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LIPID COMPOSITION OF THREE GEOGRAPHICAL SOURCES OF BRINE SHRIMP NAUPLII (*ARTEMIA* sp.)*

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Abstract—1. Percentages of triacylglycerols (TG), free fatty acids (FFA) and phospholipids (PL) in the total lipids, the fatty acid composition of each of these lipid classes, and the percentage of cholesterol were determined by gas chromatography in three geographical sources (San Francisco Bay, SFB; Chinese, CH; Colombian, COL) of brine shrimp (*Artemia* sp.) nauplii.

2. There were no significant differences among sources of brine shrimp in total lipids, TG or FFA with means for all sources of 17.8, 65.8 and 10.9%, respectively. Percentage of phospholipid was significantly higher in SFB and CH sources of brine shrimp, 25.1 and 26.5%, respectively, than in COL 18.3%.

3. Marked differences in percentages of 18:3 (*n*-3) (linolenic acid) and 20:5 (*n*-3) (eicosapentaenoic acid or EPA) were found among brine shrimp sources, and concentration of these two fatty acids were usually inversely related within sources. The CH source contained higher concentrations of EPA (>9.0%) than the COL and SFB sources (<5.0%) in all three lipid classes analyzed. No 22:6 (*n*-3) (docosahexaenoic acid or DHA) was found in any brine shrimp source.

4. Fatty acid compositions of the TG and PL were similar and did not differ among sources of brine shrimp, while the FFA had a lower percentage of polyunsaturated fatty acids, but was similar among sources of brine shrimp.

5. Differences in *n*-3 fatty acid composition indicate a difference in nutritional quality among sources of brine shrimp for feeding larval fish.

INTRODUCTION

Brine shrimp (*Artemia* sp.) nauplii are the major food source for the larvae of many cultured fishes. Variation in nutritional quality among various geographic sources of brine shrimp has been established (Wickens, 1972; Watanabe *et al.*, 1980; Seidel *et al.*, 1982; Webster and Lovell, 1990) and is suspected to be related to differences in the lipid fraction of the nauplii (Owen *et al.*, 1975; Watanabe *et al.*, 1980; Kanazawa *et al.*, 1982; Webster and Lovell, 1990). Flutcher (1982) found that the addition of a lipid soluble extract from brine shrimp to a prepared larval diet improved growth and survival of larvae of whitefish (*Coregonus lavaretus*). Watanabe *et al.* (1980) using juvenile red seabream (*Pagrus major*), and Webster and Lovell (1990) using striped bass (*Morone saxitalis*) larvae, found that growth and survival were improved when brine shrimp with a high percentage of *n*-3 highly unsaturated fatty acids (HUFA) were fed compared to brine shrimp low in *n*-3 HUFA. Other than fatty acid composition, there is limited information on comparative lipid composition among various sources of brine shrimp. The purpose of this study was to determine and compare the lipid fractions, triacylglycerols (TG), free fatty acids (FFA), phospholipids (PL), and sterols, from three geographical sources of brine shrimp which have been found to be different in nutritional value.

MATERIALS AND METHODS

Brine shrimp nauplii

Brine shrimp cysts (eggs) from three geographical sources were obtained from commercial suppliers: Chinese (Can # 686532-36; Biomarine, Inc., Hawthorne, CA), Colombian (Lot 120; Aquarium Products, Glen Burnie, MD) and San Francisco Bay (Lot 1106; San Francisco Bay Brands, Inc., Newark, CA). The cysts were hatched in water with salt (NaCl) added to give a specific gravity of 1.02, in 56 l conical hatching containers. Water was continuously aerated by airstones and an air blower and illuminated by fluorescent ceiling lights. After 30 hr incubation at 33°C, aeration was turned off and the hatched nauplii settled to the bottom of the container and were removed by opening a valve at the bottom of the container. The nauplii were immediately frozen in liquid nitrogen, placed into test tubes and sealed under nitrogen, and stored at −80°C until analyzed.

Lipid analysis

Total lipids were extracted with chloroform–methanol (2:1; v/v) and 0.5% water by the method of Bligh and Dyer (1959), and separated into TG, FFA and PL by thin-layer chromatography (TLC) as described by Kates (1986). Glass plates (20 × 20 cm) precoated (250 μm) with silica gel 60 (E. Merck, Darmstadt, Germany) were developed in hexane–diethyl ether–acetic acid (79:20:4; v/v) (Weete *et al.*, 1983). The polar lipid fraction was isolated from the silica gel at the origin of these plates and separated into individual components by two-dimensional TLC. The plates were developed first in chloroform–acetone–methanol–acetic acid–water (30:40:10:10:5, v/v) and, after drying for

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1.5 hr with nitrogen, were developed in chloroform–methanol–26% NH_4OH (65:26:5.5; v/v) (Weete *et al.*, 1983).

Lipids were visualized on the plates with iodine vapor and identified by comparison of R_f values with a standard (lecithin) containing a triacylglycerol, a free fatty acid, a sterol and wax ester, and a phospholipid fractions (Applied Science Laboratories, Deerfield, IL) which was spotted alongside the lipid samples. Silica gel was scraped from the plates, washed once with methanol–chloroform (2:1; v/v) and twice with methanol–chloroform (1:1; v/v) containing 0.5% water to obtain the lipid. The solvent was evaporated under nitrogen.

Triacylglycerol and phospholipid classes were converted to their fatty acid methyl esters by transesterification using sodium methoxide in methanol (Applied Science Laboratories). Screwcap test tubes (Teflon lined) were used, with 2 ml sodium methoxide–methanol and 1 ml of benzene as cosolvent. After heating for 20 min at 80°C and cooling, 3 ml diethyl ether and 3 ml water were added. The esters were recovered in the diethyl ether phase and dried over anhydrous sodium sulfate. The free fatty acid fraction was methylated using BF_3–methanol (Morrison and Smith, 1964). Fatty acid methyl ester analysis was conducted using a Hewlett-Packard Model 5710A (Avondale, PA) gas chromatograph equipped with a flame-ionization detector and a 30-m capillary column DB-225 (J & W Scientific, Folsom, CA). The carrier gas was nitrogen at a column pressure of 1.06 kg/cm^2. Injector and detector temperatures were both 250°C and the oven temperature was programmed from 180 to 210°C at 1°C/min. Fatty acids were recorded and quantitated on a Hewlett-Packard Model 3380A integrator-recorder and were identified by comparison of their retention times with those of standards (Nu-Chek Prep, Elysian, MN).

Sterols were obtained from the total lipid by alkaline hydrolysis (0.4 ml 33% KOH in 4 ml ethanol) for 120 min at 90°C (Kates, 1986). Three milliliters of distilled water was added to the hydrolysate. The non-saponifiable fraction, containing the sterols, was obtained by washing the hydrolysate three times with 3 ml of hexane. Sterols were acetylated with 0.25 ml acetic anhydride and 0.25 ml pyridine (Kates, 1986) and analyzed with the gas chromatograph equipped with a DB-5 capillary column (J & W Scientific) and a flame-ionization detector. The carrier gas was helium at a column pressure of 1.0 kg/cm^2. Injector and detector temperatures were both 300°C. The oven temperature was isothermic at 250°C. An internal standard of 5-dihydrocholesterol was used and sterol peaks were compared to those of standards.

Analysis of variance (ANOVA) for percentages of total lipid, TG, FFA and PL, cholesterol, and individual fatty acids in the TG, FFA and PL classes were determined (Statistical Analysis Systems, 1985). Percentages were arcsine-transformed prior to analysis (Zar, 1984); however, means are reported as untransformed data to facilitate comparison with related studies. Duncan's multiple range test was used to determine where differences existed among means.

RESULTS

Lipid classes of brine shrimp

No differences were found in the percentage of total lipids among the three sources of brine shrimp ($P > 0.05$), with an average of 17.8% (Table 1). The TG comprised the major lipid class among the sources of brine shrimp, 65.8% of the lipids, followed by the PL, 23.2% of the lipids, and the FFA, 10.9% of the lipids. No significant differences ($P > 0.05$) were found among brine shrimp sources in TG or FFA. The Colombian (COL) source contained significantly less ($P < 0.05$) PL than the Chinese (CH) source, but was not significantly different than the San Francisco Bay (SFB) source ($P > 0.05$).

Fatty acid composition of different lipid classes

Major differences in the fatty acid composition of TG in the different sources of brine shrimp were seen, especially in the percentages of 16:1 (*n*-7) (palmitoleic acid), 18:1 (*n*-9) (oleic acid), 18:1 (*n*-7) (vaccinic acid), 18:3 (*n*-3) (linolenic acid) and 20:5 (*n*-3) (eicosapentaenoic acid or EPA) (Table 2). Although the percentage of total *n*-3 fatty acids was significantly less ($P < 0.05$) in CH brine shrimp, a higher amount of EPA (9.49%) was present than in the COL and SFB sources (1.41 and 0.71%, respectively) ($P < 0.05$); however, the COL and SFB sources had significantly higher percentages of linolenic acid (24.71 and 26.83%), than the CH source (4.60%).

The TG from all sources of brine shrimp contained similar amounts of saturated and dienoic fatty acids; however, differences in the monoenoic and polyenoic fatty acids were found. The CH brine shrimp had a higher percentage of monoenoic fatty acids due to the higher amount of palmitoleic acid, while the COL and SFB brine shrimp had higher percentages of polyenoic fatty acids due to a higher percentage of linolenic acid ($P < 0.05$). The degree of unsaturation, measured by the PUFA (dienoic and polynenoic fatty acids)/saturated fatty acid ratio, was higher for the SFB source which had a ratio of 2.15:1 as compared to the CH and COL sources which were 1.14 and 1.79, respectively.

The fatty acid composition of FFA indicates a reduction in percentage of monoenoic and polyenoic fatty acids and an increase in saturated fatty acids due to increased percentages of 14:0 and 18:0 (Table 3). The COL and SFB brine shrimp had lower percentages of *n*-3 fatty acids in the FFA than in the TG due to lower percentages of linolenic acid. The CH brine shrimp had a significantly higher percentage of

Table 1. Total lipid composition in three different sources of brine shrimp nauplii*

Brine shrimp†	Total lipid (% dry wt)	Lipid classes TG‡	FFA‡ (% of lipid)	PL‡	Cholesterol (% dry wt)
CH	15.0 ± 1.0^a	64.1 ± 2.0^a	9.4 ± 1.6^a	26.5 ± 2.9^a	0.14 ± 0.03^a
COL	18.2 ± 3.6^a	70.9 ± 8.0^a	10.8 ± 2.4^a	18.3 ± 3.0^b	0.19 ± 0.05^a
SFB	20.1 ± 0.7^a	62.3 ± 3.6^a	12.6 ± 2.9^a	25.1 ± 1.8^{ab}	0.20 ± 0.08^a

*Values are means ± SE of four replications. Means with different superscripts (^{a,b}) are significantly different ($P < 0.05$).

†Sources of brine shrimp are Chinese (CH), Colombian (COL) and San Francisco Bay (SFB).

‡TG = triacylglycerols, FFA = free fatty acids, PL = phospholipids.

Table 2. Percentage of fatty acids in the triacylglycerol class in the lipid of three different sources of brine shrimp*

Fatty acid	Source† CH	COL	SFB
12:0	0.11 ± 0.04	0.30 ± 0.21	0.32 ± 0.21
14:0	1.94 ± 0.31[a]	1.31 ± 0.12[ab]	0.93 ± 0.11[b]
14:1 (*n*-5)	1.86 ± 0.40	1.29 ± 0.26	1.69 ± 0.14
15:0	1.17 ± 0.15	1.40 ± 0.29	1.53 ± 0.24
16:0	13.67 ± 0.82[ab]	14.93 ± 0.26[a]	12.59 ± 0.18[b]
16:1 (*n*-9)	0.72 ± 0.16	0.92 ± 0.06	0.70 ± 0.13
16:1 (*n*-7)	19.13 ± 1.69[a]	4.59 ± 0.69[b]	4.67 ± 0.78[b]
16:2 (*n*-4)	1.10 ± 0.22[a]	0.60 ± 0.07[b]	0.69 ± 0.07[ab]
16:3 (*n*-4)	0.88 ± 0.22	0.88 ± 0.12	0.97 ± 0.01
16:4 (*n*-3)	0.96 ± 0.29	0.92 ± 0.10	0.61 ± 0.04
17:0	2.04 ± 0.15[a]	1.17 ± 0.14[b]	1.10 ± 0.22[b]
18:0	3.76 ± 0.85	3.64 ± 0.12	3.08 ± 0.51
tr 18:1 (*n*-9)	0.23 ± 0.19	0.00 ± 0.00	0.00 ± 0.00
cis 18:1 (*n*-9)	15.29 ± 0.94[b]	19.58 ± 1.30[a]	18.67 ± 1.23[ab]
18:1 (*n*-7)	9.12 ± 1.00[a]	5.79 ± 0.28[b]	5.74 ± 0.51[b]
18:2 (*n*-6)	5.19 ± 0.10[b]	6.51 ± 0.15[a]	6.39 ± 0.11[a]
18:3 (*n*-6)	0.50 ± 0.13	0.50 ± 0.06	0.67 ± 0.10
18:3 (*n*-4)	0.61 ± 0.24	0.33 ± 0.13	0.19 ± 0.08
18:3 (*n*-3)	4.60 ± 0.51[b]	24.71 ± 0.86[a]	26.83 ± 1.30[a]
18:4 (*n*-3)	0.50 ± 0.14[c]	2.88 ± 0.13[b]	3.74 ± 0.31[a]
20:0	0.49 ± 0.13	0.33 ± 0.12	0.43 ± 0.17
20:1 (*n*-9)	0.18 ± 0.09	0.28 ± 0.12	0.23 ± 0.12
20:2 (*n*-6)	0.39 ± 0.19	0.42 ± 0.24	1.13 ± 0.90
20:3 (*n*-3)	0.17 ± 0.07	0.22 ± 0.15	0.17 ± 0.06
20:4 (*n*-6)	1.98 ± 0.51[a]	0.44 ± 0.05[b]	0.82 ± 0.11[b]
20:4 (*n*-3)	0.18 ± 0.10[b]	1.09 ± 0.30[a]	0.00 ± 0.00[b]
20:5 (*n*-6)	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.71 ± 0.18[a]
20:5 (*n*-3)	9.44 ± 0.41[a]	1.41 ± 0.33[b]	0.71 ± 0.03[b]
22:0	0.17 ± 0.13	0.17 ± 0.08	0.35 ± 0.28
22:1 (*n*-9)	0.12 ± 0.07	0.10 ± 0.05	0.03 ± 0.03
22:2 (*n*-6)	0.07 ± 0.06	0.35 ± 0.20	0.00 ± 0.00
22:3 (*n*-3)	0.01 ± 0.01	0.16 ± 0.11	0.06 ± 0.03
22:4 (*n*-6)	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.04
22:5 (*n*-3)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
22:6 (*n*-3)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Other	3.51 ± 0.79	1.53 ± 0.77	4.25 ± 1.05
% sat.	23.35 ± 1.56	23.24 ± 0.56	20.32 ± 0.88
% monoene	46.65 ± 0.87[a]	32.53 ± 1.77[b]	31.73 ± 1.28[b]
% diene	6.75 ± 0.11	7.87 ± 0.49	8.20 ± 0.92
% polyene	19.97 ± 1.32[b]	33.78 ± 0.79[a]	35.50 ± 1.29[a]
% *n*-3	15.97 ± 0.79[b]	30.29 ± 1.16[a]	32.12 ± 1.46[a]
% *n*-6	8.13 ± 0.62	7.45 ± 0.17	9.17 ± 0.83
EPA/LLA‡	2.11 ± 0.18[a]	0.06 ± 0.01[b]	0.03 ± 0.00[b]

*Values are means ± SE of four replications. Means with different superscripts ([a, b, c]) are significantly different ($P < 0.05$).
†Sources of brine shrimp are Chinese (CH), Colombian (COL) and San Francisco Bay (SFB).
‡LLA = linolenic acid.

Table 3. Percentage of fatty acids in the free fatty acid class in the lipid of three different sources of brine shrimp*

Fatty acid	Source† CH	COL	SFB
12:0	0.12 ± 0.06	0.22 ± 0.04	1.09 ± 0.78
14:0	3.54 ± 0.98	2.40 ± 0.88	3.09 ± 1.79
14:1 (*n*-5)	1.19 ± 0.26	1.07 ± 0.42	1.16 ± 0.25
15:0	1.42 ± 0.59	2.19 ± 1.24	0.67 ± 0.07
16:0	11.80 ± 1.25	13.93 ± 2.19	14.20 ± 1.81
16:1 (*n*-9)	1.10 ± 0.46	1.06 ± 0.11	1.08 ± 0.37
16:1 (*n*-7)	14.40 ± 1.91[a]	3.84 ± 1.15[b]	3.55 ± 0.64[b]
16:2 (*n*-4)	0.80 ± 0.25	0.67 ± 0.18	1.24 ± 0.22
16:3 (*n*-4)	0.60 ± 0.12	0.73 ± 0.28	0.72 ± 0.12
16:4 (*n*-3)	1.04 ± 0.32	1.74 ± 0.64	1.66 ± 0.64
17:0	1.57 ± 0.20	0.85 ± 0.32	1.24 ± 0.31
18:0	4.29 ± 0.59	4.58 ± 0.70	9.17 ± 3.47
tr 18:1 (*n*-9)	0.26 ± 0.26[ab]	0.00 ± 0.00[b]	0.54 ± 0.35[a]
cis 18:1 (*n*-9)	14.52 ± 1.92[ab]	19.97 ± 0.94[a]	10.43 ± 3.32[b]
18:1 (*n*-7)	8.86 ± 1.61[a]	8.16 ± 0.75[a]	6.57 ± 3.11[b]
18:2 (*n*-6)	4.34 ± 0.77[a]	5.02 ± 0.34[a]	2.32 ± 0.28[b]
18:3 (*n*-6)	1.04 ± 0.35[a]	0.46 ± 0.10[b]	0.52 ± 0.14[b]
18:3 (*n*-4)	0.66 ± 0.17	0.55 ± 0.08	1.32 ± 0.67
18:3 (*n*-3)	4.71 ± 0.47[c]	18.74 ± 1.76[a]	13.05 ± 1.76[b]
18:4 (*n*-3)	1.02 ± 0.13[b]	4.38 ± 0.64[a]	4.55 ± 0.60[a]
20:0	0.66 ± 0.10[b]	0.37 ± 0.09[b]	1.68 ± 0.43[a]
20:1 (*n*-9)	0.61 ± 0.18	0.27 ± 0.08	0.79 ± 0.18
20:2 (*n*-6)	0.89 ± 0.22	0.44 ± 0.13	0.70 ± 0.20
20:3 (*n*-3)	0.03 ± 0.01	0.29 ± 0.13	0.07 ± 0.04
20:4 (*n*-6)	1.84 ± 0.29	0.83 ± 0.17	1.23 ± 0.73
20:4 (*n*-3)	1.01 ± 0.39	0.25 ± 0.11	0.45 ± 0.16
20:5 (*n*-6)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:5 (*n*-3)	11.24 ± 1.04[a]	2.42 ± 0.47[b]	1.41 ± 0.65[b]
22:0	0.09 ± 0.01[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
22:1 (*n*-9)	0.23 ± 0.09	0.07 ± 0.07	0.70 ± 0.34
22:2 (*n*-6)	0.00 ± 0.00	0.08 ± 0.05	0.42 ± 0.25
22:3 (*n*-3)	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.07
22:4 (*n*-6)	0.00 ± 0.00	0.01 ± 0.01	0.26 ± 0.15
Other	6.17 ± 3.39	4.26 ± 2.32	6.94 ± 1.58
% sat.	23.48 ± 1.74[b]	24.54 ± 1.70[b]	34.10 ± 3.79[a]
% monoene	41.15 ± 1.12[a]	34.43 ± 2.17[a]	26.06 ± 3.42[b]
% diene	6.03 ± 0.62	6.35 ± 0.24	5.07 ± 0.37
% polyene	22.44 ± 1.48	30.43 ± 2.56	27.72 ± 3.61
% *n*-3	18.77 ± 1.12[b]	27.58 ± 2.64[a]	22.86 ± 3.06[ab]
% *n*-6	8.11 ± 1.23[a]	6.34 ± 0.41[ab]	5.32 ± 0.45[b]
EPA/LLA‡	2.49 ± 0.43[a]	0.15 ± 0.05[b]	0.10 ± 0.03[b]

*Values are means ± SE of four replications. Means with different superscripts ([a, b, c]) are significantly different ($P < 0.05$).
†Sources of brine shrimp are Chinese (CH), Colombian (COL) and San Francisco Bay (SFB).
‡LLA = linolenic acid.

EPA than the COL and SFB sources ($P < 0.05$). The PUFA/saturated fatty acid values were 1.21, 1.50 and 0.96 for the CH, COL and SFB sources, respectively.

Phospholipids (PL) were more unsaturated than the TG and FFA fractions. There was less saturated fatty acids in the PL than in the TG and FFA fractions (Table 4). The percentage of *n*-3 fatty acids in the PL fraction was similar to that in the TG fraction. The CH brine shrimp had a significantly higher percentage of EPA in the PL fraction than the COL and SFB sources ($P < 0.05$). The PUFA/saturated fatty acid ratio was higher for CH (2.62:1) than for the COL (2.17:1) and SFB (1.98:1) sources.

The phospholipids identified, but not quantitated, from two-dimensional TLC were phosphotidylcholine (PC), phosphotidylethanolamine (PE), two unidentified phospholipids, and a less polar lipid class (LPL) consisting mostly of sterol and wax esters.

DISCUSSION

Although the fatty acid composition of total lipids in brine shrimp nauplii from various sources of brine shrimp has been described, this is the first analysis of individual lipid classes. Since newly-hatched brine shrimp nauplii have a large amount of yolk, it would be expected that the total lipid content be high and the TG fraction comprise the largest portion of the total lipid. Lipid comprises 17.8% of the dry matter in newly-hatched nauplii and makes the nauplii a good energy source for larval fish. Adult brine shrimp would probably have a lower percentage of total lipid as the yolk content decreases and the exoskeleton and other body parts grow (Lee *et al.*, 1971). Other zooplankton species, such as copepods, have nauplii with similar total lipid contents (Takahashi and Yamada, 1976). The TG is the largest fraction of the total lipid in the nauplius of brine shrimp; however, Ackman *et al.* (1970) reported that the nauplius of some crustacean zooplankters, such as the krill, *Meganyctiphanes norvegica*, have phospholipid as the principal lipid fraction.

Table 4. Percentage of fatty acids in the phospholipid class in the lipid of three different sources of brine shrimp*

	Source†		
Fatty acid	CH	COL	SFB
12:0	0.31 ± 0.21	0.12 ± 0.03	0.17 ± 0.06
14:0	0.65 ± 0.24	1.51 ± 0.75	2.13 ± 0.90
14:1 (*n*-5)	0.80 ± 0.49	0.38 ± 0.15	0.60 ± 0.15
15:0	0.75 ± 0.35	1.37 ± 0.62	2.82 ± 0.80
16:0	4.13 ± 0.99^{b}	7.72 ± 0.63^{a}	9.22 ± 0.15^{a}
16:1 (*n*-9)	0.49 ± 0.09	0.70 ± 0.09	0.80 ± 0.11
16:1 (*n*-7)	12.95 ± 2.49^{a}	2.28 ± 0.40^{b}	2.53 ± 0.33^{b}
16:2 (*n*-4)	0.77 ± 0.22^{a}	0.20 ± 0.04^{b}	0.43 ± 0.06^{ab}
16:3 (*n*-4)	0.82 ± 0.29	0.66 ± 0.10	0.66 ± 0.06
16:4 (*n*-3)	1.16 ± 0.34	1.58 ± 0.33	0.76 ± 0.15
17:0	1.84 ± 0.16^{a}	0.77 ± 0.14^{b}	0.81 ± 0.06^{b}
18:0	5.10 ± 1.40	7.40 ± 0.21	5.86 ± 0.70
tr 18:1 (*n*-9)	0.12 ± 0.12	0.11 ± 0.11	0.00 ± 0.00
cis 18:1 (*n*-9)	17.30 ± 2.19	20.55 ± 0.39	19.71 ± 0.89
18:1 (*n*-7)	12.05 ± 2.47	9.06 ± 0.89	11.22 ± 1.03
18:2 (*n*-6)	6.07 ± 0.21	6.34 ± 0.26	5.80 ± 0.14
18:3 (*n*-6)	0.74 ± 0.08^{ab}	1.27 ± 0.06^{b}	0.65 ± 0.12^{b}
18:3 (*n*-4)	0.42 ± 0.13^{b}	1.00 ± 0.26^{a}	0.33 ± 0.07^{b}
18:3 (*n*-3)	6.59 ± 2.34^{b}	20.48 ± 1.50^{a}	20.70 ± 0.64^{a}
18:4 (*n*-3)	1.37 ± 0.29^{b}	4.99 ± 0.92^{a}	6.55 ± 0.74^{a}
20:0	0.34 ± 0.19	0.55 ± 0.24	0.39 ± 0.17
20:1 (*n*-9)	0.15 ± 0.14	0.47 ± 0.14	0.09 ± 0.04
20:2 (*n*-6)	0.56 ± 0.24	0.67 ± 0.39	0.81 ± 0.39
20:3 (*n*-3)	0.15 ± 0.08	0.14 ± 0.04	0.10 ± 0.04
20:4 (*n*-6)	2.60 ± 0.27^{a}	1.16 ± 0.17^{b}	1.14 ± 0.35^{b}
20:4 (*n*-3)	0.99 ± 0.62	0.00 ± 0.00	0.62 ± 0.17
20:5 (*n*-6)	0.28 ± 0.09^{b}	0.64 ± 0.12^{a}	0.00 ± 0.00^{b}
20:5 (*n*-3)	12.48 ± 0.50^{a}	4.92 ± 1.04^{b}	1.29 ± 0.10^{c}
22:0	0.24 ± 0.07	0.44 ± 0.18	0.13 ± 0.05
22:1 (*n*-9)	0.10 ± 0.00	0.05 ± 0.05	0.30 ± 0.18
22:2 (*n*-6)	0.15 ± 0.12	0.31 ± 0.13	0.31 ± 0.24
22:3 (*n*-3)	0.12 ± 0.12	0.00 ± 0.00	0.00 ± 0.00
Other	7.42 ± 1.74	3.21 ± 1.01	3.62 ± 1.82
% sat.	13.36 ± 2.28^{b}	19.87 ± 1.57^{a}	20.29 ± 1.77^{a}
% monoene	43.96 ± 1.88^{a}	33.57 ± 0.62^{b}	34.50 ± 0.73^{b}
% diene	7.55 ± 0.44	7.42 ± 0.67	7.34 ± 0.63
% polyene	27.47 ± 2.80^{b}	35.71 ± 2.22^{a}	32.80 ± 1.33^{ab}
% *n*-3	22.53 ± 2.62^{b}	31.23 ± 1.86^{a}	29.90 ± 0.99^{a}
% *n*-6	10.40 ± 0.31^{a}	8.23 ± 0.35^{b}	8.70 ± 0.70^{ab}
EPA/LLA‡	2.35 ± 0.64^{a}	0.24 ± 0.05^{b}	0.06 ± 0.00^{b}

*Values are means ± SE of four replications. Means with different superscripts ($^{a, b, c}$) are significantly different ($P < 0.05$).
†Sources of brine shrimp are Chinese (CH), Colombian (COL) and San Francisco Bay (SFB).
‡LLA = linolenic acid.

Brine shrimp nauplii have a relatively small amount of wax esters, less than 2% of the total lipids, as compared with calanoid copepods (*Calanus hyperboreus* and *C. helgolandicus*) which have approx. 40% of the total lipid as wax esters (Lee *et al.*, 1971). Wax esters are poorly digested by fish, especially larvae (Patton *et al.*, 1975). Other copepods (*Acartia* sp. and *Temora longcornis*) contain less than 5% wax esters in the total lipid (Kattner *et al.*, 1981; Kaitaranta *et al.*, 1986).

Within each source of brine shrimp, the fatty acid composition among the TG, FFA and PL classes was similar. Lee *et al.* (1971) stated that TG in copepods reflect the dietary fatty acids, whereas phospholipid fatty acids reflect biosynthetic pathways and membrane requirements, and do not vary with dietary changes. However, this study showed that the CH nauplii had a greater percentage of EPA in all lipid classes than the COL and SFB sources indicating that fatty acid composition of PL may be influenced by the diet of the adult brine shrimp.

The differences in the fatty acid composition of the lipids among the three sources of brine shrimp nauplii are apparently related to the environment from which the cysts (eggs) were collected. Fatty acid composition of brine shrimp cysts is derived from food consumed by the adult female (Persoone and Sorgeloos, 1980). Unlike copepods, brine shrimp do not seem to be able to elongate and desaturate C_{20-22} fatty acids from C_{18} precursors (Jezyk and Penicnak, 1966; Hinchcliffe and Riley, 1972). Algae is the primary food for adult brine shrimp, so the *n*-3 HUFA composition of the algae has a significant influence on the *n*-3 HUFA composition of the cysts and hence the nauplii (Hinchcliffe and Riley, 1972). Jeffries (1970) found that temperate algae communities change seasonally along with their fatty acid compositions. Algal fatty acid composition varies among species (Chu and Dupuy, 1980), and within species as nutrients (Moss, 1973), water temperature (Jeffries, 1970) and other environmental factors (Jassby and Goldman, 1974) change.

The presence of FFA above 1–2% of the lipid is often associated with lipid degradation due to improper storage (Kramer and Hulan, 1978). However, the extraction and storage procedures used in this study meet all the conditions for conservation of lipid (Kates, 1986), thus, enzymatic degradation of the lipid should be absent. The large amount of FFA reported here for all sources of brine shrimp is in agreement with values reported for other invertebrate lipids (Takehashi and Yamada, 1976; Napolitano *et al.*, 1988).

These results indicate that the greatest variation in the lipids among the various sources of brine shrimp was in the EPA/linolenic acid ratio. These differences in the EPA/linolenic acid ratio were present in the TG, FFA and PL classes in the lipid and may account for the difference in nutritional value among these sources. Webster and Lovell (1990) found that the CH source, which contained the highest level of EPA, allowed higher growth and survival rates for striped bass larvae than the other two sources.

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