reared penaeid post-larvae by improving feeding regimes evaluated using three different treatments (Wilkenfed *et al*., 1981, 1984; Kuban *et al*., 1987; Barros & Valenti, 2003).

The harpacticoid, *Nitocra affinis*, is a widely distributed species found commonly in coastal areas, living on fine sand or mud, in shell sand among stones and algae, in mangrove sea, in salty wells, tidal pools and shore reefs (Lang, 1948; 1965; Kunz, 1975). They are epibenthic and euryhaline with a salinity tolerance well beyond the range of most natural salinity fluctuations. Their food requirement is flexible, as they can live and thrive on a variety of food such as algae, bacteria, yeast and artificial food sources (Hicks & Coull, 1983; Gee, 1989; Weiss *et al*., 1996). Thus, *N. affinis* is a desirable organism for experimental work and a good candidate for mass cultivation. The present study was undertaken to evaluate the survival and relative growth rate of *P. monodon* post-larvae fed with *N. affinis* solely, and in combination with the traditionally used *Artemia* nauplii and artificial shrimp diet for a period of 16 days.

MATERIALS AND METHODS

Treatments

Three treatments were employed in this experiment. *Penaeus monodon* (post-larvae stages 1-15) were fed with i) *N. affinis* (size range: 50-400 µm), ii) a combination of

Artemia nauplii (size range: 410-430µm) and *N. affinis*, and iii) artificial diet (ground freeze-dried form, size range: 17-22 µm). For each treatment, three replicates were randomly assigned. The experiment was run for 16 days. The use of *Artemia*, as sole live food source, was excluded in this study due to the fact that *Artemia* is in no doubt still the prime live feed for the larviculture industry (Jones *et al*., 1989; Abelin *et al*., 1991; Samocha *et al*., 1999; Wouters & Van Horenbeeck, 2003; Robinson *et al*., 2005). In addition, it is well established that the use of Artemia can yield from 50% up to 75% survival of shrimp post larvae in commercial hatcheries throughout the world (Jones *et al*., 1989; Abelin *et al*., 1991; Samocha *et al*., 1999; Wouters & Van Horenbeeck, 2003; Robinson *et al*., 2005).

Species Isolation

Nitocra affinis californica was isolated from the coastal waters of Peninsular Malaysia. The copepods were collected using Schindler Patalas trap, along the sandy beach with water level reaching 0.35 m. The copepods were transferred into a 1-L beaker with filtered (0.20 µm filter) seawater, and provided with 24 hour-aeration for conditioning. Then, each copepod carrying egg sacs was separately transferred into a new container filled with filtered (0.20 µm filter) seawater, while mixed algae (*Chaetoceros calcitrans, Nannochloropsis oculata and Tetraselmis tetrathele*) were provided as food sources. To ensure a monospecies stock, the culture was started with one gravid female grown continuously for several generations in the laboratory.

Feed Preparation

Small scale mass production of *N. affinis* was started by transferring 150 gravid female copepods (from the original stock) in 2-L of filtered aged seawater to each culture vessel. The 3-L culture vessels were cylindrical basins (semi transparent and lightly coloured plastics with smooth surface on the inside and out) of polypropylene material, with a base area equals to 177 cm^2, a mouth area of 416 cm^2, and a height of 10 cm. The aged seawater (seawater stocked for a period of time; chlorine free and with stable water chemistry) was filtered in a 0.45 µm membrane filter paper before use. The culture was maintained at salinity 30 $g{\cdot}L^{-1}$ and at temperature levels between 25ºC to 35ºC (seawater at room temperature changed from a minimum of 25ºC, occurring in the early morning while a maximum of 35ºC usually in the mid afternoon). When salinity increased, fresh water was added to the culture vessel until the desired salinity was achieved. The cultures were exposed to natural lighting condition (ranging from 25-40 µmol m^2s^{-1}) and photoperiod (12h light:12h dark cycles). All the above parameters provided for in this study were determined to provide the maximum population growth of *N. affinis* (Matias-Peralta *et al*., 2005). Maintenance of copepod culture involves siphoning of waste water, replenishing the vessels with fresh aged water and feeding (Matias-Peralta *et*

al., 2011). In other words, copepods were harvested daily, in such a way that gravid females were separated from nauplii and copepodids (Matias-Peralta *et al*., 2011). The procedure described above was able to produce 14.9 x 10^3 copepod L^{-1} for a period of 14 days, with a total of 75 gravid females L^{-1} as inoculum (Matias-Peralta *et al*., 2011).

The *Artemia* nauplii were produced by incubating cysts for 18 hours in seawater under optimal conditions of temperature (28-30ºC), salinity (30-32 $g{\cdot}L^{-1}$), pH (7.5-8.0) and continuous illumination using the Philips white light tubes. The cysts were incubated in a 1-L capacity conical shaped container at a density of 2 g cysts L^{-1} of seawater. The production of *Artemia* nauplii without enrichment followed the standard procedure adapted by shrimp hatcheries in Malaysia (Shrimp hatchery operators in Negeri Sembilan, Malacca and Johor, Malaysia, pers. com.).

Artificial feed was prepared by weighing the recommended amount of feed according to the number of shrimp larvae used in the experiment. This procedure was done according to the manufacturer's recommendation.

Source of P. monodon Post larvae (PL)

The shrimp larvae were purchased from a private hatchery located in Port Dickson, Negeri Sembilan (approximately 100 km from the laboratory). Shrimps at their first mysis stage were transported from the hatchery to the Aquatic Animal Health Unit for acclimation and further rearing until molting to PL1 (11 days old). The mysis were fed ad libitum four times daily, with a diatom, *Skeletonema* sp., until they were stocked into the experimental tanks.

Tank Preparation

Twelve 10 L capacity plastic aquaria (made of transparent acrylic plastic) with the dimension of 31.5 cm x 16.5 cm x 24 cm were used in the experiment. The tanks were thoroughly washed with bactericidal soap, soaked in 100 mg L^{-1} chlorine, rinsed and dried to prevent any disease transfer during the culture. Then, the tanks were filled with 4 L of chlorinated (aerated under high pressure air bubbles for at least three days before use), filtered (1 µm) seawater a day before stocking. A few pieces of stones and 20 mm diameter plastic pipes cut into a length of 40 mm (which were soaked in 100 mg L^{-1} chlorine for 24 hours, washed, rinsed and dried) were placed inside the tanks to serve as refuge for the weak shrimp during the molting period. Each tank was provided with individual aeration of low pressure air bubbles.

Stocking

The shrimp post larvae (PL1), with an average initial weight of 34.24±1.02 mg, were hauled from the acclimation tank using soft net into a basin with fresh seawater. The larvae were collected from the basin using a wide mouth plastic bottle with attached soft net, counted and transferred into a tared beaker with seawater and weighed in a Vibra (Shinko Denshi) digital tuning fork scale. These procedures were done quickly and carefully so as to avoid stress that might

lead to shrimp mortality. Individual weight was calculated from the bulk weight over the total number of individuals. Each tank was stocked at a density of 45 PLs L^{-1} of seawater.

Feeding

Larval live feeds (*Artemia* nauplii, Superior 90 Brine Shrimp Eggs, USA; and *N. affinis*) and artificial shrimp diet (Mixed Feed for *P. monodon*, Higashimaru Co. Ltd.) were given ad libitum four times a day at six hours interval (0600, 1200, 1800 and 2400 hours, respectively). Live feeds were counted, while artificial diet was weighed prior to feeding. Live feeds were also washed with filtered seawater upon harvest from their respective culture containers before counting. Live feed counts were done by determining the number of individuals/ml in three 1 ml samples using graduated pipette. The counts were done using 1 ml gridded counting chamber under Leitz Diavert inverted microscope. The counts were also done daily before feeding. The dry weights of the live food were also determined to be compared with that of the artificial diet (Table 3).

Water Quality

Water parameters, such as salinity, pH, dissolved oxygen, and temperature, were measured *in situ* twice (morning and noon) daily. Salinity and temperature readings were done using YSI 30 Salinity/ Conductivity/Temperature meter, while YSI 52 Dissolved Oxygen meter and ORION portable pH meter were used for dissolved oxygen and pH reading, respectively. On the other hand, water samples for total ammonia nitrogen and nitrite nitrogen were collected (before cleaning the tanks) and analyzed daily in the laboratory following the procedures by Parsons *et al*. (1984).

Meanwhile, daily water exchange was carried out at 30%. Uneaten food and faeces were siphoned out using a 1 mm flexible hose. Using this hose, the water level was gradually reduced to provide minimal disturbance to the shrimp larvae and also to prevent the larvae from being siphoned out. Feeding was commenced as soon as the desired amount of seawater had been removed and the refilling was begun.

Feeding was stopped eight hours before harvesting. Upon harvest, all the shrimp larvae were collected, weighed and measured. All the collected samples were washed thoroughly with double distilled water to remove salt before freeze drying (prior to analysis). Protein was determined following the method described by Meyer and Walther (1988). Fatty acid methyl esters (FAME) were prepared according to the direct methylation techniques by Divakaran and Ostrowski (1989). The freeze dried samples (100 mg) were refluxed at 100ºC for 10 minutes with 10 mL of 2% methanolic NaOH. The samples were further refluxed with 6.25 mL 14% Boron Triflouride and Heptane. Then, 2 mL of saturated NaCl was added to make the FAME float, and 1 g of anhydrous Na_2SO_4 was added to absorb the remaining water in the FAME for best recovery.

The FAME of the samples were analyzed using gas liquid chromatograph (Shimadzu GC-8A) equipped with a FID and BPX-70 (SGE) or Supelco 2330 capillary column. Individual peaks of FAME were identified by comparing them with the retention times of known standards obtained from Sigma Chemicals Company and using cod liver oil as a secondary standard. A Chromatopac (SHIMADZU C-R3A) quantified the magnitude of the peaks of each chromatographic reading.

Data Analysis

The specific growth rate (SGR) was calculated from the body weight using the following formula:

$$SGR = 100\ [(expG)-1]$$

Where:

$$G = \frac{\ln(wf) - \ln(wi)}{\Delta t}$$

where:

ln(wf) is the natural logarithm of the weight at time (t),

and ln (wi) is the natural logarithm of the initial wt of the shrimp larvae.

The collected data were analyzed using one-way analysis of variance (ANOVA). Significant differences among the individual treatment effects were determined using Tukey's honestly significant different test (T-HSD) at 0.05 level of probability. The data (whenever appropriate) were arcsine-transformed to satisfy the condition of homogeneity of variance (Gomez & Gomez, 1983; Zar, 1984). Statistical analyses were done using the Statistical Analysis System (SAS Inc. 1992) computer package (version 6.07).

RESULTS

Shrimp larvae fed with *N. affinis* alone achieved the highest ($p<0.05$) survival rate (60.6%), whereas those fed with the combination of *Artemia* + *N. affinis* had the lowest survival rate (24.1%) (see Table 1). Similarly, the highest ($p<0.05$) specific growth rate (SGR) was achieved by the shrimps fed with *N. affinis* alone, while those fed with the combination of *Artemia* and harpacticoid copepod have the lowest ($p<0.05$) growth rate (14.3%) (see Table 1).

TABLE 1

Mean (± S.E.) survival, specific growth rate of shrimp larvae and total weight of different diets fed to *Penaeus monodon* larvae for a period of 15 days. Means in the rows with the same superscript are not significant ($p<0.05$).

Parameters	Treatment		
	I	II	Control
Survival (%)	$60.6^{a}\pm6.8$	$24.1^{c}\pm1.0$	$43.0^{b}\pm3.2$
Specific growth rate (% d-1)	$16.7^{a}\pm0.2$	$14.3^{c}\pm0.2$	$15.8^{b}\pm0.2$
Total weight of feed used (mg dry weight)	$94.9^{b}\pm2.4$	$53.0^{c}\pm0.6$	$109.8^{a}\pm4.5$

Note: Treatment I = fed *N. affinis* (size range 50-400 µm)
Treatment II = combination of *Artemia* nauplii (size range 410-430µm) and *N. affinis*
Treatment III = artificial diet (grounded freeze-dried form, size range 17-22 µm).

Meanwhile, significant differences in the total amount of feed consumed were found among the treatments (Table 1). The shrimp larvae consumed significantly ($p<0.05$) higher amount of artificial diet compared to the live feed used.

The results also revealed that the highest ($p<0.05$) level of protein in the copepod tissue (52% = 52.32±2.13) compared to the two other diets used (namely, *Artemia* nauplii = 34% or 33.82±0.35 and artificial diet = 40% or 39.82±0.39). The fatty acids of *N. affinis* were dominated by polyunsaturated fatty acids (PUFA), 22:6n-3 accounting 19.5% of the total PUFA identified (see Table 2). On the other hand, *Artemia* nauplii and artificial diet were dominated by the monounsaturated fatty acids (MUFA), 18:1n-9 contributing 31.3% and 21.3% of the total MUFA identified, respectively. Among the three diets, the artificial diet was found to contain the highest ($p<0.05$) amount of saturated fatty acids (SFA), whereas *Artemia* was found to contain the highest ($p<0.05$) MUFA, and *N. affinis* contained the highest ($p<0.05$) amount of PUFA (Table 2). The results also showed that *N. affinis* contained the highest ($p<0.05$) n-3/n-6 ratio compared to the other feed used.

The copepod fed shrimp showed significantly higher ($p<0.05$) amount of long chain PUFA, both of the n-3 and n-6 series fatty acids (Table 3), as compared to the artificial diet fed larvae. The fatty acid of the artificial diet fed shrimp was dominated by MUFA, specifically 18:1 and 16:1. However, the MUFA content of shrimp larvae fed with either copepod or artificial diet did not show any significant difference (Table 3).

No significant difference ($p>0.05$) was detected in the water with regards to salinity, pH, dissolved oxygen and temperature from all the treatments (Table 4). Nonetheless, significantly high ($p<0.05$) total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N) were detected from the tanks containing the artificial feed (control) (Table 4). On the other hand, the tanks with *N. affinis* (treatment II) was found to contain the lowest ($p<0.05$) concentrations of TAN and NO_2-N compared to the other treatments (Table 4).

DISCUSSION

This study illustrated that *N. affinis* was the preferred food by the *P. monodon* larvae. Meanwhile, the *Nitocra affinis* fed shrimp larvae achieved the highest survival rate and SGR compared to those fed with other food items. According to Mock *et al*. (1980), food organism in an intermediate size between algae (≤ 8-14µm) and freshly hatched *Artemia* nauplii (≤ 430µm) could increase shrimp survival and growth rates. In addition, Motoh (1981) suggested that a gradual change from algal diet during acclimatization to a diet of *Artemia* nauplii, which was fairly large (≤ 430µm) for small shrimp larvae with carapace length ranging from 0.5-2.2 mm with intermediate sized food items could reduce stress and mortality. In this study, harpacticoid copepod from copepodid to adult (ranging from 50 to 400µm) served as an appropriate sized prey

TABLE 2
Means (± S.E.) of different fatty acids (% of total fatty acids) of food sources for the post larvae *Penaeus monodon* Means in the rows with the same superscript are not significant ($p<0.05$).

Fatty acids	*Nitocra affinis*	*Artemia* nauplii	Artificial diet
C12:0	nd	nd	11.76^{a}±1.13
C14:0	0.65^{b}±0.02	0.51^{c}±0.00	1.47^{a}±0.02
C14:1	0.60^{b}±0.01	0.65^{b}±0.00	1.37^{a}±0.29
C14:2	6.65^{a}±1.14	nd	nd
C15:0	0.65^{b}±0.11	0.46^{b}±0.00	14.39^{a}±3.83
C16:0	11.83^{b}±0.16	12.55^{a}±0.00	5.06^{c}±1.31
C16:1	3.26^{a}±0.27	nd	1.14^{b}±0.21
C16:1n – 7	1.31^{b}±0.03	13.24^{a}±0.00	nd
C18:0	nd	5.57^{a}±0.00	1.54^{b}±0.27
C18:1	nd	nd	4.61^{a}±1.58
C18:1n – 9	14.10^{c}±0.10	31.30^{a}±0.00	21.26^{b}±2.99
C18:2n – 6	9.73^{a}±0.08	6.23^{a}±0.00	
C18:3n – 3	3.55^{b}±0.15	8.08^{a}±0.00	0.48^{c}±0.00
C18:3n – 6	0.37^{a}±0.15	nd	0.13^{b}±0.00
C18:4	0.76^{a} ±0.05	nd	0.37^{b}±0.00
C18:4n – 3	0.86^{b}±0.00	5.09^{a}±0.00	nd
C20:1n-9	0.15^{b}±0.02	nd	3.73^{a}±1.79
C20:2	2.32^{a}±0.26	nd	0.30^{b}±0.11
C20:4n – 6	2.32^{b}±0.26	1.41^{c}±0.00	5.13^{a}±0.06
C20:5n – 3	11.26^{a}±0.73	nd	0.64^{b}±0.19
C22:1n - 11	0.88^{b}±0.18	0.62^{a}±0.00	nd
22:5n - 3	2.57^{a}±0.23	nd	nd
C22:6n - 3	19.50^{a}±0.29	nd	8.07^{b}±1.87
Total SFA	13.13^{c}±0.14	19.09^{b}±0.00	28.73^{a}±6.11
Total MUFA	20.30^{c}±0.26	45.81^{a}±0.00	32.10^{b}±6.87
Total PUFA	59.89^{a}±1.27	20.81^{b}±0.00	15.11^{c}±1.63
Total n - 3	37.74^{a}±0.62	13.17^{b}±0.00	9.19^{c}±1.68
Total n - 6	12.42^{a}±0.80	7.64^{b}±0.00	5.26^{c}±0.06
n - 3/n - 6	3.04^{a}±0.22	1.72^{b}±0.00	1.75^{b}±0.30

nd = not detectable; SFA = saturated fatty acids;
MUFA = monounsaturated fatty acids;
PUFA = polyunsaturated fatty acids.

TABLE 3
Mean (± S.E.) fatty acid composition (% of total fatty acids) of post larvae (PL 15) *Penaeus monodon* fed with different diets. Means in the rows with the same letters are not significant ($p<0.05$).

Fatty acids	*Nitocra affinis* fed larvae	Artificial diet fed larvae
C14:0	3.25^{a}±0.00	2.24^{b}±0.12
C14:1	0.05^{b}±0.00	1.80^{a}±1.08
C15:0	0.29^{a}±0.00	Nd
C16:0	11.25^{a}±0.35	10.00^{a}±0.99
C16:1	1.23^{b}±0.00	4.10^{a}±0.46
C16:1*n* – 7	3.57^{a}±0.47	Nd
C18:0	4.90^{b}±0.00	8.05^{a}±0.46
C18:1	Nd	13.62^{a}±2.49
C18:1*n* – 9	17.43^{b}±1.54	21.17^{a}±0.21
C18:2n – 6	2.60^{a}±0.42	Nd
C18:3*n* – 3	1.80^{a}±0.00	0.95^{b}±0.03
C18:3*n* – 6	Nd	0.38^{a}±0.21
C18:4	Nd	0.47^{a}±0.01
C18:4*n* – 3	2.15^{a}±0.14	Nd
C20:1*n*-9	4.30^{a}±0.00	3.04^{b}±0.35
C20:2	Nd	0.98^{a}±0.25
C20:4*n* – 6	4.71^{a}±0.01	2.86^{b}±0.76
C20:5*n* – 3	15.62^{a}±0.47	1.08^{b}±0.11
C22:1*n* – 11	1.13^{b}±0.00	3.57^{a}±0.11
C22:5*n* – 3	1.80^{a}±0.00	Nd
C22:6*n* – 3	16.59^{a}±0.02	16.33^{a}±0.59
Total SFA	19.69^{a}±0.35	19.78^{a}±2.28
Total MUFA	27.71^{a}±2.02	28.28^{a}±1.19
Total PUFA	45.26^{a}±0.16	23.04^{b}±0.28
Total *n* – 3	37.95^{a}±0.59	18.36^{b}±0.45
Total *n* – 6	7.31^{a}±0.43	3.24^{b}±0.97

nd = not detectable; SFA = saturated fatty acids;
MUFA = monounsaturated fatty acids;
PUFA = polyunsaturated fatty acids.

TABLE 4
Mean (±S.E.) of different water quality parameters measured. Means in the rows with the same superscript are not significant ($p<0.05$).

Parameters	Treatment		
	I	II	Control
Salinity (gL-1)	30.14^{a}±0.09	30.14^{a}±0.11	30.04^{a}±0.10
Temperature (ºC)	27.51^{a}±0.10	27.52^{a}±0.04	27.51^{a}±0.10
pH (range)	7.90-8.31	7.90-8.31	7.92-8.30
Dissolved Oxygen (mg L-1)	5.85^{a}±0.05	5.88^{a}±0.04	6.20^{a}±0.08
Total Ammonia-N (µgL-1)	18.4^{c}±0.06	$19.\ 5^{a}$±0.08	19.2^{b}±0.08
Nitrite-N (µgL-1)	24.3^{c}±0.15	29.1^{a}±0.15	28.3^{b}±0.07

Note: Treatment I = fed *N. affinis* (size range 50-400 µm)
Treatment II = combination of *Artemia* nauplii (size range 410-430µm) and *N. affinis*
Treatment III = artificial diet (ground freeze-dried form, size range 17-22 µm).

for the shrimp larvae as they did not have to spend excess energy manipulating big sized prey.

Harpacticoid copepods have been widely used as a live prey for hatchery reared fish and crustaceans (Kahan *et al*., 1982; Watanabe *et al*., 1983; Szyper, 1989; Kraul *et al*., 1992; 1993; Nanton & Castell, 1998; Shields *et al*., 1999) because they have relatively high caloric content per unit weight and superior nutritional value (Kahan *et al*., 1982; Volk *et al*., 1984; Chandler, 1986; Gee, 1989). Although the absolute nutritional requirements of penaeid larvae are not fully known (Biendenbach *et al*., 1989), Colvin and Brand (1977) reported that a relatively high level of protein is important for the growth and survival of the early post-larval stages of many important penaeid species. In fact, they further suggested that larval protein requirements must be equal to or greater than 44%. In this case, the copepod *N. affinis* has provided the much needed protein requirement (52%) for the shrimp larvae. In addition, Leger *et al*. (1986), Norsker and Støttrup (1994), and McEvoy *et al*. (1995) have reported that harpacticoids are richer in essential fatty acids, most notably 22:6n-3 and 20:5n-3 and higher DHA:EPA ratio compared to *Artemia* (even after they have been fed with diet rich in DHA) and rotifer *Brachionus*. Meanwhile, several studies (e.g. Millamena *et al*., 1988; Ozkizilcik & Chu, 1994; Sorgeloos & Leger, 1992) have shown that the content of long-chain PUFA, in particular the n-3 series, is mainly responsible for the nutritional value of live feed for marine animal larvae. In this study, compared to *Artemia* (13.17 % total fatty acid) and the artificial feed (9.19 % total fatty acid) used, *N. affinis* (37.74 % total fatty acid) was found to contain significantly higher amount of long-chain PUFA, particularly of the n-3 series. According to Jones *et al*. (1979), highly unsaturated fatty acids (HUFA), specifically EPA and docosahexaenoic (DHA, 22:6n-

3), play an important role in penaeid larval nutrition and are required in larval diets. In addition, Kreeger *et al*. (1991) and Gonzalez-Felix *et al*. (2002) suggested the importance of EPA and DHA for the survival and growth of many important marine crustacean species. In the present study, it was evident that *N. affinis* provided adequate nutritional requirements for fatty acids, as reflected by the high survival and growth of the shrimp. Diets containing higher level of n-3 HUFA would improve growth and survival of penaeid larvae, since DHA is essential for penaeid larval growth (Gonzalez-Felix *et al*., 2002).

According to D'Abramo and Sheen (1993), the combined percentage of at least 15% of C-20 PUFAs of the n-3 and n-6 families (C 20:4 n-6; 20:5 n-3; 22:5 n-3; 22:6 n-3) in the whole body tissues of an animal serves as an indicator that the optimal growth requirement for essential fatty acids has been met. In this study, the combined percentage of these fatty acids for the *N. affinis* fed shrimp was >38%, while those fed with artificial diet had approximately 20%.

Merican and Shim (1996) suggested that linolenic (LNA, 18:3n-3) and DHA have been identified as essential fatty acid for *P. monodon*. Similarly, Glencross and Smith (1999) and Glencross *et al*. (2002) noted that linoleic acid (LOA, 18:2n-6) and EPA also have considerable growth-promoting value for shrimp. In fact, they suggested that additional growth would occur when two or more of the essential fatty acids were provided in an optimal combination. Growth was significantly reduced when either LNA or DHA was not provided in the diet. Moreover, Merican and Shim (1996) also showed that without any HUFA in the feed, the survival of shrimp was reduced to 52%. In this study, shrimp larvae obtained adequate supply of essential fatty acids (Table 2) from *N. affinis*, as illustrated by the significantly higher survival and growth rates compared to those fed with other diets, even with the combined Artemia + *N. affinis*.

In the present study, the tanks containing *Artemia* + *N. affinis* (treatment II) showed the highest ($P<0.05$) concentrations of TAN and NO_2-N as compared to other treatments. However, the levels found were within the range suitable for shrimp/prawn hatcheries. Olivar *et al*. (2000) reported that the unconsumed *Artemia* could have contributed to the higher ammonia levels in hatchery tanks. It is interesting to note that the treatment tanks using the artificial feed had significantly ($p<0.05$) lower levels of TAN and NO_2-N compared to those with *Artemia* + *N. affinis*, although one would have expected artificial feed to produce higher levels of the same. Moreover, artificial diet is easily consumed because of the small particles size appropriate for the requirements of the shrimp larvae with little or no excess. However, the levels of ammonia were primarily contributed by the shrimp fed with artificial diet, as reported by Shishehchian *et al*. (1999).

CONCLUSION

The results of this study suggest that *N. affinis* could be used as a live food source

for culturing penaeid shrimp larvae. This is because the shrimp larvae fed with *N. affinis* showed the highest survival percentage as compared to those fed with artificial diet and the combination of *N. affinis* and *Artemia*. In addition, the relative growth rate of shrimp larvae fed with copepod was significantly higher than that of *Artemia* nauplii combined with *N. affinis*. Furthermore, *N. affinis* was found to contain the highest amounts of protein and PUFA as compared to both *Artemia* and artificial diet. Another benefit of using copepods as shrimp larval feed is the maintenance of good water quality of the culture tanks. It is evident from this study that the amount of nitrogenous substances in the water was kept at significantly lower level compared to other treatments although similar methods of maintenance were applied in all the treatment tanks.

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