JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Variation in Fatty Acid Composition of *Artemia salina* Nauplii Enriched with Microalgae and Baker's Yeast for Use in Larviculture

REKHA D. CHAKRABORTY,[†] KAJAL CHAKRABORTY,*[,‡] AND E. V. RADHAKRISHNAN[†]

Crustacean Fisheries Division and Physiology Nutrition and Pathology Division, Central Marine Fisheries Research Institute, Ernakulam North P.O., P.B. No. 1603, Cochin-682018, Kerala, India

The high content of the essential fatty acids in some microalgae and baker's yeast has made them excellent diets for boosting the fatty acid content of livefood *Artemia*. The influences of baker's yeast (*Saccharomyces cerevisiae*) and three microalgae, viz., *Chlorella salina*, *Chaetoceros calcitrans*, and *Nannochloropsis salina*, were tested as diet components in marine livefeed brine shrimp *Artemia salina* nauplii to improve the polyunsaturated fatty acid (PUFA) composition. *Artemia* nauplii submerged in these diets for four different enrichment intervals (3, 6, 8, and 24 h) were found to incorporate essential fatty acids, and the percentage composition of different fatty acids was measured in the enriched *Artemia* nauplii and enrichment diets. *N. salina* produced higher levels of arachidonic acid (AA, 20:4n6, 9.50%), eicosapentaenoic acid (EPA, 20:5n3, 25.80%), and docosahexaenoic acid (DHA, 22:6n3, 4.18%) as compared to other diets. The total PUFA content of the enriched *Artemia* by *N. salina* increased by 56.50% with enrichment periods up to 8 h, followed by a significant reduction in the final 24 h. *N. salina* yielded *Artemia* nauplii with considerable EPA (8.05%), AA (14.15%), and DHA (1.85%) after 8 h of enrichment, which are significantly higher levels than in nauplii fed with the other three diets ($p = 0.05$). The DHA/EPA values in *Artemia* enriched for 6 h by *N. salina* and *C. calcitrans* were found to be, respectively, 88.46 and 25% higher than freshly hatched *Artemia*. *Artemia* enriched by *C. salina* and baker's yeast exhibited a reduction in PUFA content even at 6 h of enrichment. Significant relative decreases in DHA, EPA, and total PUFA in *Artemia* enriched with all of the diets were apparent, with a corresponding increase in the total saturated fatty acid content ($26.95 \pm 9.75\%$) in the final stages (24 h) of enrichment ($p = 0.05$).

KEYWORDS: Polyunsaturated fatty acid (PUFA); eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); arachidonic acid (AA); *Artemia salina*; microalgae; Baker's yeast

INTRODUCTION

Artemia salina, the brine shrimp, are the most widely used aquaculture live food organism for marine larvae primarily because they are very convenient to use and are readily available (*1*). The biochemical composition of *Artemia* is regarded as important in optimizing larval nutrition for survival and growth of aquaculture species like finfish and shellfish (*2*). Although *Artemia* are not the natural prey of these animals, they are simple to prepare and have been used effectively as the major or sole component to culture aquaculture larvae, presently making them a diet of choice (*3*, *4*). However, *Artemia* nauplii are an incomplete food source for larvae of marine finfish and crustaceans, because of their paucity of essential n3 and n6 polyunsaturated fatty acids (PUFAs), viz., arachidonic acid (AA, 20:4n6), docosahexaenoic acid (DHA, 22:6n3), and eicosapentanoic acid (EPA, 20:5n3) in particular. Nearly all mariculture production systems predominantly rely on *Artemia* nauplii, which is naturally deficient in 20:5n3, and no known strains of *Artemia* contain significant levels of 22:6n3, making n3 PUFA enrichment necessary. Research on enriching *Artemia* nauplii in increasing the amounts of long-chain PUFAs, viz., DHA, EPA, and AA, prior to their use as live prey have received considerable attention. These PUFAs, which are usually low in abundance in *Artemia*, are regarded as essential for finfish and crustaceans and must be supplied in this livefeed. The importance of PUFAs in larval finfish/shellfish nutrition has been extensively investigated during the past 20 years (*5*−*10*). DHA is one of the important PUFAs, which maintains structural and functional integrity in larval cell membranes in addition to the neural development and function, while AA and EPA are involved in, respectively, the production and modulation of eicosanoids (*11*). AA has been an essential function of producing

* To whom correspondence should be addressed. Tel: 91-484-2394867. Fax: 0091-0484-2394909. E-mail: kajal_iari@yahoo.com.
[†] Crustacean Fisheries Division.
[‡] Physiology Nutrition and Pathology Division.

10.1021/jf063654l CCC: $37.00
Published on Web 04/04/2007

eicosanoids, making it an essential fatty acid in marine fish, which has to be provided in larval and broodstock diets because prostaglandins ($PGF2_\alpha$) are produced from 20:4n6, and has roles in the natural shedding of eggs, synchronizing ovulation, and spawning. AA is the basis for cyclo-oxygenase (COX) action to produce $PGF2_\alpha$. AA, being a major component of phosphoinositol, was reported to have a vital role in the transduction signal mechanism in larvae. The roles of live food in aquaculture and enrichment of live food by PUFA-rich sources are well-established facts. However, a majority of the research works have been concentrated in increasing PUFA levels and DHA: EPA ratios in *Artemia* nauplii and other live prey species that are fortified with PUFA-rich commercial oil emulsions (e.g., DHA Selco from INVE) (*2*, *12*). The success and convenience of this technique have led to commercially produced n3 PUFA enrichment emulsions being widely used in marine hatcheries (*13*). The essential n3 PUFA content is very small in TAG micelles generated in enrichment procedures, and there is a potential chance of autoxidation of PUFAs, resulting in the formation of potentially toxic oxidation products (*14*, *15*). Moreover, the cost−benefit ratio in enrichment emulsion is fairly high.

As alternatives to fish oil fractions, there is a growing interest in the fatty acid composition of marine microalgae and baker's yeast that produce PUFAs (*16*, *17*). The importance of microalgae in aquaculture is large because they start the food chain. The n3 and n6 fatty acids with more than 18 carbon atoms are made primarily by aquatic phytoplankton and are transferred via the food chain to the higher animals like *Artemia* (*18*). In addition, to provide protein (essential amino acids) and energy, they supply other key nutrients such as vitamins, pigments, and sterols, which are transferred through the food chain. It is significant that microalgae contain high contents of essential PUFAs and are major contributors to the marine food web as a renewable source. Earlier studies demonstrated the use of microalgae of the genera *Nannochloropsis*, *Chaetoceros*, and *Chrolella* as potential diets for livefeed *Artemia* for PUFA enrichment. Also, there are reports of yeast being used to enrich the copepods (*19*). Little work has been carried out regarding in-depth biochemical and metabolic studies of PUFA enrichment in livefeed *A. salina* by different tropical microalgal species like *Nannochloropsis salina*, *Chaetoceros calcitrans*, and *Chrolella salina.* Interest in the culture of tropical microalgal species has increased over recent years in response to the growth of tropical mariculture and the resulting need for nutritious microalgae that are tolerant to tropical culture conditions. PUFAs in live organisms like microalgae are much more stable than in commercially available emulsion formulations. It is significant that the levels and ratios of 22:6n3 : 20:5n3 : 20:4n6 in live microalgal cells more closely resemble larval natural diets, and the probabilities of natural protection of PUFAs by natural antioxidants in microalgae are advantageous.

In this study, various enrichment diets that have been used to improve the nutritional composition of *Artemia* fall into two groups: (i) live microalgae, viz., the diatom *C. calcitrans*, the eustigmatophyte *N. salina*, and *C. salina*, and (*2*) baker's yeast *Saccharomyces cerevisiae*. We report the diet-induced changes in the fatty acid composition of enriched *Artemia* with consideration for the initial content of the nauplii and possible metabolic changes of the fatty acids for various durations, viz., 3, 6, 8, and 24 h, during the time−course enrichment. The objective of the feeding trials was to study the value of microalgae as live food enrichment diets for *Artemia* and to compare their efficacy with *S. cerevisiae*.

MATERIALS AND METHODS

***Artemia* Production and Enrichment with Diets.** The encapsulated *Artemia* cysts obtained from the Red Top brand, United States (0.5−1.0 g), were hatched to nauplii following established procedures (*19*, *20*). The nauplii were left in sterile filtered seawater and were enriched with four different enrichment diets, viz., three species of marine microalgae, *C. calcitrans*, *C. salina*, and *N. salina*, and baker's yeast, *S. cerevisiae*. Newly hatched *Artemia* served as a control. The microalgae were cultivated in sterile Mequil's/Conway's medium following established procedures (*21*). The microalgae were used in feeding experiments when they reached the end of the logarithmic phase, i.e., after 5−7 days of growth. The ratio of microalgae to *Artemia* was kept at about 6000−6500 algal cells per *Artemia*. Three replicates were carried out for each treatment. The enrichment diets were added at the concentration of 0.3 g/L. *Artemia* nauplii were enriched by microalgal culture (2 L) each day at 25 °C, taking into account the consumption of these microalgae by *Artemia*. Samples of *Artemia* were randomly taken at four different intervals (3, 6, 8, and 24 h) throughout the enrichment period. During feeding experiments, triplicate cultures of *Artemia* (200 mL) were collected. Uneaten microalgae and baker's yeast were separated from *Artemia* by washing with sterile filtered seawater on 120 mesh screen, rinsed with ammonium formate (NH_4-COOH, 0.5 M), freeze-dried, and stored at −20 °C prior to chemical analysis.

Extraction and Derivatization of Fatty Acids to Fatty Acid Methyl Esters (FAME) and *N*-Acyl Pyrrolidides. The freeze-dried samples of *Artemia*, microalgae, and baker's yeast were analyzed for different fatty acids. The lipids were extracted following the Bligh and Dyer method (*22*). The lipid extracts were saponified with 0.3 N KOH in CH_3OH. After removal of the nonsaponifiable material with *n*-hexane and acidification with 1 N HCl, the saponifiable materials were extracted with petroleum ether−diethyl ether (1:1, v/v) and transesterified into their methyl esters by reaction (30 min under reflux) with methylating mixture (14% BF_3/MeOH, 5 mL) (*23*). This anhydrous reaction was performed under an inert atmosphere, and the setup was assembled and cooled under dry N_2. The product was added with distilled water (20 mL) and extracted with *n*-hexane. The organic layer was concentrated under an inert atmosphere of N_2. The resulting FAMEs concentrate thus obtained was dissolved in *n*-hexane and purified by silica gel column chromatography with *n*-hexane−diethyl ether (10:1, v/v) as the eluant and by thin-layer chromatography (TLC) using the same eluant. The PUFAs were further separated by preparative argentation−TLC (1.0 mm, 20 cm × 20 cm) by double-developing with *n*-hexane−diethyl ether−acetic acid (94:4:3, v/v/v). The purified methyl esters were reconstituted in petroleum ether, flushed with N_2 in glass vials, and stored in a deep freeze (−20 °C) until required for gas chromatography (GC)/GC−mass spectrometry (MS) analyses. *N*-acyl pyrrolidides were synthesized by reaction between pyrrolidine (1 mL) and FAME (1 mg) in acetic acid (0.1 mL) under reflux (100 °C, 2 h) (*24*), and the product was solubilized in *n*-hexane−diethyl ether (1:1, v/v) and washed with distilled water (4 mL × 3). The organic phase was dried with anhydrous $MgSO_4$, filtered, and concentrated to furnish *N*-acyl pyrrolidides, which were homogeneous, by TLC.

The crude extracted lipids were separated into different classes on a small column packed with silica gel (30 mm × 5 mm i.d., Kieselgel 60, 80−200 mesh, E-Merck), previously heated to 350 °C overnight for deactivation. The first fraction was eluted with CH_2Cl_2−*n*-hexane (1:2, v/v) to remove the steryl esters. This was followed by CH_2Cl_2 (100%) eluting the triacylglycerols (TAGs), CH_2Cl_2−diethyl ether (40:1, v/v) eluting the sterols, and CH_2Cl_2−CH_3OH (9:2, v/v) eluting the free fatty acids. The fractions were monitored by TLC on silica gel using *n*-hexane−diethyl ether−acetic acid (80:20:1, v/v/v) as the developing solvent. TLC was performed on 250 μm silica gel G plates with 10% gypsum as binder, preactivated at 120 °C for 2 h, using 2,7-dichlorofluorescein (0.2% in ethanol, w/v) as the visualizing agent. The TAG was recovered from the column eluate followed by evaporation under N_2 until dryness. The resulting product was saponified and transesterified into their methyl esters to be analyzed by GC/GC-MS as illustrated earlier.

Analysis of Fatty Acids by GC and GC-MS. Quantitative and qualitative analyses of FAME obtained by transesterification were performed on a Perkin-Elmer AutoSystem XL, gas chromatograph (Perkin-Elmer, United States) equipped with an Elite-5 (crossbond 5% diphenyl−95% dimethyl polysiloxane) capillary column (30 m × 0.53 mm i.d., 0.50 μm film thickness, Supelco, Bellfonte, PA) using a flame ionization detector. The oven temperature ramp was 110 °C for 1.0 min, followed by an increase of 45 °C/min to 210 °C, where it was held for 1.0 min, followed by an increase of 30 °C/min to 250 °C, where it was held for 1.0 min, followed by an increase of 25 °C /min to 285 °C, where it was held for 2.0 min, until all peaks had appeared. For *N*-acyl pyrrolidide derivatives, the oven temperature was programmed to hold 160 °C for 1 min, and then increased at the rate of 10 °C/min up to 220 °C, and thereafter 5 °C/min up to 290 °C. Ultrahigh purity He (99.99% purity) was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 285 °C. It was equipped with a split/splitless injector, which was used in split (1:15) mode. The flow rate of He and air were maintained at 50 psi. The injection volume was 1 μL. The detector was isothermal at 290 °C. FAMEs were identified by comparison of retention times with the known standards (37 component FAME Mix, PUFA-3, PUFA-1, and BAME; Supelco). The shorthand notation used in fatty acid nomenclature is L:Bnx, where L is chain length, B is number of double bonds, and nx is the position of the ultimate double bond from the terminal methyl group. The fatty acid composition was expressed as weight percent of the total fatty acids (TFA) of each fraction. Chromatograms were analyzed using Perkin-Elmer Chromstation (version 4.02) software.

The qualitative GC-MS analyses were performed using electronic impact (EI) ionization mode in a Varian GC (CP-3800) interfaced with a Varian instrument 1200L single quaduple Mass Spectrometer for confirmation of fatty acid identification. The GC apparatus was equipped with WCOT fused silica capillary column of high polarity (DB-5; 30 m × 0.25 mm i.d., 0.39 mm o.d., and 0.25 μm film thickness; Varian). The polymeric stationary phase was nonpolar (VF-5MS, 5% phenyl-substituted methylsiloxane). The carrier gas was ultrahigh purity He (99.99% purity) with a constant flow rate of 1 mL/min. The injector (type 1079) and detector temperatures were maintained at 300 °C. The injection volume was 1 μL. Samples were injected in split (1:15) mode at 300 °C into the capillary column similar to that used for the GC analyses, and the oven was identically programmed. The ion source and transfer line were kept at 300 °C. Electron ionization (E.I.) was produced by accelerating electrons from a hot filament through a potential difference at the standard value of 70 eV. The mass spectrometer conditions were as follows: ion source temperature, 200 °C; scan, 10−800 atomic mass units (amu); centroid scan mode; scan rate, 1250 amu/s; and dwell time, 1.5 s. Mass spectra were analyzed using Varian Workstation (version 6.2) software. For example, the mass spectrometric data of methyl eicosa-5,8,11,14,17-pentaenoate/methyl eicosapentaenoate were as follows. EI-MS *m*/*z* (rel. int. %): 315 (M^+, 1.67), 175 (6.67), 161 (8.33), 145 (11.67), 131 (18.33), 119 (31.67), 108 (31.67), 91 (70.00), 79 (100), 67 (68.33), 55 (48.33). The data for methyl docosa-4,7,10,13,16,19-hexaenoate/methyl docosahexaenoate were as follows. EI-MS *m*/*z* (rel. int. %): 342 (M^+, 0.60), 145 (4.20), 131 (6.60), 119 (10.80), 108 (11.40), 91 (28.20), 79 (100), 67 (20.40). The following are the mass spectrometric data of 1-(pyrrolidin-1-yl) eicosa-5,8,11,14,17-pentaen-1-one. EI-MS *m*/*z* (rel. int. %): 355 (M^+, 3.85), 286 (7.69), 232 (7.69), 126 (13.46), 113 (100), 85 (17.31), 72 (26.92), 55 (21.15). The data for 1-(pyrrolidin-1-yl) octadeca-9,12-dien-1-one are as follows. EI-MS *m*/*z* (rel. int. %): 381 (M^+, 3.91), 312 (7.05), 272 (7.29), 232 (16.22), 218 (15.76), 192 (8.24), 166 (23.67), 153 (22.85), 113 (100), 98 (46.62), 72 (21.98).

Statistical Analyses. The percentage composition of individual FAMEs in the diets and *Artemia* sampled at different enrichment times were subjected to a one-way analysis of variance (ANOVA), and significant means were compared by Tukey's multiple range tests. Correlations between *Artemia* and microalgal fatty acids were investigated using SPSS (version 10.0) software. A significance level of 95% ($p = 0.05$) was used throughout. Arc sin transformation was used prior to statistical analyses of FAME data expressed in percentages. All measurements were performed in triplicate, and values were averaged.

RESULTS

Spectroscopic Analyses of FAMEs and *N*-Acyl Pyrrolidides. The molecular ions for saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA)−FAME were conspicuous and as obvious from the mass spectra; hence, the molecular weight was discernible from the EI-MS spectra. An ion at $(M - 31)^+$ represents the loss of the $-OCH_3$ group, thus confirming the molecular structure as methyl ester. In the mass fragmentation pattern of FAME, the base peak was assigned to be the 1-methoxyethenol moiety (*m*/*z* = 74) obtained by McLafferty rearrangement per se with the loss of the McLafferty ion (*m*/*z* = 222). The results are similar to those obtained in earlier studies (*24*). The molecular ion undergoes further rearrangement to furnish 1-methoxyprop-2-en-1-ol (*m*/*z* = 88). The spectra further contained lower *m*/*z* fragment ions at a difference of *m*/*z* = 14, definitely supporting the general structure of FAME, viz., $[-CH_3OCO(CH_2)_nCH_3]^+$ in the homologous series. The abundance of fragment ions in SFA and MUFA−FAME was found to be higher than in the EI-MS spectra of PUFAs. The EI-MS spectra of methyl octadeca-9,12-dienoate or methyl linoleate have an abundant molecular ion (*m*/*z* = 294) and base peak as the McLafferty ion (*m*/*z* = 74). In methyl octadeca-9,-12,15-trienoate or methyl α-linolenate (18:3n3), a fragment ion at *m*/*z* = 150 was obvious, which is characteristic for fatty acids with an n3 terminal moiety, while one at *m*/*z* = 108 defines an n3 terminal group as in methyl methyl eicosa-5,8,11,14,17-pentaenoate or methyl eicosapentaenoate and methyl docosa-4,7,10,13,16,19-hexaenoate or methyl docosahexaenoate. In the mass fragmentation pattern of methyl eicosa-5,8,11,14-tetraenoate or methyl arachidonate, the molecular ion is barely distinguishable, but the characteristic ion for the n3 moiety, at *m*/*z* = 108, is apparent. In the mass fragmentation pattern of PUFAs with more double bonds ($n \geq 4$), the tropylium ion (*m*/*z* = 91) does stand out as the diagnostic fragment ion. In the mass fragmentation pattern of *N*-acyl pyrrolidides, the base peak was assigned to be the McLafferty rearrangement ion, 1-(pyrrolidin-1-yl)-ethenol (*m*/*z* = 113), with the concurrent loss of the (M − 113) fragment ion. The molecular ion undergoes further rearrangement to furnish 1-(pyrrolidin-1-yl)-prop-2-en-1-ol (*m*/*z* = 127). In the mass spectrum of 1-(pyrrolidin-1-yl) octadeca-9,12,15-trien-1-one, the double bonds in positions 9 and 12 are located by the gaps of *m*/*z* = 12 units between ions at *m*/*z* = 196, 208 and 236, 248, respectively.

Fatty Acid Composition of Microalgae and Baker's Yeast. The abundance of the fatty acid content of the logarithmic phase *N. salina* was 20:5n3 > 16:1n7 > 16:0 > 18:1n9 > 20:4n6 > 22:6n3, in decreasing order (**Table 1**). Among SFAs, 16:0 was found to be the most dominant contributing 21% of TFAs in *N. salina*. Among n3 fatty acids, EPA was found to be the most abundant in the logarithmic phase of *N. salina* (25.8% TFA) and *C. calcitrans* (18.7% TFA). The high DHA (4.18%) levels in *N. salina* caused it to have a higher DHA:EPA ratio (0.16) than *C. calcitrans* diet (**Table 1**). The highest levels of PUFAs in *N. salina* were influenced by high percentages of EPA and AA, with the EPA/AA ratio of 2.72. The C_{18} fatty acids were present in small amounts in *C. calcitrans* (6.62% TFA), although significant C_{16} PUFAs (like 16:2n4, 22.5% TFA) were apparent. *Chlorella salina* had higher proportions of shorter carbon chain PUFAs (18:2n6, 18:3n3) together with lower amounts of C_{16} fatty acids (**Table 1**). The highest levels of MUFAs were found

Table 1. Fatty Acid Content (% TFA) of the Four Enrichment Diets (Three Microalgae[a] and Baker's Yeast) for *Artemia* Nauplii

	fatty acids (% TFA)			
	C. salina	*C. calcitrans*	N. *salina*	*S. cerevisiae*
		SFAs		
14:0	0.25 c	6.60 a	0.39 c	0.76 b
15:0	ND	0.32 b	3.10 a	0.28 b
16:0	21.49 a	6.90 b	21.0 a	7.09 b
18:0	2.13 a	1.50 b	0.94 b	3.10 a
24:0	ND	ND	ND	0.85
∑SFA	23.87 a	15.32 b	25.43 a	12.08 c
		MUFAs		
14:1n6	ND	7.50	ND	ND
16:1n7	22.18 a	19.20 b	20.10 b	25.58 a
16:1n9	ND	3.10 b	ND	10.46 a
18:1n7	ND	1. 50 a	0.50 b	1.78 a
18:1n9	12.11 b	4.22 c	9.40 c	41.43 a
∑MUFA	34.29 b	34.02 b	30.00 c	79.25 a
		PUFAs		
16:2n4	ND	3.60 a	0.05 b	ND
16:3n4	ND	18.90 a	0.05 b	ND
16:3n6	ND	ND	0.31	ND
18:2n6	17.20 a	0.60 c	2.10 b	4.83 b
18:3n6	ND	0.30 a	ND	0.28 a
18:3n3	21.04 a	ND	0.40 b	0.15 b
18:4n3	ND	ND	0.15 a	0.10 a
18:4n6	ND	ND	0.32	ND
20:4n6	0.92 c	2.80 b	9.50 a	0.08 c
20:5n3	ND	18.70 b	25.80 a	0.25 c
22:5n3	ND	0.19 b	0.35 a	ND
22:6n3	ND	1.80 b	4.18 a	0.05 c
∑PUFA	39.16 b	46.89 a	43.21 a	5.74 c
DHA/EPA		0.10 b	0.16 a	0.20 a
EPA/AA		6.68 a	2.72 b	3.13 b
∑n3:∑n6	1.16 b	1.85 b	2.52 a	0.11 c

[a] The three microalgae are *C. salina*, *C. calcitrans*, and *N. salina* as illustrated in the table. The individual fatty acid is expressed as the percentage of total identifiable fatty acids. Some of the fatty acid could not be identified on the GC trace; the GC-MS analysis suggested the identification. ∑SFA, total SFAs; ∑MUFA, total MUFAs; and ∑PUFA, total PUFAs. Data are the mean values of triplicate samples. Statistical comparison of some major fatty acids (%) was as outlined in the Materials and Methods. Row values with different letters (a–c) are significantly different ($p = 0.05$); ND, not determined.

in *S. cerevisiae* (79.25% TFA), which was mostly contributed by 16:1n7 (25.6% TFA) and 18:1n9 (91.4% TFA). Baker's yeast was found to contain negligible total PUFA contents (5.74% TFA).

Fatty Acid Composition of *A. salina* Enriched with Microalgae and Baker's Yeast for Different Duration. *Artemia* is well-known for its value as one of the major live foods used for the feeding of larvae of decapod crustaceans and finfish (*25*, *26*). Freshly hatched nauplii of *Artemia* in our laboratory contain a fatty acid pattern of herbivorous zooplankton. Among SFAs, 16:0 was found to be the highest (8.07%), whereas 18:1n9 and 16:1n9 recorded higher shares (24.26 and 16.30% TFA, respectively) among MUFAs (**Table 2**). Among PUFAs, high concentrations of 18:2n6 (8.50% TFA), 18:3n3 (4.10% TFA), 20:5n3 (3.18% TFA), and a negligible amount of 22:6n3 (0.1% TFA) were apparent, thus confirming earlier reports as proportions of TFAs (*4*, *26*). **Tables 2** and **3** have given the fatty acid composition of *A. salina* fed with four different diets separately for various enrichment periods (3, 6, 8, and 24 h).

SFAs. There appeared to be no significant correlation between microalgal and artemial SFA contents ($p = 0.05$). The SFA level of *Artemia* fed with *N. salina* for 8 h was found to be lower (12.36% TFA) as compared to those observed in *Artemia* fed *C. calcitrans* (14.94% TFA), *C. salina* (18.6% TFA), and baker's yeast (21.14% TFA) (**Tables 2** and **3**). The content of 18:0 was found to decline by 54.38% in *Artemia* enriched by *N. salina* up to 8 h (**Table 2**). Other SFAs like 16:0 exhibited an increase of 24.66 and 76.58% in *Artemia* fed with *N. salina* and *C. calcitrans* for 24 h. In *Artemia* enriched with *C. salina* and baker's yeast, the corresponding values were recorded to be 100.74 and 24.91%, respectively. The fatty acid 14:0 increased from 0.32% in *Artemia* nauplii to 3.25 and 4.12%, respectively, in *Artemia* enriched with *N. salina* and *C. calcitrans* for 24 h. *Artemia* enriched with baker's yeast recorded the highest value of 14:0 (18.69% TFA) of all of the feeds throughout the enrichment period (24 h).

MUFAs. *Artemia* enriched by *N. salina* for 8 h showed a decrease in 16:1n7 and 16:1n9 by 47.19 and 38.77%, respectively, whereas 18:1n7 and 18:1n9 exhibited an increase of 71.95 and 28.98% after 24 h of enrichment (**Table 2**). *C. calcitrans* followed a similar trend, although the percent increase of MUFA in the enriched *Artemia* was found to be marginally higher (**Table 2**). The total MUFA in *Artemia* enriched by baker's yeast for 8 h was found to be highest after it exhibited a decline to 37.49% after 24 h of enrichment (**Table 3**).

PUFAs. *n3 PUFAs.* The amount of total PUFA in *N. salina* enriched *A. salina* nauplii increased from 31.64% at 3 h to 43.93% at 8 h of enrichment (**Table 2**). No significant differences were apparent in the percentage of 18:3n3, 18:4n3, 16:3n4, and 16:2n4 in *Artemia* nauplii enriched by microalgal and baker's yeast diets for 48 h ($p = 0.05$). The n3 PUFAs were found to be highest in *Artemia* enriched with *N. salina* ranging from 12.38 (for 3 h of enrichment) to 16.75% (for 8 h of enrichment). Among n3 PUFAs, EPA was found to increase from 3.18% in *Artemia* nauplii to 8.05% after 8 h of enrichment with *N. salina*. By 24 h, EPA in *C. calcitrans* fed *Artemia* was reduced by 8.13%. *Artemia* enriched by baker's yeast and *C. salina* exhibited a significant decrease of n3 fatty acids, particularly EPA after 24 h of enrichment (**Table 3**). Except *N. salina*, none of the dietary treatments significantly increased the proportion of DHA in enriched *Artemia* (**Tables 2** and **3**). The DHA/EPA values in *Artemia* enriched for 6 h by *N. salina* and *C. calcitrans* were found to be, respectively, 88.46 and 25% higher than freshly hatched *Artemia*. *Artemia* enriched by *C. salina* and baker's yeast exhibited reduction in PUFA content even at 6 h of enrichment.

n6 PUFAs. The content of AA exhibited enrichment by all diets during enrichment up to 8 h, attaining 14.15% of TFA in *Artemia* enriched by *N. salina* to 2.38% TFA in *Artemia* enriched by baker's yeast. It was accompanied by a decrease of 18:2n6 in the enriched livefood (**Tables 2** and **3**). *Artemia* enriched by *C. calcitrans* exhibited an increase of 20:4n6 and 18:4n6 up to 8 h of enrichment (10.16 and 57.14% TFA, respectively) with the simultaneous reduction of 18:2n6 from 8.50% TFA in newly hatched *Artemia* to as low as 0.26% TFA in 8 h enriched *Artemia* (**Table 2**).

As evident from **Table 2**, the artemial PUFA content was found to be highly correlated with the n6 and n3 PUFAs of *N. salina* and *C. calcitrans* and indicate a negative correlation between 16:1n7, 16:1n9, and SFAs. It is apparent that increasing the time of enrichment by *N. salina* or other microalgae (from 6 to 24 h) did not appreciably increase the content of AA, EPA, or DHA. Enrichment with *C. calcitrans* for 24 h resulted in negligible PUFA content (22.61% TFA) as compared to 8 h of enriched *Artemia* (34.8% TFA).

Other PUFAs and Their Interaction. The acid 16:2n4 was gradually accumulated up to 0.92% TFA in *Artemia* nauplii

Table 2. Percentage Fatty Acid Composition of Freshly Hatched *Artemia* Nauplii and in *Artemia* Enriched with *C. calcitrans* and *N. salina* (Logarithmic Phase) for up to 24 h during This Study[a]

		Artemia enriched with							
	pre-enriched *Artemia*	*N. salina* for				*C. calcitrans* for			
		3 h	6 h	8 h	24 h	3 h	6 h	8 h	24 h
					SFAs				
14:0	0.32	0.45 c	0.68 b	0.68 b	3.25 a	0.46 c	1.69 b	1.78 b	4.12 a
15:0	0.21	0.59 b	0.85 a	1.10 a	1.69 a	0.45 b	0.58 b	0.86 a	1.06 a
16:0	8.07	8.18 b	8.32 b	8.34 b	10.06 a	8.15 b	8.46 b	9.12 b	14.25 a
18:0	4.91	4.98 a	4.09 a	2.24 b	2.30 b	6.18 a	5.57 a	3.18 b	3.12 b
∑SFA	13.51	14.2 b	13.94 b	12.36 b	17.3 a	15.24 b	16.3 b	14.94 b	22.55 a
					MUFAs				
14:1n6	ND	ND	ND	ND	ND	0.51 b	0.69 a	0.68 a	0.52 b
16:1n7	9.62	7.92 a	6.16 a	5.08 b	4.11 b	8.63 a	6.19 a	5.07 b	3.85 c
16:1n9	16.30	14.14 a	12.41 a	9.98 b	8.45 b	15.08 a	14.76 a	9.59 b	7.28 b
18:1n7	2.21	2.69 b	3.35 a	3.65 a	3.80 a	2.72 b	2.98 b	3.28 a	3.93 a
18:1n9	24.26	25.23 b	25.52 b	25.72 b	31.29 a	25.15 b	26.09 b	26.11 b	34.67 b
∑MUFA	52.39	49.98 a	47.44 b	44.43 c	47.65 b	52.09 a	50.71 b	52.39 a	49.98 b
					PUFAs				
16:2n4	ND	0.91 a	0.40 c	0.82 b	0.92 b	1.10 b	1.28 b	1.82 a	0.95 b
16:3n4	0.41	0.92 a	0.98 a	0.88 a	0.56 c	1.29 a	0.98 a	0.77 b	0.50 c
16:3n6	8.08	8.66 b	9.78 a	9.82 a	5.41 c	8.29 b	8.97 b	9.58 a	5.14 c
18:2n6	8.50	2.20 b	1.41 b	0.38 c	0.25 a	1.97 a	1.22 a	0.26 b	0.09 c
18:3n6	0.32	0.62 a	0.59 a	0.67 a	0.75 a	0.45 b	0.48 b	0.52 a	0.66 a
18:3n3	4.10	4.28 b	5.14 a	5.19 a	5.26 a	4.18 b	4.86 b	5.18 a	4.72 a
18:4n3	0.85	1.28 b	1.29 b	1.65 a	1.51 a	0.97 b	1.10 a	1.16 a	0.89 b
18:4n6	0.18	0.39 b	0.39 b	0.46 a	0.38 b	0.35 a	0.38 a	0.42 a	0.30 b
20:4n6	2.30	5.56 b	5.67 b	14.15 a	13.0 a	4.39 b	4.48 b	8.56 a	1.18 c
20:5n3	3.18	5.20 b	7.32 a	8.05 a	4.28 b	4.10 c	5.42 b	6.44 b	8.13 a
22:5n3	0.05	0.02 b	0.01 b	0.01 b	0.01 b	0.02 a	0.01 a	ND	ND
22:6n3	0.10	1.60 a	1.89 a	1.85 a	0.45 b	0.15 a	0.19 a	0.09 b	0.05 b
∑PUFA	28.07	31.64 b	34.87 b	43.93 a	32.78 b	27.26 b	29.37 b	34.8 a	22.61 c
DHA/EPA	0.03	0.31 a	0.26 a	0.23 a	0.11 b	0.04 a	0.04 a	0.01 b	0.01 b
EPA/AA	1.38	0.94 b	1.29 a	0.57 b	0.33 c	0.93 c	1.21 b	0.75 c	6.89 a
∑n3:∑n6	0.43	0.71 b	0.88 a	0.66 b	0.58 c	0.59 c	0.71 b	0.64 c	1.75 a

[a] The individual fatty acid is expressed as the percentage of total identifiable fatty acids. Some of the fatty acid could not be identified on the GC trace; the GC-MS analysis suggested the identification. ∑SFA, total SFAs; ∑MUFA, total MUFAs; and ∑PUFA, total PUFAs. Other minor and branched fatty acids included are as follows: 12:0, iso14:0, C14 PUFA, 14:1, br15:0, iso15:0, iso16:0, 16:1n5, 16:1n7t/16:2n7, 18:1n5, 18:2n4, 18:3n4, 18:3n6, iso18:0, 20:0, 21:4n6 and 21:5n3, 22:1n9, and 22:1n7, which are not listed in the table but contribute to the TFA content. Data are presented as mean values of three samples (each treatment). Different letters (a–c) within rows indicate significant differences between treatments (ANOVA, $p = 0.05$). ND, fatty acid identified on the GC trace but not integrated by the instrument.

enriched by *N. salina* for 24 h (**Table 2**). The percentage sum of PUFA was elevated over the enrichment period, reaching its highest after 8 h (43.93% TFA) in *Artemia* enriched by *N. salina*, which was at the expense of MUFA and SFA, and the composition continued to decline following 24 h. The total n3:n6 ratio of *N. salina* and *C. calcitrans* enriched *Artemia* was significantly higher up to 8 h of enrichment as compared to that in newly hatched *Artemia* (**Table 2**).

In *Artemia* enriched with *N. salina* for 8 h, the percentage composition of SFAs (14:0, 16:0, and 18:0) was found to be lower (11.6% TFA), whereas the TAG extract was found to contain comparatively higher percentage of SFAs (38.14% TFA) (**Table 4**). *Artemia* enriched with *N. salina* and *C. calcitrans* for 8 h exhibited an overall increase of unsaturation and chain length in the TFA content; the ratio between unsaturated/SFAs was 1.46 and 1.68, respectively, in terms of TFAs (**Table 4**).

DISCUSSION

Mass spectrometry of FAME with EI ionization provides meager information related to the structure of fatty acids, particularly for PUFAs. The McLafferty ion was found to be less conspicuous in EI-MS of FAME PUFAs having more double bonds ($n > 4$), which undergoes complex rearrangement under high-energy EI conditions posing difficulty to determine the location of the double bond. The reason for this is the migration of the double bond along the long aliphatic chain due to destabilization of the positive charge by E.I. in the mass spectrometer. The drawback of double bond migration was overcome by use of pyrrolidine derivatives (*N*-acyl pyrrolidides), which minimize the migration as they are able to stabilize the charge on the heterocyclic pyrrolidine moiety. A uniform distribution of fragment peaks is apparent for higher molecular weight PUFAs at every $m/z = 14$ units, except in the vicinity of the double bond, where the interval is $m/z = 12$ units.

Artemia nauplii are reported to be the most popular feed for larval cultures of finfish and crustaceans, which prefer soft-bodied prey items to meet their feed intake requirements. However, *Artemia* nauplii are an incomplete diet because the essential PUFAs, particularly EPA and DHA, which are regarded as essential n3 PUFAs for many marine larvae, are much less (*27*, *28*). Moreover, in marine larvae, the desaturase and elongase activities necessary to synthesize the long chain PUFAs from shorter carbon chain precursors are very low, so all of the essential PUFAs have to be supplied in the diet (*29*−*31*). Research on enriching *Artemia* nauplii in increasing the amounts of PUFAs, viz., n3 PUFAs, viz., EPA, 22:5n3 (DPA), and DHA; n6 PUFA, viz., AA, prior to their use as live prey for larvae has received considerable attention to increase larval survival rates (*7*). Several commercial PUFA enrichment formulations are used prior to feeding to phyllosomata. However, because of the smaller shelf life of the widely available commercial PUFA formulations, recently, there is a growing

Table 3. Percentage Fatty Acid Composition of Freshly Hatched *Artemia* Nauplii and in *Artemia* Enriched with *C. salina* and *S. cerevisiae* for up to 24 h during This Study[a]

		Artemia enriched with							
	pre-enriched *Artemia*	*C. salina* for				*S. cerevisiae* for			
		3 h	6 h	8 h	24 h	3 h	6 h	8 h	24 h
					SFAs				
14:0	0.32	0.39 c	1.10 b	2.15 b	4.26 a	1.17 c	1.29 c	3.18 b	18.69 a
15:0	0.21	0.25 c	0.30 c	1.18 b	2.69 a	1.26 a	0.30 b	1.27 a	1.77 a
16:0	8.07	9.15 c	10.29 b	10.35 b	16.20 b	8.65 b	9.09 b	9.12 b	10.08 a
18:0	4.91	5.15 a	5.25 a	4.92 b	4.76 b	6.32 b	6.19 b	6.47 b	9.23 a
24:0	ND	ND	ND	ND	ND	0.95 b	6.95 a	1.10 b	0.28 c
∑SFA	13.51	14.94 c	16.94 c	18.6 b	27.91 a	18.35 c	23.82 b	21.14 b	40.05 a
					MUFAs				
16:1n7	9.62	9.11 a	7.82 b	7.15 b	5.26 c	7.54 a	7.24 a	6.60 b	6.11 b
16:1n9	16.30	13.44 a	9.19 b	8.08 c	7.93 c	12.77 a	11.63 a	10.45 b	9.62 b
18:1n7	2.21	2.60 c	2.68 c	3.17 b	3.59 a	3.11 b	3.49 b	4.22 a	3.59 b
18:1n9	24.26	26.48 c	28.30 b	29.51 b	36.47 a	28.37 b	30.85 b	34.19 a	18.17 c
∑MUFA	52.39	51.63 a	47.99 b	47.91 b	53.25 a	51.79 a	53.21 a	55.46 a	37.49 b
					PUFAs				
16:3n4	0.41	1.42 a	1.27 a	0.92 b	0.83 b	0.18 a	0.17 a	0.10 b	0.06 c
16:3n6	8.08	8.30 b	9.11 a	9.38 a	3.72 b	8.19 b	8.78 a	9.08 a	7.82 b
18:2n6	8.50	7.14 a	5.28 b	6.69 a	2.76 c	6.61 a	1.08 b	0.07 c	ND
18:3n6	0.32	0.46 b	0.52 b	0.54 b	0.70 a	0.41 b	0.47 a	0.48 a	0.52 a
18:3n3	4.10	6.18 b	6.59 a	6.80 a	3.41 c	6.85 b	7.29 a	4.75 c	4.60 c
18:4n3	0.85	0.72 a	0.67 a	0.54 b	0.48 b	0.76 a	0.68 a	0.55 a	0.39 b
18:4n6	0.18	0.11 a	0.06 b	0.03 b	ND	0.21 a	0.17 b	0.12 c	0.12 c
20:4n6	2.30	2.34 a	2.39 a	2.40 a	0.85 b	2.32 b	2.38 a	2.38 a	2.15 c
20:5n3	3.18	3.12 a	4.17 a	1.69 b	0.92 c	3.25 b	3.26 b	3.47 a	1.28 c
22:5n3	0.05	0.01	ND	ND	ND	0.01	ND	ND	ND
22:6n3	0.10	0.09 a	0.05 b	0.01 c	ND	0.02 b	0.01 a	ND	ND
∑PUFA	28.07	29.89 a	30.11 a	29.00 a	13.67 b	28.81 a	24.29 b	21.00 b	16.94 c
DHA/EPA	0.03	0.03 a	0.01 b	0.01 b	0.00	0.01	0.00	0.00	0.00
EPA/AA	1.38	1.33 b	1.74 a	0.70 a	1.08 c	1.40 a	1.37 b	1.46 a	0.60 c
∑n3:∑n6	0.43	0.55 b	0.66 a	0.47 b	0.60 a	0.61 c	0.87 a	0.72 b	0.59 c

[a] The individual fatty acid is expressed as the percentage of total identifiable fatty acids. Some of the fatty acid could not be identified on the GC trace; the GC-MS analysis suggested the identification. ∑SFA, total SFAs; ∑MUFA, total MUFAs; and ∑PUFA, total PUFAs. Other minor and branched fatty acids included are as follows: 12:0, iso14:0, C14 PUFA, 14:1, br15:0, iso15:0, iso16:0, 16:1n5, 16:1n7t/16:2n7, 18:1n5, 18:2n4, 18:3n4, 18:3n6, iso18:0, 20:0, 21:4n6 and 21:5n3, 22:1n9, and 22:1n7, which are not listed in the table but contribute to the TFA content. Data are presented as mean values of three samples (each treatment). Different letters (a–c) within rows indicate significant differences between treatments (ANOVA, $p = 0.05$). ND, fatty acid identified on the GC trace but not integrated by the instrument.

interest in marine microalgae that produce PUFAs (*13*). Several microalgae are reported to contain considerably high contents of PUFAs and are major contributors to the marine food web as a renewable source (*14*). Microalgae may act as the feed ingredient for *Artemia*, to be used as prey item for aquacultural larvae. Earlier, it was reported that microalgae belonging to prymnesiophytes (e.g., *Pavlova* sp. and *Isochrysis* sp.) and cryptomonads are relatively rich in DHA (0.2−11% TFA), whereas eustigmatophytes (*Nannochloropsis* spp.) and diatoms (*Chaetoceros* spp.) have higher percentages of EPA and AA (*19*, *21*, *32*). Therefore, three different live microalgal enrichment diets (*N. salina*, *C. calcitrans*, and *C. salina*) were used in this study to improve the essential fatty acid composition of *Artemia* and to compare their efficacies with *S. cerevisiae*.

Among all dietary sources, *N. salina* produced higher levels of AA (9.50%), EPA (25.80%), and DHA (4.18%) followed by *C. calcitrans*. The C_{18} fatty acids were present in small amounts in *N. salina* and *C. calcitrans* but contain significant amounts of C_{16} MUFAs particularly 16:1n7, which serve as precursors in the biosynthesis of C_{18} PUFAs. It has been reported that chlorophytes (*Dunaliella* spp. and *Chlorella* spp.) are deficient in C_{20} and C_{22} PUFAs, although some species have small amounts of EPA (up to 3.2% TFA) (*17*, *33*). *C. salina* recorded higher proportions of shorter carbon chain PUFAs and SFAs. The highest levels of MUFAs contributed mainly by 16:1n7 and 18:1n9 were apparent in baker's yeast.

The study of diet-induced changes in the fatty acid composition of enriched *Artemia* for various durations (3, 6, 8, and 24 h) revealed that *N. salina* diet yielded the best performance followed by *C. calcitrans* during the whole enrichment period. The fatty acid composition of enriched *A. salina* varied and is a function of dietary treatment and enrichment time.

The percentage composition of SFAs was found to be independent of their amount in the diets indicating that *Artemia* has the ability to synthesize SFAs de novo, which was supported by earlier experiments (*1*, *5*). *Artemia* enriched by the diets exhibited a decrease of 16:1n7 and 16:1n9, with a corresponding increase of 18:1n7 and 18:1n9. The acid 16:1n7 in *Artemia* lipids reflects a dietary input, which was supported by earlier experiments (*1*, *4*, *5*, *26*). The maintenance of a low level of 16:1n7 in enriched *Artemia* is possible due to the bioconversion and elongation of 16:1n7 from diet. A decrease of the SFA 18:0 and an increase of 18:1n9 over the entire enrichment period suggest the desaturation of 18:0 to 18:1n9 by Δ9-desaturase.

N. salina yielded *Artemia* nauplii with considerable EPA (8.05%), AA (14.15%), and DHA (1.85%) after 8 h of enrichment, which are significantly higher than in nauplii fed with the other three diets ($p = 0.05$). Increasing the time of enrichment by *N. salina* or other microalgae (from 6 to 24 h) did not appreciably increase the content of AA, EPA, or DHA. The total PUFA content of the enriched *Artemia* by *N. salina* increased by 56.50% with enrichment periods up to 8 h,

Table 4. Percentage Fatty Acid Composition (in Total Extract and TAG Extract) of *Artemia* Nauplii Enriched with Microalgae and Baker's Yeast for 8 h during This Study[a]

	Artemia enriched with			
fatty acids	*N. salina*	*C. muelleri*	*C. salina*	*S. cerevisiae*
	fatty acid (total extract)			
14:0	0.68	1.78	2.15	3.18
16:0	8.34	9.12	10.35	9.12
18:0	2.24	3.18	4.92	6.47
16:1	15.06	14.67	15.23	17.05
18:1	29.37	29.39	32.68	38.41
18:2	0.38	9.58	6.69	0.07
18:3	5.86	0.78	7.34	5.23
20:4	14.15	8.56	2.40	2.38
20:5	8.05	6.44	1.69	3.47
22:6	1.85	0.09	0.01	ND
DHA/EPA	0.23	0.01	0.01	ND
unsat/sat FA	6.64	4.94	3.79	ND
	fatty acid (TAG extract)			
14:0	1.39	3.49	4.49	6.45
16:0	35.18	31.73	29.11	26.47
18:0	1.57	1.15	2.46	2.19
16:1	23.63	28.54	29.67	35.67
18:1	20.05	23.77	25.18	23.23
18:2	ND	ND	1.17	ND
18:3	1.29	2.11	0.82	0.27
20:4	1.89	5.62	1.92	0.15
20:5	8.67	0.98	0.85	0.24
22:6	0.12	ND	ND	ND
DHA/EPA	1.10	0.01	ND	ND
unsat/sat FA	1.46	1.68	1.65	1.70

[a] The individual fatty acid is expressed as the percentage of total identifiable fatty acids. Some of the fatty acids could not be identified on the GC trace; the GC-MS analysis suggested the identification. Data are presented as mean values of three samples. ND, fatty acid identified on the GC trace but not integrated by the instrument.

followed by a significant reduction in the final 24 h. A short enrichment of 6−8 h appeared to improve the efficiency in the nutritional improvement, and high *Artemia* mortalities may be remedied. At the end of enrichment period (24 h), EPA in algal-fed *Artemia* recorded a reduction possibly due to its formation occurring at an insufficient rate because of the limited capacity for the desaturation (by Δ5-fatty acid desaturase) and elongation of 18:3n3 or 18:4n3 to provide an adequate amount of essential fatty acids like EPA and DHA. A 3−5-fold increase in EPA/AA values was observed between *Artemia* in different enrichment periods irrespective of the algal diet, indicating selective EPA incorporation in *Artemia*. It is apparent that *Artemia* nauplii supplemented with PUFA from the diets preferentially catabolize DHA relative to EPA. Thus, the final ratio of DHA:EPA was found to be substantially less than the starting feed. So, it can be concluded that diets with a high ratio of DHA:EPA should be used in live feed enrichment protocols. Relative excess of EPA over DHA can be harmful in larval feeds. It was reported that EPA competitively inhibits the production of eicosanoids from AA (20:4n6) (*29*, *34*). However, the decline in DHA appears to occur at a slower rate in enriched *Artemia* by *N. salina*. It is significant that EPA to DHA conversion should take place at higher rates to meet the high demands of larval growth. It was reported that conversion of 20:5n3 to 22:6n3 is not by direct Δ-4 desaturation but by a complex pathway where 20:5n3 is chain elongated to 22:5n3 and then converted to 24:5n3, which, in turn, is converted to 24:6n3 by Δ-6 desaturase, and 24:6n3 is chain shortened to DHA. Another mechanism is by conversion from 18:2n3 to 18:4n3 by Δ-6 desaturase, and interestingly, 18:4n3 and 24:6n3 are substrates for the same enzyme. Because *Artemia* contains substantial amounts of 18:3n3, it probably competitively inhibits 20:5n3 to 22:6n3 conversion (*35*). It is apparent that to maintain a sufficient level of PUFAs in the tissue lipids, *Artemia* has to obtain n3 PUFAs directly from their diet. Because of the direct correlation between n3 PUFA with larval growth and the known limited ability of many marine larvae to synthesize several n3 essential fatty acids, they must be supplied in the diet (*29*). These observations further indicate that when *Artemia* was cultured with microalgal diets like *N. salina* and *C. calcitrans*, they were able to produce their own fatty acid content to a satisfactory level by utilizing excessive amounts of these algae as compared to other diets of relatively low PUFA content, viz., *C. salina* and baker's yeast. *Artemia* do not store DHA at similar proportions to other PUFAs and exhibit a reduction in their composition with higher enrichment time (24 h) (*5*). The inability to synthesize DHA from 18:3n3 at a rate fast enough to meet demand indicates a low level of A5-desaturase activity in *Artemia* nauplii, which must be coupled with an inability to convert 18:2n6 to 20:4n6, since this pathway requires the same enzyme (*24*). *Artemia* can biosynthesize neither 22:6n3 de novo nor from shorter chain precursors such as 18:3n3; therefore, 22:6n3 and 20:5n3 are the essential dietary constituents (*35*). Also, there is a possibility to retroconvert 22:6n3 to 20:5n3 in brine shrimp nauplii. DHA is present in very high concentrations in neural and visual cell membranes and synaptosomal membranes in marine animals. An insufficiency of 22:6n3 in marine larval fish diet is likely to impair neural and visual development with significance for a whole range of physiological and behavioral processes including those dependent on neuroendocrines.

No significant differences were apparent in the percentage of 18:3n3, 18:4n3, 16:3n4, and 16:2n4 in *Artemia* nauplii enriched by microalgal and baker's yeast diets for 48 h ($p = 0.05$). It can be concluded that their concentration range was maintained at a constant level either through input from the diet or through biosynthetic processes. Most of the n3 family of fatty acids appeared to be originated from diet, since the abundance of these components in the diet led to their increase in *Artemia* nauplii.

The high nutritional value of *N. salina* diet for *Artemia* nauplii has been related to high levels of AA (20:4n6) from *N. salina*. The content of AA exhibited enrichment by all diets during enrichment up to 8 h, attaining 14.15% of TFA in *Artemia* enriched by *N. salina* to as low as 2.38% TFA in *Artemia* enriched by baker's yeast. It was accompanied by a decrease of 18:2n6 in *Artemia* nauplii. This observation suggests a bioconversion capacity of 20:4n6 from 18:2n6 (*35*). Although *Artemia* are capable of selective accumulation of 20:4n6 to a limited extent, the levels of the same in *Artemia* mirrored that in the diet. Therefore, if DHA is an essential fatty acid in *Artemia*, it follows that AA should be also and needs to be provided in the diet. AA has been an essential function to produce COX enzyme to be used for synthesizing $PGF2_{\alpha}$, which were reported to have a vital role in synchronizing ovulation and spawning in finfish and crustaceans (*31*). Many of those from AA are proinflammatory, which serves a purpose in immune reactions to bacteria, oxidation, or injury to tissue. So, an increment in 20:4n6 during enrichment might reflect an increasing demand for AA-rich immune cells to fight surface-associated bacteria. Detailed studies examining the appropriate ratios of fatty acids, mainly 22:6n3, 20:5n3, and 20:4n6, have revealed that given a sufficiency of 22:6n3, excess of 20:5n3 is not deleterious, whereas 20:4n6 is, because of a generalized biochemically induced stress through excess eicosanoid produc-

tion. The metabolic role of AA as the main PUFA in phosphoinositol, a key molecule in transduction signal mechanism, was established (*36*). It has been established that EPA modulates eicosanoid production from AA, and failure to supply these two fatty acids in the appropriate balance may result in adverse biochemical responses. Large larval AA contents produced from the most effective diet for larval nauplii are a nutritional advantage for the larvae of aquacultural importance that ultimately consume *Artemia* feeding on microalgae. An AA dietary deficiency could act as a limiting factor restricting overall fatty acid incorporation by *Artemia* nauplii.

Artemia enriched with *N. salina* is a possibility ensuring the appropriate PUFA ratio delivery to larval marine fishes. Even though n3 PUFAs can be catabolized for energy, they are more difficult to catabolize than SFAs or MUFAs. Thus, overenrichment with PUFA could conceivably result in insufficient energy contents in the diet. However, maintenance of the levels of DHA:EPA:AA in *Artemia* till the larval fish/shellfish feeds on it has not been successful because all of these fatty acids, especially DHA, are metabolized by *Artemia* after bioencapsulation leading to a lowering of its content in the enriched organism.

The present study has very important ramifications for the use of the commercial culture of *Artemia* nauplii, as the sourcing of cheap microalgae could dramatically improve nutritional quality of *Artemia*, as compared with importing and using more expensive conventional enrichment techniques. Our analyses of diet alternatives for livefeed *Artemia* culture have basically shown reason why most of these alternatives have never been put into common commercial reality. The future direction of PUFA nutrition in mariculture is to blend the range of diets available to us to achieve economical larval survival. Microalgae have an important role in aquaculture as a means of enriching zooplankton for on-feeding to fish and other larvae and are relatively superior in terms of nutritional quality as compared with the livefood being fortified with PUFA-rich commercial oil emulsions (e.g., DHA Selco from INVE). This may be considered as an advantage of *A. salina* because it may be cultured with various microalgae locally available that have high PUFA contents and this offers opportunities for dietary manipulation to meet the needs of *Artemia* nauplii. PUFAs in live organisms like microalgae are much more stable than in emulsions like DHA Selco, which is an added advantage. Moreover, the commercial PUFA formulations are more susceptible to autoxidation because they are directly exposed to oxidizing agents like molecular oxygen (O_2), which metabolizes the olefinic double bonds in long chain PUFAs to yield undesirable hydroperoxides and peroxides. Also, there is rapid deterioration of water quality due to disintegration of micropellets of commercial PUFA formulations, which are usually fed in excess in order to achieve satisfactory growth and survival resulting in high mortality rates due to malnutrition and/or incomplete digestion of the diet components. Microalgae contain an array of essential nutrients that may be transferred through food chains, especially PUFAs. Recently, "algal-like" products such as AlgaMac 2000 and Docosa Gold (dried preparations of the thraustochytrid *Schizochytrium* sp.) have been utilized as larval and broodstock diets. These have produced similar levels of PUFA enrichment within the zooplankton as compared to the commercial oils, which are considered favorable for fish larval nutrition (*37*). Apart from improvements in the cost efficiencies of on-site algal production, an alternative is the centralization of algal production at specialized mass culture facilities, using heterotrophic methods or photobioreactors to produce cheaper algal biomasses. These technologies could be married with postharvest processing such as spray drying or algal concentration to develop off-the-shelf algal biomasses for distribution to hatcheries. Microalgal species can vary significantly in their nutritional value, and this may also change under different culture conditions (*38*). They are amenable to mass culturing and biomass scale-up through photobioreactor and/or fermentation technology, and the production of these compounds can be optimized by manipulating the culture conditions (*32*, *33*). A selected mixture of microalgae can offer an excellent nutritional package, either directly or indirectly through enrichment of PUFAs derived from microalgae, which are known to be essential for various larvae. More research is required for the development of higher shelf life concentrates of popular microalgae such as *Nannochloropsis* sp. and *Chaetoceros* sp. as diets of livefood *Artemia* and other zooplanktons.

ACKNOWLEDGMENT

We are thankful to the Director, CMFRI, Cochin, for providing necessary facilities to carry out the work. Thanks are also due to Dr. Shibu M. Eappen, Scientist B, Sophisticated Test and Instrumentation Centre, Cochin University of Science and Technology, Kerala, for recording the mass spectral data.

LITERATURE CITED

(1) Navarro, J. C.; Amat, F.; Sargent J. R. Fatty acid composition of coastal and inland *Artemia* sp. populations from Spain. *Aquaculture* **1992**, *102*, 219−230.

(2) Narciso, L.; Pousao-Ferreira, P.; Passos A.; Luis, O. HUFA content and DHA/EPA improvements of *Artemia* sp. with commercial oils during different enrichments periods. *Aquacult. Res.* **1999**, *30*, 21−24.

(3) Tong, L. J.; Moss, G. A.; Paewai, M.; Pickering, T. D. Effect of brine shrimp numbers on the growth and survival of early stage phyllosoma larvae of the rock lobster *Jasus edwardsii*. *Mar. Freshwater Res.* **1997**, *48*, 935− 940.

(4) Sargent, J. R.; McEvoy, L. A.; Bell, J. G. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* **1997**, *155*, 127−177.

(5) Navarro, J. C.; Henderson, R. J.; McEvoy, L. A.; Bell, M. V.; Amat, F. Lipid conversions during enrichment of *Artemia*. *Aquaculture* **1999**, *174*, 155− 166.

(6) Wantanabe, T. Importance of docosahexaenoic acid in marine larval fish. *J. World Aquacult. Soc.* **1993**, *24*, 152−161.

(7) Watanabe, T.; Kiron, V. Prospects in larval fish dietetics. *Aquaculture* **1994**, *124*, 223−251.

(8) Sargent, J.; McEvoy, L.; Estevez, A.; Bell, J. G.; Bell, M.; Henderson, J.; Tocher, D. Lipid nutrition of marine fish during early development: Current status and future directions. *Aquaculture* **1999**, *179*, 217−229.

(9) Cho, S. H.; Hur, S. B.; Jo, J. Y. Effect of enriched live feeds on survival and growth rates in larval Korean rockfish, *Sebastes schlegeli* Hilgendorf. *Aquacult. Res.* **2001**, *32*, 199− 208.

(10) Deering, M. J.; Fielder, D. R.; Hewit, D. R. Growth and fatty acid composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. *Aquaculture* **1997**, *151*, 131−141.

(11) Brown, M. F. Modulation of rhodopsin function by properties of the membrane bilayer. *Chem. Phys. Lipids* **1994**, *73*, 159− 180.

(12) Coutteau, P.; Castell, J. D.; Ackman, R. G.; Sorgeloos, P. The use of lipid emulsions as carriers for essential fatty acids in bivalves: A test case with juvenile *Placopecten magellanicus*. *J. Shellfish Res.* **1996**, *15*, 259−264.

(13) Refsgaard, H. H. F.; Brockhoff, P. B.; Jensen, B. Biological variation of lipid constituents and distribution of tocopherols and

astaxanthin in farmed Atlantic salmon (*Salmo salar*). *J. Agric. Food Chem.* **1998**, *46*, 808−812.
(14) Hartvigsen, K.; Lund, P.; Hansen, L. F.; Holmer, G. Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. *J. Agric. Food Chem.* **2000**, *48*, 4858−4867.
(15) Refsgaard, H. H. F.; Brockhoff, P. M. B.; Jensen, B. Free polyunsaturated fatty acids cause taste deterioration of salmon during frozen storage. *J. Agric. Food Chem.* **2000**, *48*, 3280−3285.
(16) Vazhappilly, R.; Chen, F. Heterotrophic production potential of omega-3 polyunsaturated fatty acids by microalgae and algae-like microorganisms. *Bot. Mar.* **1998**, *41*, 553−558.
(17) Volkman, J. K.; Jeffrey, S. W.; Nichols, P. D.; Rogers, G. I.; Garland, C. D. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **1989**, *128*, 219−240.
(18) Lubzens, E.; Gibson, O.; Zmora, O.; Sukenik, A. Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. *Aquaculture* **1995**, *133*, 295−309.
(19) Lavens, P.; Sorgeloos, P. Production of *Artemia* in culture tanks. In *Artemia Biology*; Browne, R. A., Sorgeloos, P., Trotman, C. N. A., Eds.; CRC Press: Boca Raton, FL, 1991; pp 317−350.
(20) Lavens, P.; Sorgeloos, P. The history, present status and prospect of the availability of the *Artemia* cysts for aquaculture. *Aquaculture* **2000**, *181*, 397.
(21) Renaud, S. M.; Parry, D. L.; Thinh, L. V. Microalgae for use in tropical aquaculture. 1. Gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. *J. Appl. Phycol.* **1994**, *6*, 337−345.
(22) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem.* **1959**, *37*, 911−917.
(23) Morrison, W. R.; Smith, L. M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **1964**, *5*, 600−608.
(24) Andersson, B. A. Mass spectrometry of fatty acid pyrrolidides. *Prog. Chem. Fats Other Lipids* **1978**, *16*, 279−308.
(25) Gara, B.; Shields, R. J.; McEvoy, L. Feeding strategies to achieve correct metamorphosis of Atlantic halibut, *Hippoglossus hippoglossus* L., using enriched *Artemia*. *Aquacult. Res.* **1998**, *29*, 935−948.
(26) Sorgeloos, P.; Coutteau, P.; Dhert, P.; Merchie, G.; Lavens, P. Use of brine shrimp, *Artemia* spp. *in* larval crustacean nutrition: A review. *Rev. Fish. Sci.* **1998**, *6* (1−2), 55−68.
(27) Castell, J. D. Fatty acid metabolism in crustaceans. In *Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition*; Pruder, G. D., Langdon, C. J., Conklin, D. E., Eds.; Louisiana State University: Baton Rouge, Louisiana, 1982; pp 124−145.
(28) Kanazawa, A. Fatty acid metabolism in crustaceans. In *Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition*; Pruder, G. D., Langdon, C. J., Conklin, D. E., Eds.; Louisiana State University: Baton Rouge, Louisiana, 1982; pp 87−105.
(29) Mourente, G.; Tocher, D. R. *In vivo* metabolism of [1-14C]-linolenic acid (18:3n-3) and [1-14C] eicosapentaenoic acid (20:5n3) in a marine fish: Time-course of the desaturation/elongation pathway. *Biochim. Biophys. Acta* **1994**, *1212*, 109−118.
(30) Tocher, D. R.; Ghioni, C. Fatty acid metabolism in marine fish: low activity of fatty acyl D5-desaturation in gilthead seabream (*Sparus aurata*) cells. *Lipids* **1999**, *34*, 433−440.
(31) Bell, J. G.; Sargent, J. R. Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture* **2003**, *218*, 491−499.
(32) Nichols, P. D.; Holdsworth, D. G.; Volkman, J. K.; Daintith, M.; Allanson, S. High incorporation of essential fatty acids by the rotifer *Brachionus plicatilis* fed on the prymnesiophyte alga *Pavlova lutheri*. *Aust. J. Mar. Freshwater Res.* **1989**, *40*, 645−655.
(33) Martínez-Fernández, E.; Acosta-Salmón, H.; Southgate, P. C. The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera* L.) larvae. *Aquaculture* **2006**, *257*, 491−503.
(34) Van Anholt, R. D.; Spanings, F. A. T.; Koven, W. M.; Nixon, O.; Wendelaar, S. E. Arachidonic acid reduces the stress response of gilthead seabream *Sparus aurata* L. *J. Exp. Biol.* **2004**, *207*, 3419−3430.
(35) Kanazawa, A.; Teshima, S.; Ono, K. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **1979**, *63*, 295−298.
(36) Smith, M. L.; Murphy, R. C. The eicosanoids: Cyclooxygenase, lipoxygenase and epoxygenase pathways. In *Biochemistry of Lipids, Lipoproteins and Membranes*; Vance, D. E., Vance, J., Eds.; Elsevier: Amsterdam, 2002; pp 341−371.
(37) Rodríguez, C.; Pérez, J. A.; Badía, P.; Izquierdo, M. S.; Fernández-Palacios, H.; Lorenzo Hernández, A. The n-3 highly unsaturated fatty acid requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet. *Aquaculture* **1998**, *169*, 9−23.
(38) Brown, M. R.; Skabo, S.; Wilkinson, B. The enrichment and retention of ascorbic acid in rotifers fed microalgal diets. *Aquacult. Nutr.* **1998**, *4*, 151−156.

Received for review December 16, 2006. Revised manuscript received March 2, 2007. Accepted March 5, 2007.

JF063654L