

Full Length Research Paper

Small-scale continuous production of a tropical marine copepod, *Nitocra affinis californica* Lang and its potential as live food for aquaculture

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A study to establish a relatively simple and reliable small scale mass production and the nutritional profile of *Nitocra affinis* was undertaken. The result confirmed the feasibility of maintaining *N. affinis* on a small scale for a long period using cheaply available culture vessels. Within six weeks (42 days) a minimum harvest of 43.6×10^3 copepod $\cdot L^{-1}$ and maximum of 44.5×10^3 copepod $\cdot L^{-1}$ were obtained. Using several numbers of vessels (2L-capacity) for mass production, it was feasible to harvest 15.0×10^3 copepod $\cdot L^{-1}$ daily after two weeks of inoculation. *Nitocra affinis* has a great potential as live food because it contained high protein (39 to 52%), lipid (13 to 23%) and carbohydrates (8 to 11%). It also contained high level of n-3 HUFA (up to 44%) and n-6 HUFA (up to 14%). In addition it contained high amount of DHA (up to 19%) and EPA (up to 25%). Moreover, levels and ratios of fatty acids closely matched both the requirements of marine finfish and shrimp larvae.

Key words: Mariculture, live food, marine copepod, *Nitocra affinis*, fatty acids.

INTRODUCTION

For decades, the aquaculture industry has relied primarily on brine shrimp and rotifers to provide the necessary nutrition for rearing the early life stages of fish and crustaceans (Millamena et al., 1998; Szyper, 1989; Marcus and Murray, 2001). However, the continued supply of *Artemia* cysts sufficient to meet demands of the aquaculture industry is in question due to poor yields (Lavens and Sorgeloos, 2000). In addition, brine shrimp and rotifers are almost always unsuitable as first feed for fish larvae due to inappropriate size (too large or too little) (van der Meeren, 1991; Pepin and Penney, 1997; Knuckey et al., 2005). Moreover, both food items may not elicit a feeding response, are difficult to capture and their

swimming behavior makes them less susceptible to predation (Buskey et al., 1993; von Herbing and Gallagher, 2000). Because of these problems, there has been a great interest in the identification of alternative live feeds to increase the variety and survival of aquaculture species that can be cultivated. There have been a number of studies done providing evidence of the effectiveness of copepods as a food item, and many investigators have obtained good growth and survival of fish larvae and crustaceans when fed with copepods as test food organisms (Fukusho, 1980; Fukusho et al., 1980; van de Meeren, 1991; Holmefjord et al., 1993; Naess et al., 1995; Stottrup and Nosker, 1997; Drillet et al., 2006; VanderLugt and Lenz, 2008).

Despite the growing interest and success obtained using cultured copepods, still they are not routinely used by the aquaculture industry. This was due to several reasons but mainly, that copepod cultures are difficult to

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Table 1. Summary of experimental design used for the culture experiment.

| Types of culture vessels (Treatment) | No. of replicates per treatment | No. of gravid female inoculums per culture vessels | Feeding density (cells/ml) |
|--------------------------------------|---------------------------------|--|----------------------------|
| Acrylic plastic aquarium | 3 | 150 | 1×10^6 |
| Polypropylene plastic basin | 3 | 150 | 1×10^6 |
| Borosilicate glass beaker | 3 | 150 | 1×10^6 |

maintain in ponds or in the laboratory on a continuous basis, and the procedures for their large-scale cultivation are still in the developmental stage or have not yet been established (Marcus and Murray, 2001; Støttrup, 2003; O'Bryen and Lee, 2005). As copepods form an important component in the food chain of fish larvae and crustaceans, culture trials aimed at establishing a reliable mass production system were attempted by several earlier workers (Huntly, 1982; Arnott et al., 1986; Szyper, 1989; Støttrup et al., 1990; Millamena et al., 1998). Successful culture of copepods in few liters of culture medium was also reported by Parrish and Wilson (1978) and Bretler (1980). In addition, Fukusho (1980) reported that 2 to 3 kg (wet weight) of planktonic *Tigriopus japonicus* can be harvested daily in large outdoor tanks. Moreover, Uhlig (1984) found that mass cultivation of the harpacticoid, *Tisbe holoturiae*, could satisfy the requirements of mariculture. Furthermore, Sun and Fleeger (1995) succeeded in yielding millions of harpacticoid, *Amphiascoides atopus*, per day in a relatively small basal surface area of four square meters.

Nutritional quality of food offered to culture marine organisms is crucial during the first few weeks of larval life (Ben-Amotz et al., 1987). Several reports (Fujita, 1979; Scott and Middleton, 1979; Watanabe et al., 1983) have suggested that lipids in general and specifically n-3 highly unsaturated fatty acids (HUFA) have an essential role in the larval diet. According to Norsker and Støttrup (1994), the fatty acids in the copepods reflected largely to that of their diets. Hence the quality of food, whether natural (algal diet) or artificial is important priority since the nutritional content of food is transferred directly or indirectly through the copepod mediation to the cultured organisms.

With the purpose of evaluating the potential of *Nitocra affinis* as a new candidate species for mariculture, this study was undertaken with the following objectives:

- To describe an easy and economical method for small scale mass culture of this species.
- Evaluate the nutritional quality of the species fed with different food items.

MATERIALS AND METHODS

Culture vessel

Three different culture vessels ranging from glass to plastic

materials and of different shapes were used; however, all the vessels were maintained at uniform volume of two liters. The first culture vessel was a small semi transparent plastic basin of polypropylene material with a base area equal to 176.7 cm^2 , mouth area of 415.5 cm^2 , and a height of 10.0 cm. The basin was cylindrical, with smooth surface from inside and out. The second was a small acrylic plastic aquarium commonly used to raise ornamental fishes. The aquarium was rectangular having a base area of 185.0 cm^2 , mouth area of 240.0 cm^2 and height of 15.0 cm. The third vessel was a borosilicate glass beaker with base area of 122.7 cm^2 , which is equal to the mouth area and height of 18.5 cm. Three replicates for each culture vessel were used in this experiment (Table 1). The experiment was repeated several times to validate and confirm the results.

The experiment was set up in an outdoor laboratory at the Aquatic Animal Health Unit of the Veterinary Faculty, University Putra Malaysia. The temperature inside the laboratory ranged from 25 to 35°C (average of 30°C most of the time) depending on the time of the day. The culture was started by transferring 150 gravid female copepods (from the original stock) in 2 L of filtered aged seawater to each culture vessel (Figure 1). The cultures were maintained at 30 ppt salinity and at temperatures between 25 to 35°C (seawater at room temperature changed from a minimum of 25°C in the early morning, while a maximum of 35°C usually in the mid afternoon). When the salinity level increased, fresh water was added to the medium until the desired salinity was achieved. The cultures were provided with natural lighting conditions (ranging from 25 to 40 $\mu\text{mol s}^{-2}$) and photoperiod (12 h light: 12 h dark cycles). All the above parameters provided for in this study have been determined to provide the maximum population growth of *N. affinis* (Matias-Peralta et al., 2005). The cultures were provided with mixed algal food, that is, *Chaetoceros calcitrans*, *Nannochloropsis oculata* and *Tetraselmis tetrahele* which were maintained at $1.0 \times 10^6 \text{ cells ml}^{-1}$ throughout the 14day culture cycle. Daily salinity and temperature readings were determined using YSI 30 salinity-conductivity-temperature meter.

Nutritional profile

After choosing the best culture vessel to be used for the small-scale production, a semi-continuous culture was started. The culture was started by isolating 150 gravid female copepods (from the stock culture) in the culture vessel with 2 L of aged seawater. The protocols established from the previous experiment were followed for the mass production of *N. affinis* for nutritional analysis. Ten different food items were provided, which include three types of algae (that is, *C. calcitrans*, *N. oculata* and *T. tetrahele*) at different combinations and three artificial food (that is, baker's yeast (*Saccharomyces cerevisiae*), shrimp feed (Mixed Feed for *Penaeus monodon*, Higashimaru Co. Ltd.) and rice bran) (Table 2). Feeding densities were maintained at $1.0 \times 10^6 \text{ cells ml}^{-1}$ and 0.06 mg/ml for algal food and artificial diet, respectively, for the whole duration of the study (Table 2).

Copepods were collected and counted every 14 days following which, new batch was started (collection/harvest was done for four

A. Culture maintenance (150 gravid female inoculums)

Partial water replacement (approximately 30%) is carried out every two days (for 14 days) before feeding commenced

Water from the basin is siphoned into a plastic bottle (10 cm diameter) with attached net of 50 μm mesh size

Any copepods attached to the sieve net were flooded with filtered seawater and replaced in the same vessels

The vessels were then filled up back to 2 L with freshly filtered aged seawater

Cultures were provided with mixed algal food at 1.0×10^6 cells mL⁻¹

B. Harvesting (14 days after inoculation)

Total harvest is accomplished in such a way that gravid females are separated from nauplii and copepodids

Water is poured from the culture vessel through a series of nets with different pore sizes of 300 μm (trapped mostly the adults and gravid females) and 50 μm (trapped all the nauplii and copepodids)

All the remaining copepods inside the basin were washed with filtered seawater and passed again through the nets until all the attached copepods were in the sieve nets

The trapped gravid copepods were then transferred into new basins and used to start new batch while the rest of the copepods are counted and used as live feed for fish or shrimp

Figure 1. Flow chart showing the procedure for maintaining *Nitocra affinis*' culture.

culture cycles, until there were enough samples for nutritional analysis). Copepods were harvested in such a way that adult copepods (gravid females) were separated from nauplii and copepodids (Figure 1). Gravid females were counted in concave watch glass (15 mm diameter) under Wild Heerbrugg dissecting

microscope. After counting, the gravid copepods were then transferred into new basins and used to start new batch.

Prior to analysis, the collected copepods were concentrated and washed three times with distilled deionize water (thorough and careful washing was done to remove all traces of salts, which may

Table 2. The different food items, feeding densities and number inoculums used for each food item.

| Food items | Feeding density | No. of gravid female used |
|--|----------------------------|---------------------------|
| <i>C. calcitrans</i> | 1.0×10^6 cells/ml | 150/basin |
| <i>T. tetrahele</i> | -----do----- | -----do----- |
| <i>N. oculata</i> | -----do----- | -----do----- |
| <i>C. calcitrans</i> + <i>T. tetrahele</i> | -----do----- | -----do----- |
| <i>C. calcitrans</i> + <i>N. oculata</i> | -----do----- | -----do----- |
| <i>T. tetrahele</i> + <i>N. oculata</i> | -----do----- | -----do----- |
| <i>C. calcitrans</i> + <i>T. tetrahele</i> + <i>N. oculata</i> | -----do----- | -----do----- |
| Baker's yeast | 0.06 mg/ml | -----do----- |
| Shrimp food | -----do----- | -----do----- |
| Rice bran | -----do----- | -----do----- |

accumulate after freeze drying and later on hinder the analyses) using centrifugation. The concentrated copepods were then transferred in plastic vials (50 ml capacity) and freeze-dried. Proximate contents of freeze-dried copepod samples fed with different feeds were analyzed following the method described by Meyer and Walter (1988) with some modifications following the basic analytical scheme from Zöllner and Kirsch (1962), Itzhaki and Gill (1964), Herbert et al. (1971), Holland and Gabbott (1971) and Rausch (1981). The fatty acid methyl esters (FAME) were prepared according to the direct methylation techniques (Divakaran and Ostrowski, 1989). The FAME of the samples was analyzed with a gas liquid chromatograph (Shimadzu GC-8A) equipped with a FID and BPX-70 (SGE) or Supelco 2330 capillary column. The fatty acids were identified by comparison with 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.

Analysis

The maximum specific growth rate (K) of copepod was calculated at the end of each culture cycle following the method defined by Omori and Ikeda (1984) as follows:

$$K = \frac{\ln(x_2) - \ln(x_1)}{t_2 - t_1}$$

where:

x_1 = number of copepods at the initial of the selected time interval.
 x_2 = number of copepods at the final of the selected time interval.
 $t_2 - t_1$ = selected time (in days) for determination of number of copepods (in this experiment 14 days which was the day of harvest).

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

RESULTS

Culture method

High and sustained production of *N. affinis* was maintained in all the culture vessels used (Figure 2). Although during the second trial, the number of harvested copepod was reduced in all the culture vessels, the values overall were insignificant ($P > 0.05$) (Figure 2). The highest mean total production obtained were 29.80×10^3 copepod $\cdot L^{-1}$ for a period of 14 days with a total of 75 gravid females $\cdot L^{-1}$ as inoculum. Whereas the lowest total production obtained was 14.55×10^3 copepod $\cdot L^{-1}$. In all the trials performed, no significant differences were found in the copepod production regardless of the type of culture vessel used (Figure 2). Similarly, the specific growth rate of *N. affinis* ($K = 0.42 \pm 0.05$) did not vary significantly ($p > 0.05$) among the different culture vessel used in all the trials performed (Figure 2).

Nutritional profile of copepod *N. affinis* fed with different food items

The highest ($p < 0.05$) protein content was obtained with the copepod fed with mixed algal food, followed by *C. calcitrans* singly and yeast (Table 3). Likewise, copepod fed with mixed algal diet, *C. calcitrans*, *T. tetrahele*, and their combination was found to contain the highest ($p < 0.05$) percentage of lipid, while, the lowest percentage of lipid was found in copepod fed with yeast (Table 3). Generally, carbohydrate content was high in copepod fed with algal diet, except for those fed with *N. oculata* and combine *N. oculata* and *C. calcitrans*. Moisture and ash content was highest ($p < 0.05$) in copepod fed with shrimp feed and rice bran.

The result showed that the fatty acid composition of *N. affinis* was readily affected by the fatty acids of their diet. Although at different proportion, all the fatty acids present in the diet were detected in the copepod. The same proportion of saturates, monounsaturates and

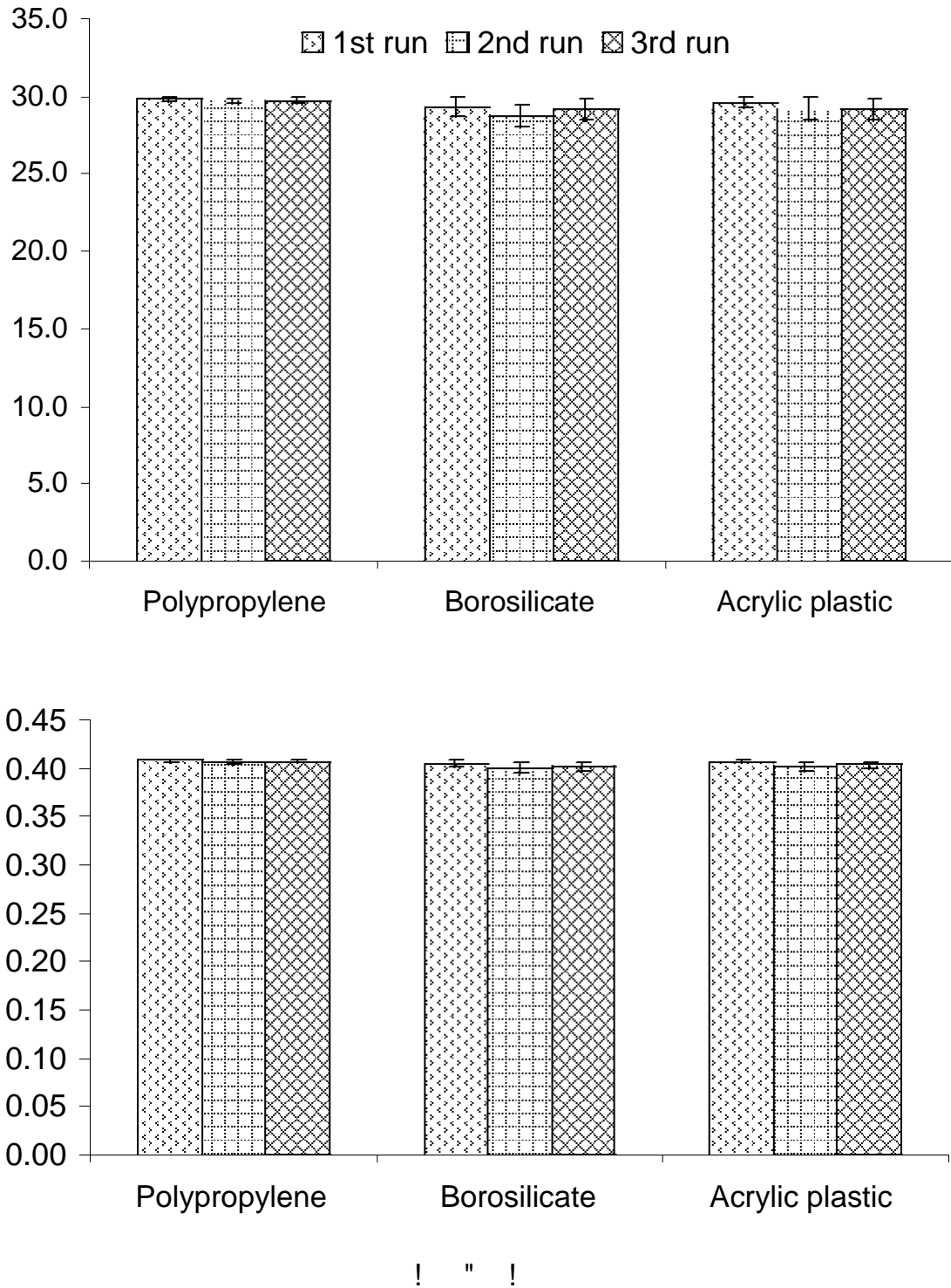


Figure 2. The density (copepod production/L) (a) and maximum specific growth rate (b) of harpacticoid copepod, *N. affinis* reared in different types of culture vessels after two weeks of culture. Bars indicate standard error of the mean.

polyunsaturates in the diet were also obtained from the copepods. The major fatty acids present in *N. affinis* fed with algal diet were 14:0, 16:0, 18:0, 18:2n6, 20:0,

20:4n6, 20:5n3, 22:5n3 and 22:6n3 (Table 4). For copepod fed with artificial food, the common fatty acids present were 14:0, 16:0, 18:0, 18:1n9 and 20:5n3. *N. affinis*

Table 3. Proximate composition (% dry weight) of harpacticoid copepod *Nitocra affinis* fed with different food items. Values are means \pm standard error of the means. Means in the column with the same superscript are not significantly different ($p > 0.05$, $n = 3$).

| Food items | Parameters | | | | | |
|-------------|---|---------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|
| | Protein | Lipid | Carbohydrates | Moisture | Ash | |
| Algal food | <i>C. calcitrans</i> | 48.53 ^{ab} \pm 0.11 | 21.40 ^a \pm 0.57 | 11.25 ^a \pm 0.34 | 5.53 ^e \pm 0.11 | 8.57 ^c \pm 0.20 |
| | <i>T. tetrahele</i> | 44.24 ^{cd} \pm 1.17 | 19.88 ^a \pm 1.14 | 11.00 ^a \pm 0.01 | 8.27 ^b \pm 0.47 | 9.40 ^{bc} \pm 0.23 |
| | <i>N. oculata</i> | 44.95 ^{bcd} \pm 1.85 | 18.98 ^{ab} \pm 1.19 | 9.08 ^b \pm 0.31 | 8.34 ^b \pm 0.30 | 8.93 ^c \pm 0.47 |
| | <i>C. calcitrans</i> : <i>T. tetrahele</i> | 46.02 ^{bcd} \pm 0.80 | 21.42 ^a \pm 1.37 | 10.73 ^a \pm 0.12 | 7.53 ^{bcd} \pm 0.11 | 10.47 ^b \pm 0.06 |
| | <i>C. calcitrans</i> : <i>N. oculata</i> | 47.44 ^{bc} \pm 1.08 | 19.91 ^a \pm 0.27 | 9.26 ^b \pm 0.40 | 7.61 ^{bc} \pm 0.07 | 10.40 ^b \pm 0.23 |
| | <i>T. tetrahele</i> : <i>N. oculata</i> | 48.22 ^b \pm 0.79 | 18.23 ^{ab} \pm 0.28 | 10.72 ^a \pm 0.38 | 6.62 ^{cd} \pm 0.23 | 8.93 ^c \pm 0.47 |
| | Mixed (<i>C. calcitrans</i> : <i>T. tetrahele</i> : <i>N. oculata</i>) | 52.32 ^a \pm 2.13 | 22.82 ^a \pm 0.54 | 10.69 ^a \pm 0.11 | 6.56 ^d \pm 0.17 | 7.57 ^{bc} \pm 0.17 |
| | Other food items | Baker's yeast | 46.72 ^{bc} \pm 0.38 | 13.46 ^c \pm 0.97 | 10.82 ^a \pm 0.01 | 7.33 ^{bcd} \pm 0.27 |
| Shrimp feed | | 39.98 ^u \pm 0.27 | 18.27 ^{du} \pm 0.29 | 8.78 ^u \pm 0.55 | 12.27 ^d \pm 0.21 | 14.48 ^d \pm 0.15 |
| Rice bran | | 39.22 ^e \pm 0.22 | 16.21 ^{bc} \pm 0.73 | 8.36 ^b \pm 0.17 | 10.54 ^a \pm 0.25 | 12.48 ^a \pm 0.18 |

fed algal diet consistently had high amount of 22:6n3 (docosahexaenoic acid or DHA) and 20:5n3 (eicosapentaenoic acid or EPA) in their fatty acid profile, except for those fed with the algal diet *N. oculata* which contained untraceable amount of DHA. Although the yeast does not contain any EPA, this fatty acid was detected in minimal amount from copepod fed with this diet (Table 4).

DISCUSSION

Culture method

The main objectives in developing a method for the culture of *N. affinis* were to establish a system that is reliable, highly productive, less labor intensive and cheap. For this method, within six weeks (42 days) operations we could harvest a minimum of 43.6×10^3 copepod L^{-1} and maximum of 44.5×10^3 copepod L^{-1} . Our present work on harpacticoid copepods appears to be easier and less intensive compared with previous studies (Chandler, 1986; Sun and Fleeger, 1995; Souza-Santos et al., 1999; Payne and Rippingale, 2001).

In this study, three different culture vessels of different sizes and shapes were used. Using the same initial densities of gravid female inoculums, this study showed that copepod production was the same, regardless of the culture vessel used. Three trial runs were performed for this study to verify the effectiveness of the method. The copepod production obtained from all the trials showed no significant difference. Although during the second trial the production decreased (Figure 2), the difference compared from the first and third runs were insignificant. All the culture vessels that were used for this study were available locally anytime. However, glass beakers

command high price and very susceptible to breakage, therefore, proper care and handling should be observed when working with them. The acrylic plastic aquarium tanks were cheaper compared to the glass beaker; however, since acrylic plastics are brittle and cracks over time, they are also prone to breakage if not handled properly. On the other hand, plastic basin was the cheapest available material in the market compared to the rest of the vessels used. This type of plastic is easier to manage and no special treatment was needed. Among the three types of culture vessels, the plastic basin was chosen for the small-scale production of *N. affinis*.

One main advantage of this method is the ease of maintenance, since *N. affinis* can be grown without the need for aeration. In addition, the culture vessels were small, therefore harvesting can be done anytime with less effort and labor cost. Harvesting time for three basins takes 30 min, which included the filtering, collection and feeling the basins with fresh seawater for the new inoculums. Using several numbers of basins for mass³ production it was feasible to harvest 15.0×10^3 copepod L^{-1} daily after two weeks of inoculations. Moreover, contamination of culture and deteriorating water quality was not significant problems in this study. Partial water change and replenishing every two days effectively prevented water quality deterioration from occurring.

Many meiobenthic harpacticoid species have been cultured in quantities with promise for mariculture applications (Tseng and Hsu, 1984; Kahan, 1979; Kahan, 1980). However, it is difficult to compare the result of our study with other published works because they usually report growth rates rather than yield and culture densities. Nevertheless, the results of this study suggest a potential for aquaculture application because the yields of more than $15,000 L^{-1}$ copepods daily could be produced

Table 4. Fatty acid (% of total fatty acids) profile of harpacticoid copepod *Nitocra affinis* fed with different food items. Values are means \pm SE. Means in the row with the same superscript are not significantly different ($p > 0.05$, $n = 3$).

| Fatty acids | Food items | | | | | | | | | |
|--------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
| | C | T | N | CT | CN | TN | Mixed | Yeast | Shrimp food | Rice bran |
| C12:0 | nd | nd | nd | nd | nd | nd | nd | nd | 11.76 ^a \pm 1.13 | nd |
| C14:0 | 8.17 ^a \pm 0.10 | 8.97 ^a \pm 0.57 | 0.89 ^e \pm 0.06 | 8.13 ^a \pm 0.15 | 8.60 ^a \pm 0.07 | 5.56 ^b \pm 0.46 | 0.65 ^f \pm 0.02 | 3.29 ^c \pm 0.49 | 1.47 ^e \pm 0.02 | 2.31 ^{cd} \pm 0.31 |
| C14:1 | 1.44 ^a \pm 0.06 | nd | nd | 2.25 ^a \pm 0.74 | 1.04 ^a \pm 0.02 | nd | 0.60 ^c \pm 0.01 | nd | 1.37 ^b \pm 0.29 | nd |
| C15:0 | nd | 0.39 ^e \pm 0.02 | nd | 5.40 ^b \pm 0.19 | nd | 2.23 ^c \pm 0.59 | 0.65 ^d \pm 0.11 | 1.45 ^c \pm 0.65 | nd | 17.61 ^a \pm 1.28 |
| C16:0 | 8.13 ^e \pm 0.09 | 6.55 ^f \pm 0.09 | 25.44 ^b \pm 0.06 | 6.36 ^f \pm 0.26 | 9.09 ^d \pm 0.38 | 6.55 ^f \pm 0.09 | 5.45 ^g \pm 0.80 | 18.26 ^c \pm 0.29 | 5.05 ^g \pm 1.31 | 27.03 ^a \pm 0.42 |
| C16:1 | nd | nd | nd | nd | nd | nd | nd | nd | 1.14 ^a \pm 0.21 | nd |
| C16:1 n7 | 7.35 ^d \pm 0.06 | 5.29 ^e \pm 0.14 | 24.55 ^b \pm 0.05 | 5.46 ^e \pm 0.20 | 8.22 ^c \pm 0.12 | 5.20 ^e \pm 0.08 | 1.31 ^f \pm 0.03 | 30.28 ^a \pm 0.25 | nd | nd |
| C17:0 | nd | nd | 2.72 ^a \pm 0.08 | nd | nd | nd | nd | 2.63 ^a \pm 0.06 | nd | nd |
| C17:1 | 5.92 ^b \pm 0.15 | 3.98 ^c \pm 0.19 | 5.82 ^b \pm 0.13 | nd | 6.44 ^b \pm 0.22 | 7.64 ^a \pm 0.30 | nd | nd | nd | nd |
| C18:0 | 5.53 ^c \pm 0.77 | 8.97 ^b \pm 0.29 | 7.41 ^c \pm 0.12 | 4.40 ^c \pm 0.06 | 5.50 ^c \pm 0.84 | 8.97 ^b \pm 0.29 | nd | 2.83 ^d \pm 0.10 | 1.54 ^e \pm 0.27 | 13.66 ^a \pm 0.17 |
| C18:1 | nd | nd | nd | nd | nd | nd | nd | nd | 4.61 ^a \pm 1.58 | nd |
| C18:1n7 | nd | 3.12 ^a \pm 0.24 | nd | 1.76 ^b \pm 0.25 | nd | 3.12 ^a \pm 0.24 | nd | nd | nd | nd |
| C18:1n9 | 4.63 ^e \pm 0.30 | nd | 5.88 ^d \pm 0.13 | 3.59 ^e \pm 0.14 | 4.57 ^e \pm 0.36 | 2.48 ^e \pm 0.09 | 14.10 ^a \pm 0.10 | 13.10 ^c \pm 0.01 | 21.26 ^a \pm 2.99 | 23.55 ^a \pm 0.40 |
| C18:2n6 | 1.21 ^c \pm 0.12 | 3.48 ^a \pm 0.09 | 2.83 ^{ab} \pm 0.54 | 2.45 ^b \pm 0.32 | 2.49 ^b \pm 0.08 | 1.77 ^b \pm 0.27 | 1.73 ^b \pm 0.08 | nd | nd | nd |
| C18:3n3 | 1.61 ^b \pm 0.30 | 1.77 ^b \pm 0.27 | nd | 1.35 ^b \pm 0.27 | 1.56 ^b \pm 0.04 | 0.82 ^c \pm 0.14 | 3.55 \pm 0.15 | 5.84 ^a \pm 0.11 | 0.48 ^d \pm 0.00 | nd |
| C18:3n6 | nd | 1.22 ^a \pm 0.24 | nd | 0.47 ^b \pm 0.02 | 0.89 ^{ab} \pm 0.07 | nd | nd | nd | 0.13 ^c \pm 0.00 | nd |
| C18:4 | nd | nd | nd | nd | nd | nd | nd | nd | 0.37 ^a \pm 0.00 | nd |
| C18:4n3 | 1.86 ^b \pm 0.06 | nd | nd | 1.10 ^c \pm 0.12 | 4.05 ^a \pm 0.28 | nd | 0.86 ^d \pm 0.00 | nd | nd | nd |
| C20:0 | 4.38 ^a \pm 0.34 | 1.82 ^b \pm 0.20 | 4.02 ^a \pm 0.12 | 4.88 ^a \pm 0.17 | nd | 4.70 ^a \pm 0.17 | nd | nd | nd | nd |
| C20:1n9 | nd | nd | 1.16 ^b \pm 0.08 | nd | nd | nd | nd | nd | 3.73 ^a \pm 1.79 | nd |
| C20:2 | nd | nd | 1.10 ^c \pm 0.07 | nd | nd | nd | 2.32 ^a \pm 0.26 | 1.67 ^b \pm 0.11 | 0.30 ^d \pm 0.11 | nd |
| C20:4n3 | nd | nd | 2.61 ^a \pm 0.30 | nd | nd | nd | nd | nd | nd | nd |
| C20:4n6 | 10.15 ^a \pm 0.39 | 9.73 ^{ab} \pm 0.34 | 2.99 ^d \pm 0.06 | 8.97 ^b \pm 0.03 | 10.08 ^a \pm 0.15 | 9.40 ^{ab} \pm 0.15 | 2.32 ^e \pm 0.26 | 4.38 ^d \pm 0.19 | 5.13 ^c \pm 0.06 | nd |
| C20:5n3 | 22.62 \pm 1.04 | 25.06 ^a \pm 0.34 | 12.15 ^c \pm 0.36 | 23.36 ^{ab} \pm 0.62 | 23.94 ^{ab} \pm 0.59 | 25.06 ^a \pm 0.34 | 11.26 ^c \pm 0.73 | 0.76 ^e \pm 0.14 | 0.64 ^e \pm 0.19 | 5.90 ^d \pm 0.36 |
| C22:1n11 | nd | 6.09 ^a \pm 0.19 | nd | 3.65 ^b \pm 0.31 | nd | 3.09 ^b \pm 0.19 | 0.88 ^c \pm 0.18 | nd | nd | nd |
| C22:5n3 | 6.32 ^a \pm 0.38 | 4.38 ^b \pm 0.12 | nd | 6.17 ^a \pm 0.16 | 6.66 ^a \pm 0.12 | 4.38 ^b \pm 0.12 | 2.57 ^c \pm 0.23 | nd | nd | nd |
| C22:6n3 | 10.36 ^b \pm 0.33 | 8.62 ^c \pm 0.04 | nd | 10.32 ^b \pm 0.71 | 10.54 ^b \pm 0.33 | 8.62 ^c \pm 0.04 | 19.50 ^a \pm 0.29 | nd | 8.07 ^c \pm 1.87 | nd |
| SFA | 26.21 ^d \pm 1.15 | 26.70 ^{cd} \pm 0.60 | 40.48 ^b \pm 0.16 | 29.17 ^c \pm 0.14 | 23.19 ^e \pm 0.66 | 28.01 ^{cd} \pm 0.58 | 6.74 ^f \pm 0.77 | 28.47 ^{cd} \pm 0.31 | 8.06 ^e \pm 0.57 | 60.61 ^a \pm 1.24 |
| MUFA | 19.34 ^{ed} \pm 0.11 | 18.47 ^e \pm 0.38 | 37.41 ^b \pm 0.13 | 16.71 ^f \pm 0.51 | 20.27 ^d \pm 0.62 | 19.05 ^{ed} \pm 0.14 | 16.82 ^f \pm 0.14 | 43.38 ^a \pm 0.24 | 32.10 \pm 7.57 | 23.55 ^c \pm 0.40 |
| PUFA | 54.13 ^{ab} \pm 1.11 | 54.26 ^{ab} \pm 0.59 | 21.68 ^c \pm 0.33 | 53.71 ^{ab} \pm 0.83 | 56.15 ^a \pm 1.08 | 52.53 ^b \pm 0.29 | 41.37 ^c \pm 1.08 | 12.65 ^e \pm 0.23 | 15.11 ^d \pm 1.63 | 5.90 ^f \pm 0.36 |
| Unidentified | 0.32 \pm 0.15 | 0.57 \pm 0.24 | 0.43 \pm 0.33 | 0.41 \pm 0.20 | 0.38 \pm 0.05 | 0.41 \pm 0.28 | 34.56 \pm 1.30 | 15.50 \pm 0.17 | 44.74 \pm 7.44 | 9.95 \pm 1.24 |
| n3 | 28.40 ^d \pm 3.01 | 39.83 ^{ab} \pm 0.42 | 14.76 ^d \pm 0.54 | 42.29 ^a \pm 0.49 | 43.58 ^a \pm 0.95 | 39.83 ^{ab} \pm 0.42 | 37.32 ^c \pm 0.80 | 5.84 ^f \pm 0.11 | 9.19 ^e \pm 1.68 | 5.90 ^f \pm 0.36 |
| n6 | 11.36 ^c \pm 0.29 | 14.43 ^a \pm 0.26 | 5.82 ^d \pm 0.60 | 11.43 ^c \pm 0.35 | 12.57 ^b \pm 0.14 | 12.70 ^b \pm 0.28 | 4.05 ^e \pm 0.29 | 4.38 ^e \pm 0.19 | 5.26 ^d \pm 0.6 | nd |

Table 4. Contd.

| | | | | | | | | | | |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-----|
| n3/n6 | 2.50 ^d ±0.25 | 2.76 ^d ±0.04 | 2.59 ^d ±0.36 | 3.70 ^b ±0.08 | 3.47 ^b ±0.04 | 3.14 ^{bc} ±0.10 | 9.26 ^a ±0.46 | 1.34 ^f ±0.07 | 1.75 ^e ±0.30 | nd |
| Total Identified | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

nd = non detectable level; SFA = Saturated Fatty Acids (12:0,14:0,15:0,16:0,17:0,18:0,20:0); MUFA = Monounsaturated Fatty Acids (4:1,16:1,16:1 n7,17:1,18:1,18:1n7,18:1n9,20:1n9,22:1n11). PUFA = Polyunsaturated Fatty Acids (18:2n6,18:3n3,18:3n6,18:4,18:4n3,20:2,20:4n3,20:4n6,20:5n3,22:5n3,22:6n3).

in a relatively small culture vessel (<200 cm²).

Nutritional value of *N. affinis*

The result of this study showed that *N. affinis* contained relatively high amount of protein, which was more than 40% except for those fed with rice bran, which contained only 39%. In fact, copepods fed with mixed algal diet contained more than 50% protein (Table 3). The copepods fed mixed algal diet was found to contain 52.3% protein followed by those fed *C. calcitrans* with 48.5%. Similarly, mixed algal fed *N. affinis* was found to contain the highest amount of lipid, together with those fed with *C. calcitrans*. In general, the chemical composition of an organism depends greatly on the dietary input. According to Båmstedt (1986) and Lavens and Sorgeloos (1996), generally the amount of protein content of copepod ranged from a minimum of 44% to a maximum of 82% depending on the geographical locations. Proteins are continually required for growth and tissue repair, and thus its continuous supply is needed in the diet. A study by Roman (1983) noted that the growth and reproduction of marine invertebrates are often dependent on the amount of protein ingested and that the protein content in the diet can affect their biochemical composition as well as their growth rate.

Since the yeast contained the lowest lipid among the food provided, the lowest ($p<0.05$) lipid content was found in the copepod fed with yeast.

According to Sargent and Falk-Petersen (1988) and Græve et al. (1994), the variability of lipid in the copepod often reflects the lipid composition of algae, which varies between taxonomic groups. Watanabe et al. (1983) suggested that lipids, specifically the HUFA have an essential role in the larval diet. In addition, these fatty acids are crucial to the survival, growth and development of cultured marine organisms (Whyte, 1988).

A few differences were noted in the fatty acid composition within the different diets of *N. affinis*. These differences were best indicated by the summary of saturated, monounsaturated and polyunsaturated fatty acids and the calculated ratios (Table 4). Among the algal diets, the highest ($p<0.05$) SFA (40%) and MUFA (37%) and lowest PUFA (22%) was found in the copepod fed with *N. oculata*. Whereas, among artificial diets, rice bran fed copepod contained the highest ($p<0.05$) SFA of 61% and the lowest ($p<0.05$) PUFA of 6%. According to Sargent and Falk-Petersen (1988) and Græve et al. (1994), levels and ratios of fatty acids in copepods vary depending on the dietary input. The result of the present study suggested a close relationship between the percentage of HUFA (specifically n-3 and n-6) content of the diet and the copepod. The algal food with highest n-3 and n-6 HUFA content also gave the highest percentage in the copepod. The high ratio of n-3 to n-6 PUFA was used as an indication of high nutritional value of potential food organisms in aquaculture. In this study, the copepod *N. affinis* fed with mixed algal diet was

found to contain a significantly high proportion of n-3 to n-6 PUFA (9.25), followed by copepod fed with combined *C. calcitrans* + *T. tetrahele* and *C. calcitrans* + *N. oculata* with 3.7 and 3.5, respectively.

The nutritional composition of a good live food species should match the nutritional requirements of the organisms being cultured (Watanabe et al., 1983). Fatty acids are important components of biomembranes in fish, as well as providing a source of energy. Similarly, fatty acids primarily the n-3 HUFA are required by most marine shrimp species for growth and survival (Sorgeloos and Leger, 1992). The result of this study showed that the copepod, *N. affinis* has a nutritional composition that closely matches both the requirements of marine finfish larvae, which are predominantly HUFAs including EPA (20:5 n-3), DHA (22:6 n-3) and arachidonic acid (ARA:20:4 n-6) and of marine shrimp, which are mostly n-3 HUFA.

Conclusion

It was feasible to maintain *N. affinis* on a small scale for a long period (> 6 months) under the culture conditions described in the present study. For this method, within a six week period (42 days) we were able to harvest a maximum of nearly 50.0×10^3 copepod L⁻¹. Using several numbers of vessels for mass production, it was feasible to harvest 15.0×10^3 copepod L⁻¹ daily

after two weeks of inoculations. The ease of maintenance may indicate that this species could be a good candidate as a live food organism for mariculture industry. Furthermore, *N. affinis* has a great potential as live food because they contain high protein (39% to 52%), lipid (13 to 23%) and carbohydrates (8 to 11%). It also contains high level of n-3 HUFA (up to 44%) and n-6 HUFA (up to 14%). In addition, it contains high amount of DHA (up to 19%) and EPA (up to 25%). More importantly, levels and ratios of fatty acids closely match both the requirements of marine finfish larvae and of marine shrimp.

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REFERENCES

- Arnott GH, Brand GW, Kos LC (1986). Effect of food quality and quantity on the survival, development and egg production of *Gladioferens pectinatus* (Brady). (Copepoda: Calanoida). Australian J. Mar. Freshwater Res., 37: 467-473
- Båmstedt ULF (1986). Chemical composition and energy content. In: Corner EDS, O'Hara SCM (eds) The Biological Chemistry of Marine Copepod, Clarendon Press, Oxford, pp. 1-58
- Ben-Amotz A, Fisher R, Shneller A (1987). Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar. Biol., 95: 31-36.
- Bretler WCMK (1980). Continuous breeding of marine pelagic copepod in the presence of heterotrophic dinoflagellates. Mar. Ecol. Progress Ser., 2: 229-233.
- Buskey EJ, Coulter C, Strom S (1993). Locomotory patterns of microzooplankton: potential effects on food selectivity of larval fish. Bull. Mar. Sci., 53: 29-43.
- Chandler TG (1986). High-density culture of meiobenthic harpacticoid copepods within a muddy sediment substrate. Canadian J. Fish. Aquat. Sci., 43: 53-59
- Divakaran S, Ostrowski AC (1989). Fatty acid analysis of fish eggs without solvent extraction. Aquaculture, 80: 371-375.
- Drillet G, Jorgensen NOG, Soresnsen TF, Ramlov H, Hansen BW (2006). Biochemical and technical observations supporting the use of copepods as previous term live next term feed organisms in previous term marine next term larviculture. Aquaculture Res., 37: 756-772.
- Fujita S (1979). Culture of red sea bream, *Pagrus major* and its food. In: Styczynska-Jurewicz C, Jasper E, Parsoone G (eds) Cultivation of fish fry and its live food, European Mariculture Society, Special Publication No. 4 Bredene, Belgium, pp 183-197
- Fukusho K (1980). Mass production of a copepod *Tigriopus japonicus* in combination culture with a rotifer, *Brachionus olicatilis*, fed -yeast as food source. Bull. Japanese Soc. Fish., 46: 625-629.
- Fukusho K, Arakawa T, Watanabe T (1980). *Tigriopus japonicus* cultured with -yeast for larvae and juveniles of mud dab *Limanda yokohamae*. Bull. Japanese Soc. Fish., 46: 625-629.
- Gomez KA, Gomez AA (1983). Statistical Procedure for Agricultural Research (2nd edition). John Wiley and Sons New York, NY, p. 680.
- Græve M, Kattner G, Hagen W (1994). Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. J. Exp. Mar. Biol. Ecol., 182: 97-100.
- Holmejord I, Gulbrandsen J, Lein I, Refstie T, Leger P, Harboe T, Huse I, Sorgeloos P, Bolla S, Olsen Y, Reitan KI, Vadstein O, Oie G (1993). An intensive approach to Atlantic halibut fry production. J. World Aquaculture Soc., 24: 275-284.
- Herbert D, Phipps PJ, Strange RE (1971). Chemical analysis of microbial cells. In: Norris JR, Ribbons DW (eds) Methods in Microbiology. Academic Press London, 5B: 209-344.
- Holland DL, Gabbott PA (1971). A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. J. Marine Biolo. Assoc. United Kingdom, 51: 659-668.
- Huntley ME (1982). Yellow water in La Jolla Bay, California, July 1980. II. Suppression of zooplankton grazing. J. Exp. Mar. Biol. Ecol., 63: 81-91.
- Itzhaki RF, Gill DM (1964). A micro-biuret method for estimating proteins. Anal. Biochem., 9: 401-410.
- Kahan D (1979). Vegetable as food for marine harpacticoid copepods. Aquaculture, 16: 345-350.
- Kahan D (1980). Mass cultivation of food organisms in hatcheries-problems and proposed solutions. Symposium on Coastal Aquaculture 12-18 January 1980. Cochin India.
- Knuckey RM, Semmens GL, Mayer RJ, Rimmer MA (2005). Development of an optimal microalgal diet for the culture of the calanoid copepod *Acartia sinjiensis*: effect of algal species and feed concentration on copepod development. Aquaculture, 249: 339-351.
- Lavens P, Sorgeloos P (1996). Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper. No. 361. Rome, FAO, p. 295.
- Lavens P, Sorgeloos P (2000). The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. Aquaculture, 181: 397-403.
- Marcus NH, Murray M (2001). Copepod diapause eggs: a potential source of nauplii for aquaculture. Aquaculture, 201: 107-115.
- Matias-Peralta H, Yusoff FM, Shariff M, Arshad A (2005). Effects of some environmental parameters on the reproduction and development of a tropical marine harpacticoid copepod *Nitocra affinis californica* Lang. Mar. Poll. Bull., 51: 722-728.
- Meyer E, Walther A (1988). Methods for estimation of protein, lipid, carbohydrates and chitin levels in freshwater invertebrates. Archivium fur Hydrobiologie, 113: 161-177.
- Millamena OM, Bautista-Teruel MN, Reyes OS, Kanazawa A (1998). Requirements of juvenile marine shrimp, *Penaeus monodon* (Fabricius) for lysine and arginine. Aquaculture, 164: 95-104.
- Naess T, Germain-Henry M, Naas KE (1995). First feeding of Atlantic halibut (*Hippoglossus hippoglossus*) using different combination of *Artemia* and wild zooplankton. Aquaculture, 130: 235-250.
- Norsker NH, Støttrup JG (1994). The importance of dietary HUFAs for fecundity and HUFA content in the harpacticoid, *Tisbe holothuriae* Humes. Aquaculture, 125: 155-166.
- O'Bryen PJ, Lee CS (2005). Culture of copepods and applications to marine finfish larval rearing workshop discussion summary. In: Lee CS, O'Bryen PJ, Marcus NH (eds) Copepods in Aquaculture, Blackwell, Melbourne, pp. 245-255.
- Omori M, Ikeda T (1984). Methods in Marine Zooplankton Ecology. Wiley, New York, p. 332.
- Parrish KK, Wilson DF (1978). Fecundity studies on *Acartia tonsa* (Copepoda: Calanoida) in standardized culture. Mar. Biol., 46: 65-81.
- Payne MF, Rippingale RJ (2001). Effects of salinity, cold storage and enrichment on the calanoid copepod *Gladioferens imparipes*. Aquaculture, 201: 251-262.
- Pepin P, Penney RW (1997). Patterns of prey size and taxonomic composition in larval fish: are there general size dependent models? J. Fish Biol., 51(Supplement A): 84-100
- Roman MR (1983). Nitrogenous nutrition of marine invertebrates. In Carpenter EJ, Capone DG (eds). Nitrogen in the Marine Environment, pp 347-383.
- Rausch T (1981). The estimation of micro-algal protein content and its meaning to the evaluation of algal biomass I. Comparison of methods for extracting protein. Hydrobiologia, 78:237-251.
- Sargent JR, Falk-Petersen S (1988). The lipid biochemistry of calanoid copepods. Hydrobiologia, 167/168: 101-114.
- SAS (1992). Statistical Analysis System. SAS Institute Inc. Cary NC, USA. Science and technology, Marcel Dekker, Inc. New York and

- Basel USA, pp. 25-39.
- Scott AP, Middleton C (1979). Unicellular algae as food for turbot (*Scophthalmus maximus* L.). The importance of dietary long chain polyunsaturated fatty acids. *Aquaculture*, 18:227-240.
- Sorgeloos P, Leger P (1992). Improve larviculture outputs of marine fish, shrimp and prawn. *J. World Aquaculture Soc.*, 23: 251-264
- Souza-Santos LP, Santos PJP, Castel J (1999). Development and population dynamics of *Amonardia normani* Brady reared on axenic and non-axenic diatoms. *J. Experi. Marine Biol. Ecol.*, 235: 167-182.
- Støttrup J (2003). Production and nutritional value of copepods. In: Støttrup J, McEvoy LA (eds) *Live Feeds in Marine Aquaculture* Blackwell Publishing, Oxford, pp. 145-205.
- Støttrup JG, Norsker NH (1997). Production and use of copepods in marine fish larvae culture. *Aquaculture*, 155: 231-247.
- Støttrup JG, Richardson K, Kirkegaard E, Pihl HJ (1990). The cultivation of *Acartia tonsa* Dana for use as live food source for marine fish larvae. *Aquaculture*, 52:87-96
- Sun B, Fleeger JW (1995). Sustained mass culture of *Amphipisacoides atopus* a marine harpacticoid copepod in a recirculating system. *Aquaculture*, 136: 313-321
- Szyper JP (1989). Nutritional depletion of the aquaculture feed organisms *Euterpina acutifrons*, *Artemia* sp. and *Brachionus plicatilis* during starvation. *J. World Aquaculture Soc.*, 20: 162-169.
- Tseng W-Y, Hsu C-K (1984). Studies on the culture of the marine copepod *Tigriopus japonicus* Mori and its value as a food for juveniles of the yellow-fin sea bream *Acanthopagrus latus* (Houttuyn). *Crustaceana. Int. J. Crustacean Res., Supplement 7*: 381-389
- Uhlig G (1984). Progress in mass cultivation of harpacticoid copepods for mariculture. *Special Publication of the European Mariculture Society*, 8: 261-273.
- VanderLugt K, Lenz PH (2008). Management of nauplius production in the paracalanoid, *Bestiolina similes* (Crustacean: Copepoda): Effect of stocking densities and culture dilution. *Aquaculture*, 276: 69-77.
- van der Meeren T (1991). Selective feeding and prediction of food consumption in turbot larvae (*Scophthalmus maximus* L.) reared in rotifer *Brachionus plicatilis* and natural zooplankton. *Aquaculture*, 93: 35-55
- von Herbing H, Gallagher, S (2000). Foraging behavior in early Atlantic cod larvae (*Gadus morhua*) feeding on a protozoan (*Balanion* sp.) and a copepod nauplius (*Pseudodiaptomus* sp.). *Mar. Biol.*, 136: 591-602.
- Watanabe T, Kitajima C, Fujita S (1983). Nutritional values of live food organisms used in Japan for mass propagation of fish: a review. *Aquaculture*, 34: 115-143.
- Whyte J (1988). Fatty acid profiles from direct methanolysis of lipids in tissue of cultured species. *Aquacultur*, 75:193-203
- Zar JH (1984). *Biostatistical Analysis* (2nd edition) Prentice Hall Inc. Englewood Cliffs, New York, p. 718.