FATTY ACID PROFILES OF CYCLOPOID COPEPOD NAUPLII *Apocyclops panamensis* AND THE EFFECTS OF SALINITY CHANGE

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ABSTRACT

Lipid and fatty acid profiles were described for copepod nauplii *Apocyclops panamensis* from fertilized brackish water ponds, and after being acclimated to full-sea water salinity. Mean total lipid content of copepod nauplii collected from ponds fertilized with inorganic fertilizer combined either with alfalfa meal, rice bran, wheat bran, and a combination of these fertilizers ranged from 5.66 ± 0.15 to 7.76% ± 0.27%. Non-polar (neutral) lipid fraction of pond copepod nauplii was a significantly higher percentage of the total lipid content (74.5 ± 1.8 - 93.5% ± 1.0%) compared to those of polar lipid (6.5 ± 1.0 - 21.3% ± 1.8%) (P= 0.000). DHA/EPA ratio in neutral lipids ranged from 1.8 ± 0.2 - 2.0 ± 0.1 with no significant differences in three fertilization regimes. DHA was 27.5% ± 0.56% of the neutral lipids and EPA 14.8% ± 0.8%. Acclimation of copepod nauplii for six hours from brackish to full-sea water salinity reduced their lipid content and individual dry weight significantly. Mean total lipid content was reduced 44.2%, non-polar lipid was reduced 46.9% and polar lipid was reduced 24.4%. Acclimation altered the DHA/EPA ratio, in the neutral fraction the ratio increased 26.3% but in the polar fraction it decreased 25%.

KEYWORDS: ***Apocyclops panamensis*, copepod naupii, fatty acid, salinity change**

INTRODUCTION

Copepod nauplii have been used as a food for the culture of a number of marine finfish larvae resulting in increased performance of the larvae (Taniguchi *et al.*, 1982; van der Meeren & Naess, 1993; Nanton & Castell, 1999; Toledo *et al.,* 1999). Daily specific growth rates of cod *Gadus morhua* have been shown to greatly increase from 2.8% to 21% with an increase in availability of copepod nauplii (van der Meeren & Naess, 1993). Survival of turbot *Scophthalmus maximus* larvae was 73% when fed *Artemia* nauplii, and 93% when fed copepod nauplii (Kuhlman *et al.*, 1979). Copepod nauplii contain suitable amounts of essential elements needed for the survival and growth of fish larvae. In particular, copepods often contain a higher amount of highly unsaturated fatty acids making them nutritional better than other live food items (Stottrup & Norsker, 1997; Stottrup *et al.*, 1998).

Although the importance of copepods as a live food for larval marine fish has been recognized, laboratory techniques are not well established for their mass production and the densities that have been obtained are relatively low (Stottrup *et al.*, 1998). Producing copepods under a controlled environment requires complex facilities and sophisticate skill (Stottrup & Norsker, 1997) and may not be economical for certain hatchery applications. An alternative approach to obtain large quantity of live food organisms is to collect zooplankton from ponds. Outdoor tanks and ponds given both organic and chemical fertilizers have been used for copepod production (Colura *et al.*, 1987; Ohno & Okamura, 1988; Harrell % Bukowski, 1990; Doi *et al.*, 1994b; Bootes, 1998). By using various combinations of organic and chemical fertilizers, these workers were able to produce copepod nauplii and adult copepods with maximum densities up to 1,225 individuals/L

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and 679 individuals/L, respectively within three to 33 days. Nystrom (1999) and Lan (2001) developed trapping techniques to collect copepod nauplii from ponds that were able to trap 167,000—690,000 copepod nauplii per hour. The advantages of extensive culture of copepod are that it can be an inexpensive and practical method to provide a large number of nauplii. However, there is often considerable variation in zooplankton composition and abundance from pond to pond. The pond environment may differ in salinity and other environmental parameters relative to the settling where the food organism is to be given as a food.

A given species of copepod may be found over a range of salinities. The cyclopoid copepod *Apocyclops* occurs at salinities below 30 ppt (Cheng *et al.*, 2001), they can thrive in water with salinities up to 69 ppt (Dexter, 1993). However, there is little information as to how salinity changes may directly or indirectly affect the nutritional characteristics of copepods. Changes in salinity can affect food sources and the nutritional profile of the copepods. Furthermore, changes in salinity stimulate crustaceans to adjust their osmoregulation. It has been observed in adult calanoid copepod *Eurytemora affinis* and zoea 1 of decapod crustacean larvae *Cancer pagurus, Homarus gammarus, Carcinus maenas,* and *Chasmagnathus granulata* that extracellular osmoregulation is associated with energy expenditure as part of active ion transport, involving degradation of energy-rich compounds such as lipid and protein (Gonzales & Bradley, 1994; Kimmel & Bradley, 2001; Torres *et at.*, 2002). When copepod nauplii are to be used as feed for larval fish, changes in salinity may affect their nutrient quality.

This present study was designed to evaluate fatty acid profiles of cyclopoid copepod nauplii *Apocyclops panamensis* harvested from brackish water ponds fertilized using different preparation protocols, as well as after acclimation into full-strength sea water salinity.

MATERIALS AND METHODS

Production and Collection of Copepod Nauplii

Fatty acid profiles were determined for copepod nauplii *Apocyclops panamensis* collected from brackish water ponds fertilized with rice, wheat bran or a mixture of organic fertilizers. Ponds fertilized with either rice bran or wheat bran received an initial application of 250 kg/ha along with two liquid fertilizers 32-2-0 (N:P:K) and 10-34-0 combined to give a 38-8-0 and applied at an initial dose of 20 L/ha. Three ponds were used for each type of organic fertilizer. Ponds were subsequently given half initial dose of both organic and inorganic fertilizers weekly.

An additional pond was prepared initially fertilized with 50 kg/ha of each of the following organic fertilizers: alfalfa meal, menhaden fish meal and rice bran. In addition, two liquid fertilizers of 32-2-0 (N:P:K) and 10-34-0 were combined to give a 38-8-0 and the combination was applied to each pond at initial dose of 20 L/ha. This pond was subsequently given at half the initial dose of both organic and inorganic fertilizers weekly. Organic fertilizers were applied to the pond bottom just before filling with 300 µm mesh sock-filtered 12 ppt brackish water while the liquid fertilizer was applied when the pond was full.

The ponds were sampled for zooplankton every morning (0700—0900 h) taking a 25 L composite sample of pond water. The sample was concentrated over a 35-mm Nitex® plankton net and the volume adjusted to one liter and fixed in Lugol's solution. Zooplankton were enumerated as to the abundance per liter of copepod nauplii, adult copepods, rotifers and other organisms using a binocular Olympus® CH-30 microscope at magnification of 10x4 and a 1 mL modified plastic-grid Sedgewick® Rafter counting cell. Identification of copepod nauplii was conducted according to Dr. K. Stuck of University of Southern Mississippi and Dr. J. Reid of Department of Systematic Biology, Museum of Natural History, Smithsonian Institution.

Phytoplankton sampling was conducted in the afternoon (1500—1600 h) at the beginning, middle and end of the study. Five liters of pond water were sieved through 5-mL Nitex® plankton net and concentrated into 50 mL and preserved in Lugol's solution (Sournia, 1978; Tomas, 1997). Phytoplankton was counted using a Reichert® Improved Neubauer Haemacytometer with the plankton density expressed in cells/mL from an average of two counts (Sournia, 1978; Hoff & Snell, 1993).

When copepod nauplii were found to be more than 90% of the total zooplankton organisms in the pond, they were collected

following the trapping method described by Lan (2001). The trap consisted of three cylindrical containers (140 L each) connected in series by 5.08-cm pipe. A 5.08 cm diameter PVC water supply pipe was placed above the containers and had two water outlets (1.9 cm PVC T's) extending to each container. A set of 40-mm and 100-mm nylon filter bags with drawstrings around the mouth, 80-cm long and 17.5-cm diameter, were tied to each water outlet. The 100-mm mesh bag was placed inside the 40-mm mesh bag. A sump pump (1/3 HP-115 volt) with a mean pumping rate of 133.81 ± 8.5 L/min. was connected to water supply pipe and suspended in the water column of the pond. Water passed through the 100 mm bag then the 40 mm bag into the surrounding container, where the water level was maintained at 17 cm below the top of the containers, then back to the pond. Filtered water flowed back to the pond through the containers. The discharge was approximately 2 m away from the pump intake.

Copepod nauplii *A. panamensis* used in the acclimation trials were obtained only from the pond receiving mixed organic fertilizers. Acclimation was accomplished by dilution of the initial holding water (8—12 ppt) with 32 ppt seawater to achieve an end point of 32 ppt. Copepod nauplii were siphoned from the 20-L transport bucket and equal volumes added to each 20 L acclimator bucket. The dilution water flow rate was determined by calculating the salinity increase divided by acclimation period (6, 12, 24 hours). Salinity was measured using an Atago refractometer (± 1 ppt). Water flow and salinity was monitored hourly to maintain the salinity increase evenly. Temperature and dissolved oxygen was measuring hourly using YSI dissolved oxygen meter (Model #8510). Aeration was adjusted to maintain the D.O. level above 4 mg/L. All acclimation trials were conducted in the laboratory and acclimated to the same temperature of 23°C. Copepod nauplii were not fed during the experiments.

Prior to acclimation, at least 50 million of the collected copepod nauplii (approximately 5.0 g wet weight) were sieved through 21µm Nitex® plankton net, rinsed with approximately one liter of distilled water, placed in screwed-cap glass jars and stored at –20°C (Kates, 1986; Christie, 1987). After acclimation, a minimum sample of 5.0 g (wet weight) of copepod nauplii were collected and frozen following the same procedures. All samples were held for lipid extraction and fatty acid analysis.

Lipid Extraction and Fatty Acid Analysis

Lipid extraction was conducted at the Fish Nutrition Laboratory of Department of Fisheries and Allied Aquacultures, Auburn University, Alabama. The extraction of lipid from copepod nauplii and oils was carried out with a mixture of chloroform and methanol (2:1, v/v) containing 0.01% butylhydroxytoluene (BHT) following the method of Folch *et al.* (1957). Total lipids were separated to polar and neutral lipids with Whatman solid phase extraction silica cartridge as described by Juaneda & Rocquelin (1985). The lipids were saponificated with potassium hydrate. The relative fatty acid methyl esters (FAME) were prepared by transesterification with borontrifluoride in methanol. FAME were analyzed on a gas liquid chromatograph (GC-17A; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector (FID) and an Supelco Omegawax® fused silica capillary column (30 m x 0.53 id x 0.50 mm film thickness; Supelco Inc., Bellefonte, Pennsylvania). The column temperature was initially held at 140°C for 5 min, followed by an increase at a rate of 3 C/min to a final temperature of 260°C and held for two minutes at the end of each run. The carrier gas was helium and the pressure was 100 kPa. Individual FAME was quantified with an integrator (Shimadzu Class VP™ Data System Version 4.3, Shimadzu Scientific Instrument). Flow rates were: helium at 3.4 mL/min, hydrogen 7.4 mL/min and air 2.8 mL/min. The air was passed through Supelcarb® HC (Supelco Inc., Bellefonte, Pennsylvania) and a carrier gas drying tube. Total analysis time in each run was 42 minutes. Retention times were determined relative to that of FAME standards of Supelco® PUFA-3 (Catalog No. 47085-U) and Supelco® 37 Component FAME Mix (Catalog No. 47885-U). The flame ionization detector (FID) and injector were set at 270°C and 260°C, respectively with sample size of 1.0 mL in 10:1 split and splitless mode. The pressure of gas carrier was set at 26 kPa to 30 kPa at 0.1 kPa/minute and also held for two minutes at the end of each run. Fatty acid values were expressed as area percent of the total identified fatty acids. Each sample was analyzed in duplicates.

Statistical Analyses

Values are presented in mean ± standard error. If required, some data were transformed into square root for count data and arcsin

square root for proportions before analyses. Data were analyzed using *t-test* and one-way analysis of variance (ANOVA) model procedure of SAS® Version 6.12 for Windows® (Statistical Analysis System, 1996). When significant differences among treatment means were detected, a least significant difference (LSD) multiple range test was applied (Sokal & Rohlf, 1981). The differences were considered to be significant at probability level of P d" 0.05.

RESULTS

Copepod Nauplii

Results of non polar and polar fraction of HUFA acid profiles (ARA, EPA, and DHA), total lipid content and individual weight of post harvest copepod nauplii *A. panamensis* trapped from several fertilization pond regimes are shown in Table 1 and 2, respectively. Total lipid content (g/100 g dry weight) of copepod

Table 1. Non-polar fatty acid profile, total ω3 and ω6, ω3/ω6 and DHA/EPA ratios, total unsaturated and saturated fatty acid (% total fatty acid) and its ratio, non-polar lipid content (g/100g dry weight) and % non-polar lipid of total lipids, total lipid content (g/100g dry weight), and individual copepod naupliar weight (µg) of post harvest copepod nauplii *A. panamensis* trapped from rice bran, wheat bran and mixed meals fertilization of brackish water ponds (mean ± standard error). Only parameters with significant differences (P<0.05) are noted

Fatty acid	Rice bran	Wheat bran	Mixed meals
14:0	17.5 ± 0.4	11.8 ± 0.7	13.0 ± 1.6
16:0	12.8 ± 0.5	13.9 ± 0.5	16.2 ± 0.6
16:1(n-7)	1.5 ± 0.1	0.9 ± 0.0	0.8 ± 0.1
16:2(n-4)	0.6 ± 0.0	0.7 ± 0.1	0.4 ± 0.0
16:3(n-4)	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.2
18:0	4.0 ± 0.0	4.9 ± 0.4	5.2 ± 0.1
18:1(n-9)	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1
18:1(n-7)	1.4 ± 0.2	1.2 ± 0.1	1.5 ± 0.2
18:2(n-6)	0.3 ± 0.0	0.7 ± 0.1	0.7 ± 0.1
18:3(n-4)	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
18:3(n-3)	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
18:4(n-3)	0.3 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
20:1(n-9)	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.0
20:4(n-6)	1.3 ± 0.0	1.3 ± 0.2	1.4 ± 0.0
20:4(n-3)	0.4 ± 0.0	0.4 ± 0.1	0.2 ± 0.0
20:5(n-3)	14.0 ± 0.1	15.6 ± 3.2	14.8 ± 0.4
22:5(n-3)	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1
22:6(n-3)	27.4 ± 1.6	27.0 ± 1.8	28.1 ± 2.3
Σω3[1]	42.3 ± 1.6	43.7 ± 4.9	44.1 ± 1.8
Σω6[2]	1.5 ± 0.1	1.9 ± 0.3	2.1 ± 0.1
ω3/ω6	28.2 ± 0.1[a]	23.1 ± 0.5[b]	21.5 ± 0.4[b]
DHA/EPA	2.0 ± 0.1	1.8 ± 0.2	1.9 ± 0.2
Σidentified fatty acids	83.0 ± 0.8	80.6 ± 3.6	84.5 ± 3.8
Non-polar lipid	7.76 ± 0.27[a]	6.35 ± 0.34[b]	6.90 ± 0.52[ab]
% non-polar lipid	91.8 ± 1.0	74.5 ± 1.8	84.6 ± 6.0
Total lipid	7.76 ± 0.27	6.35 ± 0.34	6.90 ± 0.52
Naupliar weight	0.16 ± 0.01	0.13 ± 0.02	0.13 ± 0.01

[1] Sum included 18:3(n-3), 18:4(n-3), 20:4(n-3), 20:5(n-3), 22:5(n-3), and 22:6(n-3)
[2] Sum includes 18:2(n-6), and 20:4(n-6)

Table 2. Polar fatty acid profile, total ω3 and ω6, ω3/ω6 and DHA/EPA ratios, total unsaturated and saturated fatty acid (% total fatty acid) and its ratio and polar lipid content (g/100g dry weight) and % polar lipid of total lipids of post harvest copepod nauplii *A. panamensis* trapped from rice bran, wheat bran and mixed meals fertilization of brackish water ponds (mean ± standard error). Only parameters with significant differences ($P<0.05$) are noted

Fatty acid	Rice bran	Wheat bran	Mixed meals
14:0	12.3 ± 1.1	12.7 ± 0.7	11.9 ± 0.7
16:0	25.5 ± 0.6	16.7 ± 0.8	18.6 ± 0.4
16:1(n-7)	1.5 ± 0.1	1.2 ± 0.2	0.9 ± 0.0
16:2(n-4)	1.4 ± 0.3	1.3 ± 0.0	0.5 ± 0.0
16:3(n-4)	0.8 ± 0.2	1.6 ± 0.0	0.4 ± 0.1
18:0	5.9 ± 0.4	7.9 ± 0.5	6.6 ± 0.8
18:1(n-9)	1.4 ± 0.1	1.2 ± 0.0	1.3 ± 0.0
18:1(n-7)	1.2 ± 0.1	1.2 ± 0.2	1.1 ± 0.2
18:2(n-6)	1.1 ± 0.0	2.1 ± 0.0	2.2 ± 0.2
18:3(n-4)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
18:3(n-3)	0.1 ± 0.0	0.1 ± 0.0	1.1 ± 0.2
18:4(n-3)	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:1(n-9)	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
20:4(n-6)	1.5 ± 0.3[a]	1.0 ± 0.1[b]	1.4 ± 0.0[a]
20:4(n-3)	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1
20:5(n-3)	9.8 ± 0.4[a]	12.6 ± 0.8[b]	13.8 ± 0.8[b]
22:5(n-3)	0.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.1
22:6(n-3)	23.0 ± 2.2	22.9 ± 0.3	22.4 ± 0.4
Σω3[1]	33.4 ± 2.6	36.2 ± 1.0	38.1 ± 1.4
Σω6[2]	2.5 ± 0.3	3.1 ± 0.1	3.6 ± 0.2
ω3/ω6	13.3 ± 0.7	11.8 ± 0.6	10.8 ± 1.0[b]
DHA/EPA	2.4 ± 0.1[a]	1.8 ± 0.1[b]	1.6 ± 0.1[b]
Σidentified fatty acids	86.7 ± 3.5	83.0 ± 2.9	84.1 ± 1.7
Polar lipid	0.51 ± 0.09	0.36 ± 0.19	0.82 ± 0.48
% polar lipid	6.5 ± 1.0	21.3 ± 1.8	11.4 ± 6.0

[1] Sum included 18:3(n-3), 18:4(n-3), 20:4(n-3), 20:5(n-3), 22:5(n-3), and 22:6(n-3)
[2] Sum includes 18:2(n-6), and 20:4(n-6)

nauplii ranged from 6.35% in a wheat bran-fertilized pond to 7.76% in rice bran fertilized pond. There was no difference in mean lipid content of copepod nauplii trapped from rice bran, wheat bran and mixed meals fertilized ponds ($P= 0.182$). Means of non-polar lipid content of copepod nauplii ranged from 4.72% (74.5% ± 1.8% of total lipid content in wheat bran fertilized ponds) to 7.12% (91.8% ± 1.0% of total lipid content in rice bran fertilized ponds). Mean non-polar lipid content of copepod nauplii trapped from wheat bran fertilized ponds was lower than those from other fertilization regimes ($P= 0.003$).

Ranges of mean polar lipid content of copepod nauplii trapped from various fertilization regimes were 0.51 to 0.82 g/100g dry wt, with percentages of the total lipid 6.5% to 21.3%. There were significant differences in ARA, EPA, and DHA/EPA ratio for copepods collected from the different fertilization regimes. Copepod nauplii from wheat bran ponds had significant lower mean content of ARA than those of other regimes ($P= 0.022$) while mean content of EPA in copepod nauplii trapped from the rice bran regime had a significant lower level than those of other regimes ($P= 0.004$). There was no significant

different in mean individual copepod nauplii weight (P= 0.294).

Pre- and Post-acclimation Fatty Acid Profiles

HUFA profiles, survival rate and individual dry weight of post harvest and six-hour post acclimation are presented in Table 3 and 4. Acclimation from an average initial salinity of 19.7 ± 0.2 ppt up to full seawater salinity of 32.0 ppt was conducted over six hours at a copepod nauplii density of 200 individuals/L. Mean total lipid content was significantly lower (P= 0.029) after acclimation decreasing from

Table 3. Non-polar fatty acid profile, total ω3 and ω6, ω3/ω6 and DHA/EPA ratios, total unsaturated and saturated fatty acid (% total fatty acid) and its ratio, non-polar lipid content (% dry weight and % non-polar lipid), total lipid content (% dry weight), and individual copepod naupliar weight (µg), survival rate (%), and change values (%) of post harvest and six-hour post acclimation of copepod nauplii *A. panamensis* trapped from mixed meals fertilized brackish water ponds (mean ± standard error). Values followed by the same letter in the same row are not significantly different (P>0.05)

Fatty acid	Post harvest	Six-hour post acclimation	Change (%)
14:0	13.0 ± 1.6	14.1 ± 1.2	8.5
16:0	16.2 ± 0.6	19.9 ± 0.8	22.8
16:1(n-7)	0.8 ± 0.1	2.0 ± 0.5	150
16:2(n-4)	0.4 ± 0.0	0.5 ± 0.4	25
16:3(n-4)	0.2 ± 0.2	0.4 ± 0.1	20
18:0	5.2 ± 0.1	6.0 ± 0.3	15.4
18:1(n-9)	0.4 ± 0.1	0.5 ± 0.2	25
18:1(n-7)	1.5 ± 0.2	3.2 ± 0.4	113.3
18:2(n-6)	0.7 ± 0.1	0.8 ± 0.1	14
18:3(n-4)	0.2 ± 0.0	0.2 ± 0.0	0
18:3(n-3)	0.3 ± 0.0	0.2 ± 0.1	33.3
18:4(n-3)	0.6 ± 0.0	0.6 ± 0.1	0
20:1(n-9)	0.6 ± 0.0	0.8 ± 0.0	33.3
20:4(n-6)	1.4 ± 0.0	1.6 ± 0.1	14.3
20:4(n-3)	0.2 ± 0.0	0.2 ± 0.2	0
20:5(n-3)	14.8 ± 0.4[a]	8.5 ± 0.6[b]	57.4
22:5(n-3)	0.2 ± 0.1	0.2 ± 0.1	0
22:6(n-3)	28.1 ± 2.3	20.5 ± 2.1	27
Σω3[1]	44.1 ± 1.8[a]	30.2 ± 1.4[b]	31.5
Σω6[2]	2.1 ± 0.1	2.4 ± 0.3	14.3
ω3/ω6	21.5 ± 0.4[a]	12.6 ± 0.5[b]	41.4
DHA/EPA	1.9 ± 0.2	2.4 ± 0.9	26.3
Σidentified fatty acids	84.5 ± 3.8	80.1 ± 3.2	5.2
Non-polar lipid	5.83 ± 0.05[a]	3.15 ± 0.06[b]	46
% non-polar lipid	84.6 ± 6.0[a]	82.1 ± 4.4[a]	3
Total lipid	6.90 ± 0.52[a]	3.85 ± 0.26[b]	44.2
Naupliar weight	0.13 ± 0.01[a]	0.08 ± 0.01[b]	38.5
Survival rate		90.4 ± 3.4	

[1] Sum included 18:3(n-3), 18:4(n-3), 20:4(n-3), 20:5(n-3), 22:5(n-3), and 22:6(n-3)
[2] Sum includes 18:2(n-6), and 20:4(n-6)

Table 4. Polar fatty acid, total ω3 and ω6, ω3/ω6 and DHA/EPA ratios, total unsaturated and saturated fatty acid (% total fatty acid) and its ratio and polar lipid content (% dry weight and % polar, and change values (%) of post harvest and six-hour post acclimation of copepod nauplii *A. panamensis* trapped from mixed meals fertilization in brackish water ponds (mean ± standard error). Values followed by the same letter in the same row are not significantly different (P>0.05)

Fatty acid	Post harvest	Six-hour post acclimation	Change (%)
14:0	11.9 ± 0.7	13.3 ± 0.7	11.8
16:0	18.6 ± 0.4	20.3 ± 0.8	9.1
16:1(n-7)	0.9 ± 0.0	0.8 ± 0.2	-11.1
16:2(n-4)	0.5 ± 0.0	0.6 ± 0.1	20
16:3(n-4)	0.4 ± 0.1	0.4 ± 0.0	0
18:0	6.6 ± 0.8	7.5 ± 0.9	13.6
18:1(n-9)	1.3 ± 0.0	1.2 ± 0.1	-7.7
18:1(n-7)	1.1 ± 0.2	2.1 ± 0.3	90.1
18:2(n-6)	2.2 ± 0.2	2.3 ± 0.2	4.5
18:3(n-4)	0.4 ± 0.0	0.3 ± 0.1	-25
18:3(n-3)	1.1 ± 0.2	0.9 ± 0.1	-18.2
18:4(n-3)	0.3 ± 0.0	0.1 ± 0.1	-66.7
20:1(n-9)	0.9 ± 0.1	0.7 ± 0.1	-22.2
20:4(n-6)	1.4 ± 0.0	1.2 ± 0.0	-14.3
20:4(n-3)	0.3 ± 0.1	0.1 ± 0.1	-66.7
20:5(n-3)	13.8 ± 0.8	12.0 ± 0.6	-13
22:5(n-3)	0.4 ± 0.1	0.3 ± 0.1	-25
22:6(n-3)	22.4 ± 0.4[a]	12.5 ± 0.7[b]	-36.1
Σω3[1]	38.1 ± 1.4[a]	27.8 ± 2.3	-27
Σω6[2]	3.6 ± 0.2[a]	3.4 ± 0.4[a]	-5.6
ω3/ω6	10.8 ± 1.0[a]	8.2 ± 0.4[a]	-24.1
DHA/EPA	1.6 ± 0.1[a]	1.2 ± 0.0[b]	-25
Σidentified fatty acids	84.1 ± 1.7[a]	78.5 ± 2.1[b]	-18.8
Polar lipid	0.82 ± 0.48	0.62 ± 0.21	-24.4
% polar lipid	11.4 ± 6.0	16.1 ± 4.4	41.2

[1] Sum included 18:3(n-3), 18:4(n-3), 20:4(n-3), 20:5(n-3), 22:5(n-3), and 22:6(n-3)
[2] Sum includes 18:2(n-6), and 20:4(n-6)

6.90 ± 0.52 to 3.85% ± 0.26% after six hours, a 44.2% decrease. Non-polar lipid content was significantly lower after acclimation (P<0.001) with a 46.9% decrease. There was a trend towards a small increase in relative abundance for many of the fatty acids. The fatty acid 16:1(n-7) increased from 0.8% to 2.0% and 18:1 (n-7) increased from 1.5% to 3.2%. In contrast, the relative abundance of DHA decreased 27.45 and EPA was statistically less, a 57.4% decrease. The degree of decrease in EPA being greater than that for DHA resulted in the DHA/EPA ratio increasing from 1.9 ± 0.2 to 2.4 ± 0.4.

In the polar fraction there were minor increases or decreases in the relative abundance for the majority of the fatty acids. Similar to the non-polar fraction, 18:1 (n-7) increased, in this case from 1.1% to 2.1%. The change in DHA and EPA showed the opposite trend to that seen in the non-polar fraction. In the polar fraction DHA decreased significantly

from 22.4% ± 0.4% to 14.3% ± 0.9% while EPA changed from 13.8% ± 0.8% to 12.0% ± 0.6% and as a result the DHA/EPA ratio was decreased significantly from 1.6 ± 0.1 to 1.2 ± 0.0. Mean survival rate during acclimation was 90.4% ± 3.4%.

DISCUSSION

Fatty Acid Profiles of Copepod Nauplii from Ponds

Copepod nauplii are nutritionally more suitable than rotifer (*Brachionus plicatilis*), brine shrimp (*Artemia* sp.) (Stottrup *et al.*, 1998) and oyster trochophores (Lim, 1991; Doi & Singhagraiwan, 1993; Su *et al.*, 1997) as a feed for larval marine fish. Copepod nauplii have high contents of PUFA (polyunsaturated fatty acids) and other fatty acids needed to meet the nutritional requirements of a fish's early ontogeny (Witt *et al.*, 1984). Moreover, the small size of copepod nauplii favors its acceptance (Last, 1978) as often other commonly produced food organisms may be too large for many marine fish larvae.

Nauplii can be obtained in large quantities from prepared production ponds (Lam, 2001; Sumiarsa, 2003). Differences in pond preparation procedures used in this study had little effect on the nutrient profile of nauplii, resulting in a high quality live food.

The pond produced copepod nauplii of *A. panamensis* in this study (Tables 5) are slightly higher in lipid content than those of copepod nauplii in South China Sea (Lokman, 1994; Shansuddin *et al.*, 1997) but much lower than those of adult calanoid copepod *Calanus hyperboreas* found in Arctic Ocean (Lee, 1974) and copepodite stage *Calanus finmarchicus, Pseudocalanus* sp. and *Temora longicornis* in nutrient-enriched seawater enclosure (Fraser *et al.*, 1989), and similar to seasonal variations of subarctic adult copepod *Acartia longiremis* and *Pseudocalanus acuspes* (Norrbin *et al.*, 1990).

As with other animals, zooplankton contains both polar lipids (largely phospholipids present in cell membranes) and non-polar (neutral) lipids that are fundamentally metabolic energy reserves (Sargent *et al.*, 1989). The neutral lipid in zooplankton can be present in much greater amounts than polar lipids and can account for up to one-half or more of the animals' dry body weigh. Fraser *et al.* (1989) found neutral lipids to be 55.7% ± 1.4% to 79.0% ± 1.7% of total lipid in late copepodites and adult copepods *C. finmarchicus, Pseudocalanus* sp. and *T. longicornis*. Norrbin *et al.* (1990) found neutral lipids of two adult calanoid copepod species to be 55% to 72% of total lipid. In the laboratory, Stottrup *et al.* (1986) found the proportion of polar lipids was comparable to that of neutral lipids based upon different provided diets and proportion of polar lipid content of naupliar and adult calanoid copepod *A. tonsa* were 40%—59% of the total lipid content (Table 5). Early stage nauplii often have high lipid levels but drops as the nauplii continue to develop (Sargent & Henderson, 1986). Nauplii of *A.panamensis* from fertilized ponds had an average of 83,6% of the total lipids as neutral lipids.

DHA/EPA ratio in neutral lipids ranged from 1.7 ± 0.4—2.0 ± 0.1 with no significant differences in three fertilization regimes. DHA was 27.5% ± 0.56% of the neutral lipids and EPA 14.8% ± 0.8%. Lokman (1994) and Shansudin *et al.* (1997) reported DHA/EPA ratio of natural zooplankton (> 60% was *Oithona* sp.) of between 1.0 and 2.3 with mean contents of DHA and EPA in dry and wet seasons of 6.8% to 28.7%, and 3.2% to 19.7% of total fatty acids, respectively. Fraser *et al.* (1989) reported DHA/EPA ratio of *C. finmarchicus, Pseudocalanus* sp. and *T. longicornis* in natural waters of between 0.7 and 2.4 in non-polar fatty acids, while Norrbin *et al.* (1990) found the ratio in adult calanoid species *Pseudocalanus acuspes* and *Acartia longiremis* in natural waters to be 0.2 and 0.7, respectively.

Ranges of DHA/EPA ratios of polar lipid class in copepod nauplii in this study were 1.6 ± 0.1 to 2.4 ± 0.1 with the ratio in rice bran regime being the highest level compared to the other regimes (P= 0.035). Fraser *et al.* (1989) and Norrbin *et al.* (1990) reported ratios in polar lipid fatty acids of adult copepods *A. longiremis, C. finmarchicus, Pseudocalanus* sp., *P. acuspes* and *T. longicornis* to be 1.3—1.4 and 0.8—1.2, respectively. In general, Sargent *et al.* (1999) suggested that the optimal DHA/EPA ratio in lipids for marine finfish larval diets was about 2.0. Copepod nauplii from the various fertilization regimes had a DHA/EPA ratio for both lipid classes of approximately 2.0.

Table 5. Summary of fatty acid profiles of copepods from natural waters, laboratory produced, and from this study

Sources[1] Natural waters	Fatty acid profiles							
	ARA (20:4n-6)	EPA (20:5n-3)	DHA (22:6n-3)	DHA/EPA	Fatty acids	Non-polar lipid	Polar lipid	Total lipid
Lee (1974)	1-13 (% DW)	12-35 (% DW)	3-42 (% DW)	1.9 –2.3		34 –91 (% DW)	1-7 (% DW)	29-74 (% DW)
Fraser *et al*. (1989)	0.1 –0.3 (% FA)	6.3 – 23.1 (% FA)	2.2 - 30.9 (% FA)	0.7 –2.4	87.2 –96.9 (% FA)	55.7 –79.0 (% lipid)	21.0 –44.3 (% lipid)	2.1 –31.5 (% DW)
Norrbin *et al*. (1990)	0 –1.2 (% FA)	4.7 –31.6 (% FA)	1.9 –24.3 (% FA)	0.8 –1.2	47.6 –62.3 (% FA)	55 –72 (% lipid)	28 –45 (% lipid)	
Lokman (1994)		4.3 –21.9 (% FA)	9.5 –33.6 (% FA)	1.5 –2.3	19.0 –56.9 (% FA)			1.5 –6.4 (% DW)
Shansudin *et al*. (1997)	0.1 –0.2 (% FA)	3.2 –19.7 (% FA)	6.8 –28.7 (% FA)	1.0 –2.3	14.4 –51.9 (% FA)			1.2 –5.9 (% DW)
Laboratory produced Vilela (1992)	0.1 – 0.6 (% FA)	0.6 16.5 (% FA)	0.4 11.7 (% FA)					
Nanton and Castell (1998)	0.2 –1.0 (% FA)	0.1-33.7 (% FA)	0.3-25.7 (% FA)	0.1-12.2	99-100 (% FA)			0.2 –0.3 (µg/copepod)
Nanton and Castell (1999)	0.1 –0.3 (% FA)	0.8-2.7 (% FA)	13.6-16.4 (% FA)	6.6-24.0	99-100 (% FA)			0.31-0.51 (µg/copepod)
Stottrup *et al*. (1999)	0.5 –2.3 (% FA)	6.6-23.2 (% FA)	28.5-41.6 (% FA)	1.2-6.3	98-100 (% FA)	50.3-54.4 (% lipid)	46.2-49.9 (% lipid)	
Payne *et al*. (1998)	0.0 – 1.5 (% FA)	3.2 – 9.5 (% FA)	7.0 - 16.4 (% FA)	1.7 –2.2				

Table 5. continued

Sources[1] Natural waters	Fatty acid profiles							
	ARA (20:4n-6)	EPA (20:5n-3)	DHA (22:6n-3)	DHA/EPA	Fatty acids	Non-polar lipid	Polar lipid	Total lipid
Payne and Rippingale (2001)	0.0 –0.9 (mg/g DW)	1.4 –2.8 (mg/g DW)	6.9 –10.1 (mg/g DW)	3.6 –7.0	42.9 –59.5 (mg/g DW)			
Payne *et al*. (2001)	0.9 (mg/g DW)	2.8 (mg/g DW)	10.1 (mg/g DW)	3.6				
Vilela (1992)								
Nanton and Castell (1998)								0.2 –0.3 (µg/copepod)
Nanton and Castell (1999)								0.31-0.51 (µg/copepod)
Stottrup *et al*. (1999)	0.5 –2.3 (% FA)	6.6-23.2 (% FA)	28.5-41.6 (% FA)	1.2-6.3	98-100 (% FA)	50.3-54.4 (% lipid)	46.2-49.9 (% lipid)	
Payne *et al*. (1998)	0.0 – 1.5 (% FA)	3.2 – 9.5 (% FA)	7.0 - 16.4 (% FA)	1.7 – 2.2				
Payne and Rippingale (2001)	0.0 –0.9 (mg/g DW)	1.4 –2.8 (mg/g DW)	6.9 –10.1 (mg/g DW)	3.6 –7.0	42.9 –59.5 (mg/g DW)			
Payne *et al*. (2001)	0.9 (mg/g DW)	2.8 (mg/g DW)	10.1 (mg/g DW)	3.6				
Laabir *et al*. (2001)	1.0 –2.0	12.0 –14.0	26.0 –28.0	1.9 –2.3				
Copepod nauplii from this study	0.5 –1.4 (% FA)	9.8 –16.4 (% FA)	22.4 –28.1 (% FA)	1.6 –2.4	80.6 –89.5 (% FA)	78.7 –92.8 (% lipid)	7.2 –21.3 (% lipid)	5.7 –7.8 (% DW)

[1] See text for explanation of life stage, copepod species, and treatment

Fatty Acid Profiles of Acclimated Copepod Nauplii

Species in the genera *Apocyclops* have a wide range of salinity tolerance. Dexter (1993) reported that the cyclopoid copepod *Apocyclops dengizicus* can thrive in natural waters with salinities of 0.5—68 ppt. Cheng *et al.* (2001) demonstrated that optimal salinity for another cyclopoid copepod *A. royi* was between 10 and 20 ppt in laboratory. Salinity in the fertilized ponds averaged 19.0 ± 0.2 ppt, respectively.

Fertilized brackish water ponds are a good source of nauplii with densities of >1,000/L were in common (Sumiarsa, 2003) but such nauplii must be acclimated to full strength salinity if they are to be used as a live food for many marine fish larvae. Lan (2001) found that without acclimation, nauplii mortality was often 100% within 6 h when directly transferred to sea water, but when acclimated from 8 to 32 ppt over a 6 h period the survival was 69.6%. In this study survival averaged 90.4% ± 3.4% after a 6 h acclimation from 19.7 to 32 ppt. Holding nauplii in a crowded environment with little opportunity to feed while they adjust to salinity changes can impact nutrient reserves as was evident in this study. Mean total lipid content was reduced 44.2 %, non-polar lipid was reduced 46.9% and polar lipid was reduced 24.4%.

Copepod nauplii were not fed during acclimation and this may have contributed to the reduced fatty acid levels (Bourdier & Amblard, 1989). Rippingale & Crossland (1993) found that differences in salinity had little effect on survival of copepods provided with excess food, but for animals that were starved, significant differences in survival did occur with another estuarine calanoid copepod *Eurytemora affinis*. Gonzales & Bradley (1994) suggested that osmoregulation in these copepods require energy. Evjemo *et al.* (2003) found that when *T. longicornis* and *Eurytemora* sp. were starved total lipids declined 18and 12%/d respectively and the fatty acid content declined 24 and 16%/d.

The decrease in copepod nauplii neutral lipids after acclimation was significantly more than that of the polar lipid (P= 0.043). It is suggested that energy reserves from triglycerides and wax ester (neutral lipids) were used in much greater proportion during unfed and crowded conditions of acclimation. Bourdier & Amblard (1989) reported that almost all the neutral lipid in the calanoid copepod *Acanthodiaptomus denticornis* was used up after 20 days of starvation while the polar lipids were reduced only about 5%—16%. Torres *et al.* (2002) acclimated four decapods crustacean larvae (*Cancer pagurus, Homarus gammarus, Carcinus maenas*, and *Chasmagnathus granulate*) from 32 ppt to 25, 20, and 15 ppt, and they found that lipid content decreased 5%—55% after short (16 hours) and long (>16 hours) exposures. Effects of salinity on copepod nauplii biomass may have occurred as metabolic adjustments induced by osmotic stress or due to physiological strategies to face such stress (Kinne, 1971). Osmotic stress due to disturbance of the water and mineral balance may cause critical variation in the metabolic rate or disharmonizing effects on mechanisms of organism integration. Under osmotic stress, metabolism increases and the energy necessary for this process must have originated primarily from catabolism of neutral lipids explaining negative growth (lost of dry weight) and reduced lipid content after acclimation.

Evjemo *et al.* (2003) found that when *T. longicornis* and *Eurytemora* sp. were starved 96 h the absolute DHA content (mg/g) declined 14 and 10% respectively. However, they found that the DHA/EPA ratio increased. With *A. panamensis* the DHA/EPA ratio in the neutral fraction had increased 26.3% after acclimation but in the polar fraction the ratio declined 25%. DHA in the neutral fraction declined 27.0% and 36.1% in the polar fraction after acclimation. The relative abundance of EPA was reduced 57% in the neutral fraction but only 13% in the polar fraction. These results suggest a selective utilization of EPA in the neutral fraction.

CONCLUSIONS

Mean total lipid contents and fatty acid profiles of copepod nauplii *A. panamensis* trapped from inorganic fertilizer combined either with alfalfa meal, rice bran or wheat bran, and combinations of these organic fertilizers were similar. DHA/EPA ratios of both non-polar and polar lipids were between 1.6 and 2.4, and non-polar lipid fraction was significantly larger than that of the polar lipid.

Acclimation of copepod nauplii from brackish to full-strength seawater salinity was the major bottleneck in their propagation in

brackish water ponds for marine finfish hatchery. Not only did it reduce mean total lipid content to almost half of its origin level but also generally reduced other fatty acid profiles significantly. In addition, acclimation delayed delivery of copepod nauplii from ponds to the hatchery for at least six hours and reduced the total supply of live copepod nauplii by ten percent or more.

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