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FATTY ACID COMPOSITION OF *ARTEMIA* (BRANCHIOPODA: ANOSTRACA) CYSTS FROM TROPICAL SALTERNS OF SOUTHERN MÉXICO AND CUBA

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ABSTRACT

The growing demands and high costs of commercial *Artemia* cysts and the establishment of new shrimp hatcheries and farms have caused people to search for local sources of *Artemia*, putting special attention on their nutritional characteristics. As an essential step to determine the biochemical composition of *Artemia*, the fatty acid profiles of decapsulated cysts from six tropical salterns of southern México [Campeche (1), Oaxaca (1), and Yucatán (4)], two of Cuba (Camagüey and Guantánamo), and from a temperate site (San Francisco Bay, USA) (SFB) were analysed using direct transesterification and gas chromatography-mass spectrometry. Of 51 fatty acids identified, C16:0 (hexadecanoic), C16:1 n5 (hexadecenoic), C18:1 n9 (octadecenoic), C18:1 n7 (octadecenoic), and C18:2 n6 (octadecadienoic) were the major compounds found. The SFB strain from a temperate area showed significant differences from the rest of the samples of tropical origin, having a greater concentration in the fatty acids 18:2 n5 (10, 13-octadecadienoic), 18:3 n3 (octadecatrienoic), and 18:4 n3 (6, 9, 12, 15-octadecatetraenoic). The SFB strain showed the lowest proportion of mono-unsaturated fatty acids. Based on the fatty acid composition, the *Artemia* strains studied can be assessed as "freshwater" type, except for the one from Oaxaca that had a "marine" type profile characterized by 3% to 4% of the fatty acid C20:5 n3 (eicosapentaenoic).

INTRODUCTION

The brine shrimp *Artemia* occurs in all continents except the Arctic and Antarctic (Belk, 1984). It lives in hypersaline lakes, temporary desert pools, and in coastal lagoons, saltern ponds, and saltmarshes (Triantaphyllidis et al., 1998). *Artemia* diapausing embryos (cysts) are specialized to withstand adverse environmental stress and to develop and hatch when there are proper conditions of light, salinity, oxygen concentration, temperature, and pH (Lavens and Sorgeloos, 1987). The use of *Artemia* cysts and nauplii in aquaculture is well-known, especially in fish and shrimp larviculture. Reproducible results may not be guaranteed because of the variable nutritional value of the cysts. This variability has been related to the habitat conditions where the cysts were produced, and particularly related to the content of essential amino acids and fatty acids provided from the phytoplankton available in the environment (Watanabe et al., 1978; Sorgeloos et al., 1998), though a genetic component influencing the biochemical composition of the cysts cannot be excluded (Navarro and Amat, 1992).

Studies indicate that the nauplii of *Artemia* from different origins show similar protein and carbohydrate contents (Leger et al., 1986; Fernández-Reiriz et al., 1991), but their fatty acid composition may vary considerably (Leger et al., 1986). The fatty acid (FA) composition of *Artemia* is one of the most studied aspects after the establishment was made of a high relation between survival of fish larvae and the fatty acids C20:5 n3 and C22:6 n3 (Watanabe, 1978). Watanabe et al. (1978, 1980) found significant differences in the content of polyunsaturated fatty acids (PUFA) of the type n3 within several strains of *Artemia*. These strains were classified into two types; the "marine" type, rich in C20:5 n3 giving good results in the culture of marine fish larvae, and the "freshwater" type with a low content of this compound and yielding low survival in these marine organisms, but showing good results for the survival of freshwater fish larvae (Watanabe et al., 1978; Tacon, 1987; Torrentera and Tacon, 1989). The importance of the C22:6 n3 FA has also been demonstrated for crustaceans and fish during the larval phase, though a certain combination of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) appears even more important than the individual content of each of these fatty acids (Watanabe, 1993; Kanazawa, 1994).

The fast development of the aquaculture industry has led to an increasing demand for the cysts of *Artemia* worldwide. The Great Salt Lake, USA, producer of 95% of the marketable cysts, faces important variability in harvests from year to year because of, among others, climate changes and anthropogenic activities (Dhont and Sorgeloos, 2002). The shortage of cysts and the high costs of commercial cysts are expected to considerably affect the aquaculture industry. Local strains of *Artemia* could be used as an alternative source to meet the demand of the increasing number of

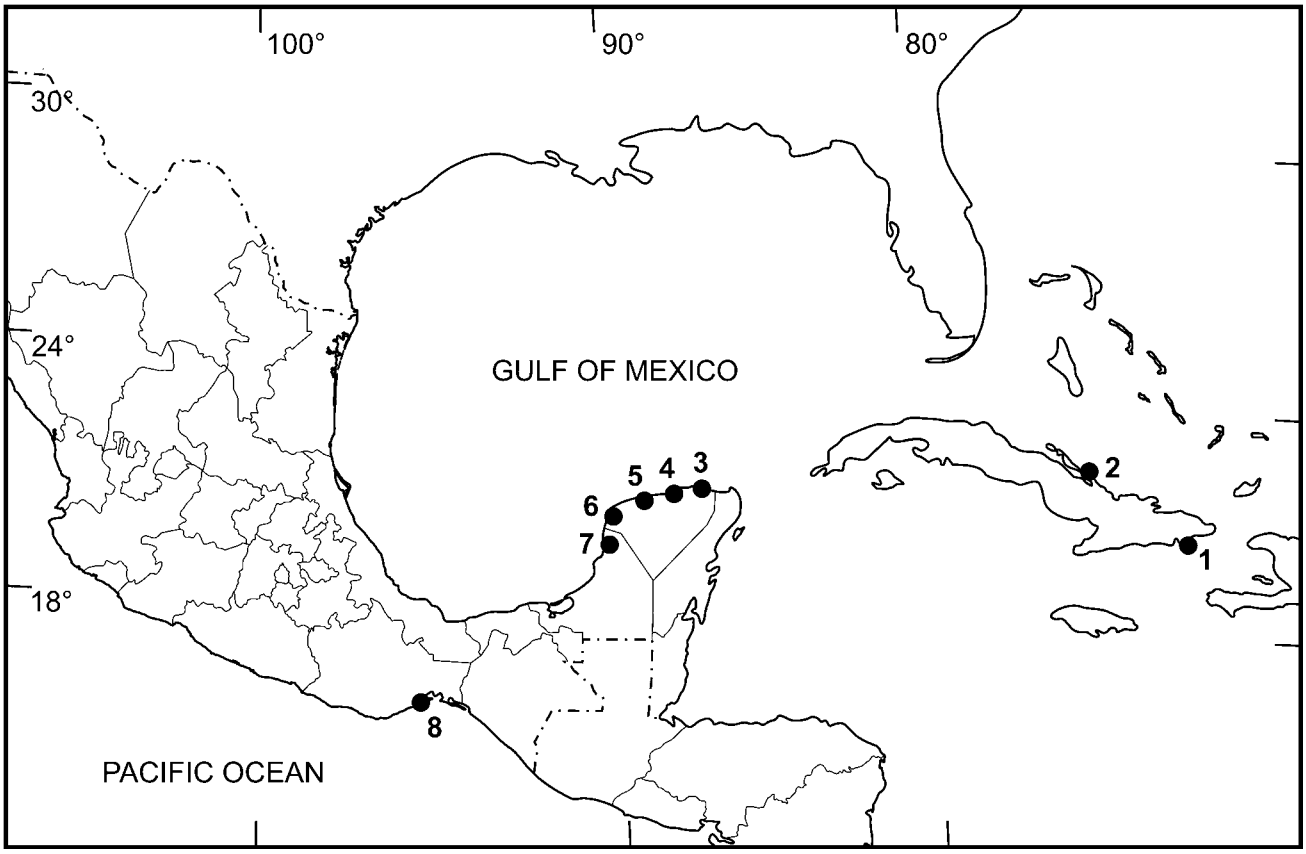

Fig. 1. Locations in Cuba and southern México of the tropical populations of *Artemia* used for the fatty acid characterization. Cuba: Frank País, Guantánamo province (1); El Real, Santa Lucía, Camagüey province (2). México: Xtampú (3), San Crisanto (4), Chuburna (5), and Celestún (6), Yucatán; Real de Salinas, Campeche (7); La Colorada, Oaxaca (8).

shrimp hatcheries and farms of México and Cuba after the establishment of their nutritional characteristics. By using direct transesterification and gas chromatography-mass spectrometry we analyzed and compared the fatty acid composition of *Artemia* cysts from eight tropical salterns distributed in southern México and Cuba (Fig. 1), and from a temperate site of California, USA.

Material and Methods

Sampling Sites

Cuba

Guantánamo Province

Frank País site (CM). Permanent commercial saltwork of about 772 ha in a coastal mangrove area situated at 21°23′N, 88°53′W. The site is in a semiarid region with a mean annual precipitation of about 450 mm.

Camagüey Province

El Real, Santa Lucía site (CG). Temporary commercial saltwork of about 814 ha in a coastal mangrove area situated at 20°52′N, 90°22′W. The mean annual precipitation is about 1070 mm. There are two dry periods, one from December to May and a shorter one from July to middle September. The rainy season occurs during October and November (Bruno, 1988).

México

Campeche State

Real de Salinas site (CP). Commercial salt work in coastal mangrove area of about 60 ha situated at 20°02′N, 90°14′W. The climate is characterized by a rainy summer season and a dry winter. Average annual rainfall is about 760 mm and the mean annual temperature is 26.4°C. Saltern phytoplankton is dominated by microalgae such as *Dunaliella* sp.

Oaxaca State

La Colorada site (OX). Commercial salt work in a shallow hypersaline coastal lagoon of 132 ha, surrounded by mangrove, and situated at 15°33′N, 95°32′W. The climate is characterized by a rainy summer season and dry winter. Average annual rainfall is about 820 mm, and the annual temperature ranges from 12 to 42°C. Saltern phytoplankton is dominated by microalgae such as *Dunaliella* sp.

Yucatán State

The sampled sites in this state are located on the northern coast (Fig. 1), which is characterized by alkaline hypersaline lagoons, saltmarshes, and temporary salterns (Weidie, 1984; Lesser, 1974; Stringfield and Legrand, 1974). Salterns provide specialized hypersaline habitats in which salinity ranges from that of seawater (35 psu) to about 150 psu or more. The weather in the Yucatán Peninsula is characterized by three seasons; rain from the end of June to October, cold storms from October-November to March, and the dry season from March to June. Average annual rainfall is about 1050 mm and the annual mean temperature is 26°C (Stringfield and Legrand, 1974; Lesser, 1974). Saltern phytoplankton is dominated by microalgae such as *Dunaliella* sp., *Rhodomonas* sp., and halophytic purple sulfur bacteria, and cyanobacteria (Torrentera, unpublished data).

Celestún Site (YCE).—Natural highly alkaline temporary shallow ponds situated at 20°52′N, 90°22′W. The ponds dry out totally during the dry season, and have some of the highest and lowest temperatures (20 to 37.5 °C), salinity (32 to 400 psu) and pH (6.5 to 9.5) observed in the region (Torrentera and Dodson, 2004).

Chuburna Site (YC).—Large semipermanent pools in a natural salt marsh in an estuarine coastal area situated at 21°15′N, 89°48′W. The site is

characterized by a moderate and relatively stable salinity (45 to 151 psu) showing the lowest mean value (94 psu) in the region (Torrentera and Dodson, 2004).

San Crisanto Site (YSC).—Experimental *Artemia* culture ponds in a old abandoned commercial saltwork in a salt marsh area situated at 21°20′N, 89°10′W. The climate is hot and semiarid with a mean annual precipitation of 540 mm and a temperature range of 15 to 33°C.

Xtampú Site (YX).—Natural alkaline temporary shallow ponds situated at 21°23′N, 88°53′W. The ponds partially dry up during the dry season, and reach the highest mean values of temperature (32.2°C) and salinity (224 psu) and the lowest pH mean value (7.9) observed in the region (Torrentera and Dodson, 2004).

United States of America

California State

San Francisco Bay site. Commercial *Artemia franciscana* Kellogg, 1906 culture ponds. Argentemia, pond No. 4, lot BP03051, San Francisco Bay National Wildlife Refuge, situated at 37°28′N, 122°30′W. Average annual rainfall is about 530 mm (Tsai et al., 2001); the water temperature range is 6 to 29°C, salinity 25 to 130 psu, and pH 8 to 9 (Miles et al., 2004).

Cyst Sampling

Field-sampled cysts were separated from debris using a 1000-μm nylon mesh and collected in a 125-μm nylon mesh. The cysts were placed in an inverted conical container with freshwater and after five minutes the cysts deposited at the bottom were separated and placed in a container with brine. Finally, the cysts were dried in an oven at 35°C for three days and stored in a sealed flask in a refrigerator at 2 to 4°C until analysis.

Fatty Acid Analysis

Cyst samples of 50 mg, taken in triplicate, were decapsulated by using the techniques of Bruggeman et al. (1980) and Lavens et al. (1989), and then lyophilyzed (Virtis, SL Gardiner NY). Extraction and methanolysis of fatty acids were done by direct transesterification (Lapage and Roy, 1984, 1986; Barnung and Grahl-Nielsen, 1987). Dry methanol (500 μL) containing 2N HCl (Supelco Bellefonte, PA) was added to samples (20 to 38 mg) and then sonicated three times during 7 min. Thick-walled glass tubes were tightly closed with Teflon-lined caps and heated at 90°C for 2 h. The excess hydrochloric acid was removed by using a stream of nitrogen. The residues were dissolved in 4 mL of hexane and 0.5 mL of distilled water, vortexed for 1 minute, and centrifuged for 5 min at 4000 rpm and 10°C. The upper phase (hexane with fatty acids) was separated and 10 μL of butylhydroxyltoluene (1%) was added to prevent oxidation. Samples were washed three times with distilled water and vortexed. The lower phase was extracted and centrifuged for 5 min at 4000 rpm and 10°C, a few granules of $NaSO_4$ were added to eliminate the excess water, and the residual liquid stored at −20°C until injection into the chromatograph. The samples were processed in a gas chromatograph-mass spectrometer (GCD System of Hewlett Packard G1800) by automatic injection into a Omegawax 250 capillary column 30-m long, 25-mm diameter, and 0.25-μm liquid phase with an initial oven temperature of 110°C. After a 3.3-min solvent time delay, the temperature was increased at 30°C/min to 165°C maintaining it there for 2 min. The temperature was then increased at 14°C/min to 220°C maintaining that temperature for 10 min. The carrier gas was helium with a constant flow of 0.9 mL/min. The chromatograms were processed with the software program GCD of Extra ChemStation (G1074B, A.01.00 version). Using the retention time, the NBS75K and NIST98 libraries, and a data base of 29 fatty acid standards (PUFA-CIBNOR), the molecular weight and the detection of the characteristic ions for each compound were determined. The final area percentage under the peaks for each compound was determined for quantitative analysis. From the areas, we reported the concentration values in μg/mg dry weight (DW) based on a calibration curve and the corresponding percentage value of each replicate. Data, composed of the mean of three replicates, were examined by a one-way analysis of variance (ANOVA) and by a Tukey's multiple range test to detect significant differences among means ($P \leq 0.05$). Before statistical analysis, percentage values were transformed to the Arc-sin of the square root of the percentage divided by 100.

Results

A total of 51 fatty acids were identified (Appendix 1). Table 1 shows the average concentration for 29 selected compounds (concentration > 0.1 μg/mg DW). The most representative fatty acid methyl esters (FAME) were C16:0 and C18:0. The hexadecanoic acid (C16:0) was over 16 μg/mg DW in all samples with greater amounts in the strains CM, CP, YX, and SF. The lowest values (< 8 μg/mg DW) of cis-11-hexadecenoic acid (C16:1 n5) were present in OX, YCE, and SF samples. The C18:0 FAMEs, 9-octadecenoic (C18:1 n9), 11-octadecenoic (C18:1 n7), and 9, 12-octadecadienoic (C18:2 n6) acids, were present in all samples. The minimum and maximum concentrations measured for the C18:1 n9 acid were 13.5 (YCE) and 42.8 μg/mg DW (YX). In all samples the concentration of C18:1 n7 was >6.5 μg/mg DW, with the CP sample having the highest concentration of 19.1 μg/mg DW. The C18:3 n3 acid was present in all samples with a maximum of 42.9 μg/mg DW in SF, with lower concentrations in the samples from OX (8.5 μg/mg DW), CP (8.1 μg/mg dw), and YCE (7.7 μg/mg dw) and the lowest for CG with 0.7 μg/mg DW. The C22:0 (docosanoic acid) was present in all samples but in concentrations <1.2 μg/mg DW, whereas the C22:6 n3 (docosahexaenoic acid) was not found in any sample.

Discussion

A number of different methods for lipid extraction have been used to obtain the needed amount and quality of the sample to be used for chromatography. The traditional extraction techniques, like those used by Folch et al. (1957) and Bligh and Dyer (1959), are widely used, although a number of variations to the technique have been made to analyze samples with particular characteristics (Kates, 1972; Sato and Murata, 1988). The direct transesterification technique used in this study has added precision (Lapage and Roy, 1984, 1986) in comparison to traditional extraction techniques (Folch et al., 1957; Bligh and Dyer, 1959). This technique is applicable to simple and complex lipids and is particularly advantageous for the recovery of triglycerides (Lepage and Roy, 1984, 1986). The use of gas chromatography-mass spectrometry analysis, compared to simpler gas chromatography analysis, allows the detection of compounds at lower concentrations (ng/mg) (Appendix 1).

The results obtained suggest that the fatty acid composition, especially the PUFA content of *Artemia* cysts, may be related to the latitudinal distribution of the population. The strain SF from a temperate area showed significant differences ($P < 0.05$) from the rest of the samples of tropical origin, having a greater concentration in the fatty acids 18:2 n5 (10, 13-octadecadienoic), 18:3 n3 (octadecatrienoic) and 18:4 n3 (6, 9, 12, 15-octadecatetraenoic) (Table 1). There was a higher total content of PUFAs in SF (65.3 μg/mg DW) compared to the tropical samples (14.3 to 40.3 μg/mg DW) (Table 1). Narciso and Morais (2001) also reported a high PUFA concentration of 102.8 μg/mg DW in the nauplii of *Artemia franciscana* from another temperate site, the Great Salt Lake, Utah, USA (Coutteau and Mourente, 1997). The SF strain showed the lowest proportion of the monounsaturated fatty acids (Table 2).

The variability in fatty acid composition of cysts and nauplii from salt works are probably correlated with changes of the existing water conditions, which are common in these extreme hypersaline habitats, such as temperature, salinity, and dissolved oxygen variation that affect the quality and

Table 1. Average concentration (μg/mg DW) ($n = 3$) for 29 selected fatty acids of *Artemia* decapsulated cysts. The populations analysed were from Cuba, El Real, Santa Lucía, Camagüey province (CG), and Frank País, Guantánamo province (CM), from México, Real de Salinas, Campeche (CP), La Colorada, Oaxaca (OX), Chuburna, Yucatán (YC), Celestún, Yucatán (YCE), Xtampú, Yucatán (YX), San Crisanto, Yucatán (YSC), and from the United States of America, San Francisco Bay, California (SF). Different superscripts in the same row denote significant differences ($P < 0.05$).

Fatty acid	CG[a]	CM[b]	CP[c]	OX[d]	YC[e]	YCE[f]	YX[g]	YSC[h]	SF[i]
14:0	1.86[f]	2.54	2.08	1.92	1.39	1.27[a]	2.10	1.60	1.64
iso 14:0	2.29[c]	2.32[c]	4.89[abdefghi]	1.25[ch]	2.01[c]	2.31[c]	1.81[c]	3.16[cd]	2.06[c]
15:0	1.78[dfghi]	2.01[defghi]	1.89[dfghi]	0.91[abc]	1.24[bfi]	0.23[abceh]	0.69[abc]	1.00[abcf]	0.33[abce]
iso 15:0	1.32[c]	1.50[c]	2.93[abdefghi]	0.61[ch]	1.22[c]	1.37[c]	0.98[c]	1.92[d]	1.22[c]
16:0	16.40[bc]	37.14[aef]	41.13[aef]	24.47	18.02[bc]	19.41[bc]	26.84	24.07	30.81
16:1 n7	2.74[bcg]	7.96[adefhi]	5.99[adefi]	2.35[bg]	1.80[bcg]	0.58[bcg]	7.83[aefhi]	2.97[bg]	1.94[bcg]
16:1 n5	19.31[dfi]	14.56	21.99[dfgi]	8.52[ac]	16.36[fi]	5.51[ace]	11.28[c]	14.36	5.13[ace]
iso 16:0	1.76[c]	1.97[c]	4.02[abdefghi]	0.74[ch]	1.88[c]	1.75[c]	1.29[c]	2.30[c]	1.24[c]
16:2	1.43[ghi]	1.96[dfghi]	1.32[gh]	0.57[b]	1.14	0.80[b]	0.45[abc]	0.46[abc]	0.50[ab]
17:0	3.49[fi]	3.31[fi]	3.70[fi]	1.87	3.25[fi]	0.82[abce]	1.82	2.63	1.00[abce]
17:1	3.98[fi]	5.44[defghi]	4.40[fghi]	2.29[bc]	2.71[b]	0.83[abc]	2.49[b]	2.15[bc]	0.88[abc]
18:0	5.73	5.19	8.15	4.55	5.43	5.59	5.20	6.54	8.59
18:1 n9	24.53	41.92[ef]	42.00[ef]	21.74	18.14[bcg]	13.59[bcg]	42.86[ef]	25.25	30.92
18:1 n7	16.37[d]	9.31[c]	19.18[bdfgi]	6.95[ac]	12.83	6.52[ac]	11.01	13.88	7.66[ac]
18:2 n5	0.03[fi]	0.02[fi]	0.09[i]	0.03[fi]	0.04[i]	0.12[abdi]	0.06[i]	0.07[i]	0.56[abcdefgh]
18:2 n6	16.22[bfghi]	27.80[adefghi]	20.46[defghi]	11.80[bc]	11.28[bc]	3.54[abc]	6.80[abc]	7.44[abc]	7.02[abc]
18:3 n6	0.25[ci]	0.41[fg]	0.50[adfgh]	0.26[ci]	0.34	0.18[bci]	0.21[bci]	0.30[ci]	0.52[adfgh]
18:3 n3	0.79[cdfi]	2.16[cdfi]	8.17[abegi]	8.55[abegi]	2.20[cdfi]	7.7 6[abei]	3.84[cdi]	4.63[i]	42.94[abcdefgh]
18:4 n3	0.29[cfgi]	0.48[cfi]	1.7[abdei]	0.66[ci]	0.61[ci]	1.35[abi]	1.15[ai]	0.99[i]	7.40[abcdefgh]
19:0	0.13	0.17[f]	0.13	0.06	0.10	0.03[b]	0.07	0.08	0.04
20:0	0.22	0.24	0.45	0.24	0.30	0.21	0.30	0.28	0.27
20:1 cis 11	0.58	0.81[f]	0.88[efh]	0.43[i]	0.38[ci]	0.17[bcgi]	0.80[fh]	0.22[bcgi]	1.04[defh]
20:1 cis 13	0.32	0.15	0.41[dfh]	0.10[c]	0.26	0.11[c]	0.21	0.13[c]	0.20
20:2 n6	0.11[b]	0.31[adegh]	0.19	0.12[b]	0.09[b]	0.19	0.11[b]	0.12[b]	0.25
20:3 n6	0.08	0.18[g]	0.17[g]	0.11	0.11	0.09	0.07[bc]	0.12	0.11
20:4 n6	0.86[b]	2.64[acefghi]	0.9[b]	2.04[fg]	1.05[b]	0.05[bdi]	0.37[bd]	1.11[b]	1.36[bf]
20:5 n3	1.87[bdi]	4.06[acefgh]	2.03[bdfi]	4.28[acefgh]	1.95[bfi]	0.23[bcdehi]	1.08[bdi]	2.02[bdfi]	4.48[acefgh]
22:0	0.70	0.85	1.12	0.59	0.72	0.70	0.79	0.75	0.80
24:0	0.15	0.27	0.33	0.11	0.14	0.22	0.29	0.23	0.28
Total saturated	38.4	59.4	75.0	38.8	38.1	35.7	44.3	48.2	51.4
Total mono - unsaturated	71.4	85.2	100.2	45.0	55.8	30.6	79.9	63.4	51.5
Total PUFA	22.2	40.3	35.9	28.6	19.2	14.8	14.3	17.4	65.3

quantities of phytoplankton blooms (Carpelan, 1957; Haynes and Hammer, 1978; Torrentera and Dodson, 2004). Mura et al. (1997, 1998, 2000), concluded that particular habitat conditions and biological factors such as food (quality and quantity), temperature, life stage, and sex have a direct impact on the fatty acid profile of fresh water anostracans.

According to the water regime, the tropical salterns studied can be divided in two types; those always having water present, such as El Real (CG), Frank País (CM), Campeche (CP), Oaxaca (OX), Chuburna (YC), and San Crisanto (YSC), and those that temporally become dry, such as Celestún (YCE) and Xtampú (YX). The Cuban samples (CG and CM)

Table 2. Comparison of the proportions (% of the total) of saturated, monounsaturated, and polyunsaturated fatty acids of *Artemia* of this study with those reported by different authors. The populations analysed in this study were from Cuba, El Real, Santa Lucía, Camagüey province (CG), and Frank País, Guantánamo province (CM), from México, Real de Salinas, Campeche (CP), La Colorada, Oaxaca (OX), Chuburna, Yucatán (YC), Celestún, Yucatán (YCE), Xtampú, Yucatán (YX), San Crisanto, Yucatán (YSC), and from the United States of America, San Francisco Bay, California (SF). 1. Direct transesterification with hydrochloric acid-methanol (Lapage and Roy, 1984, 1986). 2. Transmethylation with potassium hydroxide-methanol (Bligh and Dyer, 1959). 3. Homogenization in chloroform-methanol (Folch et al., 1957). 4. Transmethylation with toluene-methanol (Bligh and Dyer, 1959). 5. Homogenization in chloroform-methanol (Folch et al., 1957). [a] Bisexual *Artemia* cyst , [b] *A. franciscana* nauplii, [c] Decapsulated cyst, [d] *A. franciscana* nauplii.

Strain	Saturated	Monounsaturated	Polyunsaturated	Extraction methodoloy	Reference
CG	29.1	54.1	16.8	1	this study
CM	32.1	46.1	21.8	1	this study
CP	35.5	47.5	17.0	1	this study
OX	34.5	40.0	24.4	1	this study
YC	33.7	49.3	16.6	1	this study
YCE	44.2	37.5	18.3	1	this study
YX	32.0	57.7	10.3	1	this study
YSC	37.3	49.1	13.5	1	this study
SF	30.5	30.6	38.9	1	this study
ATP S.A.[a]	22.6	38.8	35.2	2	Navarro and Amat, 1992
INVE[b]	17.9	31.0	—	3	McEvoy et al., 1995
Frank País, Cuba[c]	34.1	42.9	20.3	4	Navarro et al., 1997
Great Salt Lake, USA[d]	19.7	36.9	—	5	Coutteau and Mourente, 1997

from two different types of water bodies showed significant differences ($P < 0.05$) in the concentration of six fatty acids (16:0, 16:1 n7, 18:2 n6, 20:2 n6, 20:4 n6 and 20:5 n3) (Table 1). This might be explained by environmental local differences at both locations despite their relatively small geographical separation. The Frank País, a permanent salt work (CM) is characterized by an annual precipitation of only about 450 mm, whereas the El Real salt work (CG) is affected every year by the rainy season, which has a direct impact on salinity, temperature, and nutrient flow (Menu, 1988). Before our study, Navarro et al. (1997) reported 4.9% (*Artemia* nauplii) and 8.1% (decapsulated cysts) of the fatty acid C20:5 n3 (eicosapentaenoic) in samples from the Cuban salt works of Frank País, but did not find the C22:0 acid (docosanoic).

Within the Mexican populations, the one from Campeche (CP) had the major differences in the fatty acids iso14:0, 15:0, iso15:0, 16:1 n7, iso16:0, and 16:1 n5 (Table 1). However, for the general fatty acid profile, the Campeche sample (CP) is closer to the Oaxaca (OX), Chuburna (YC), and San Crisanto (YSC) samples, which also come from permanent or semipermanent water bodies, thus not exhibiting extreme environmental conditions (Torrentera and Dodson, 2004). Mura et al. (1999, 2000), found considerable amounts of 20:5 n3 and 22:6 n3 in cysts of the fresh water fairy shrimp *Chirocephalus ruffoi* Cottarelli and Mura, 1984, and *C. kerkyrensis* Pesta, 1936, and concluded that individuals from different environments or fed different diets were characterized by different fatty acid profile. Of the studied *Artemia* cysts the quantities of 20:5 n3 are low and for 22:6 n3 not found at all.

In general, the fatty acids 16:0, 16:1 n7, 18:1 n9, 18:2 n6, 18:3 n3, and 20:5 n3 were found to make up to 80% of the fatty acid profile in strains of *Artemia* (Leger et al., 1986; Mura and Fancello, 2005). In our *Artemia* cysts, these fatty acids constituted from 57% to 87% of the fatty acid profile, whilst in fresh water fairy shrimp (*Branchipus*, *Chirocephalus*, and *Tanymastix*) were from 18.9% to 59.3% (Mura and Fancello, 2005).

The presence of iso-and anteiso-branched fatty acids in *Artemia* can be explained by the ingestion of halophytic bacteria, which are the predominant microbial populations in the last stages of the salt production systems (Suutari and Laakso, 1994). The fatty acid profile of marine algae and bacteria and the amount of different compounds may vary greatly depending on the species, season, and nutrient present (Dunstan et al., 1993; Intriago and Jones, 1993). The production of cysts and nauplii with a high content of n3 PUFA seems to be limited to natural biotopes where the water intake comes from a mangrove zone with a dominant presence of halophytic purple sulfur bacteria, and cyanobacteria, which are the main component of the brine shrimp diet (Sorgeloos et al., 1986).

Although compounds of great interest for aquaculture such as the PUFA were found, the concentration of C20:5 n3 (eicosapentaenoic acid) in the samples was low. The populations from CM, OX, and SF had concentrations of this compound >4 μg/mg DW. Usually the content of this fatty acid is low in *Artemia*, except in strains as those from Chaplin Lake, Canada and San Francisco Bay, USA (Leger et al., 1986). Quynh et al. (1988) also found large amounts (over 10%) of C20:5 n3 in *Artemia* inoculated in the salt works of Cam Ranh Bay, Vietnam without fertilizing the habitat. In aquaculture, nutritional deficiencies can be solved through the use of enrichment techniques developed by Watanabe et al. (1982, 1983) and now used for nutritional manipulation (Estévez and Kanazawa, 1996; McEvoy et al., 1996; Tocher et al., 1997). Of the three populations (CM, OX, and SF), only OX showed C20:5 n3 between 3% and 4% of the total of fatty acid content, therefore this population can be assessed as a "marine" type using the classification of Watanabe et al. (1978). Although an influence of the natural food on the fatty acid profile should be expected, the high level of C20:5 n3 in the OX strain may be an indication that a genetic component may exist that could favor the accumulation of certain compounds, which is worth being investigated. Navarro and Amat (1992) suggested that besides the phenotypic influence of live food in the fatty acid transfer process, the genotypic influence and the bioconversion capacity in *Artemia* are also factors that should be studied. Torrentera and coworkers proposed that the Yucatán populations are composed of distinct sibling species ecologically separated by habitat partitioning (Torrentera and Dodson, 1995; Torrentera and Abreu-Grobois, 2002; Torrentera and Dodson, 2004). Results of a phylogenetic analysis indicate that the two populations of *Artemia* from Cuba are closely related to *A. franciscana* of San Francisco Bay, whilst the six populations from México form a divergent group (Tizol-Correa et al., 2006).

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Appendix 1. Systematic name, short name, and concentration range (μg/mg DW) of 51 fatty acids identified from *Artemia* decapsulated cysts of eight tropical sites (El Real, Santa Lucía, Camagüey province, Cuba; Frank País, Guantánamo province, Cuba; Real de Salinas, Campeche, México; La Colorada, Oaxaca, México; Chuburna, Yucatán, México; Celestún, Yucatán, México; Xtampú, Yucatán, México; San Crisanto, Yucatán, México), and one temperate site (San Francisco Bay, California, USA).

		Range	
Systematic name	Short name	Min	Max
dodecanoic	12:0	0.06	0.16
11-methyl dodecanoic	iso 12:0	0.07	0.14
tridecanoic	13:0	0.03	0.16
12-methyl tridecanoic	iso 13:0	0.23	0.38
tetradecanoic	14:0	1.27	2.54
tetradecenoic	14:1	0.12	0.65
tetradecenoic	14:1	0.15	0.35
methyl 13-methyl tetradecanoic	iso 14:0	0.01	0.18
methyl 19-methyl tetradecanoic	iso 14:0	1.25	4.89
methyl 12-methyl tetradecanoic	ante iso 14:0	0.45	0.80
pentadecanoic	15:0	0.23	2.01
pentadecenoic	15:1	0.13	2.33
pentadecenoic	15:1	0.04	1.13
pentadecenoic	15:1	0.02	0.14
14-methyl pentadecanoic	iso 15:0	0.61	2.93
13-methyl pentadecanoic	iso 15:0	0.01	0.05
tetramethyl 2,6,10,14-pentadecanoic	iso 15:0	0.01	0.22
hexadecanoic	16:0	16.40	41.13
cis 9-hexadecenoic	16:1 n7	0.58	7.96
cis 11-hexadecenoic	16:1 n5	5.13	21.99
hexadecenoic	16:1	0.11	0. 52
methyl 15-hexadecanoic	iso 16:0	0.74	4.02
hexadecadienoic	16:2	0.45	1.96
14-methyl hexadecanoic	ante iso 16:0	0.21	1.67
hexadecadienoic	16:2	0.07	0.47
heptadecanoic	17:0	0.82	3.70
heptadecenoic	17:1	0.83	5.44
heptadecenoic	17:1	0.04	1.23
heptadecenoic	17:1	0.04	0.17
heptadecenoic	17:1	0.04	1.81
16-methyl heptadecanoic	iso 17:0	0.02	0.10
heptadecadienoic	17:2	0.02	0.19
octadecanoic	18:0	4.55	8.59
9-octadecenoic	18:1 n9	13.59	42.86
11-octadecenoic	18:1 n7	6.52	19.18
octadecenoic	18:1	0.08	0.19
9,12-octadecadienoic	18:2 n6	0.02	0.12
octadecadienoic	18:2 n3	3.54	27.80
octadecatrienoic	18:3 n6	0.21	0.52
nonadecanoic	19:0	0.04	0.17
octadecatrienoic	18:3 n3	0.79	42.94
6,9,12,15-octadecatetraenoic	18:4	0.29	7.40
eicosanoic	20:0	0.21	0.45
cis 11-docosenoic	20:1 cis 11	0.17	1.04
cis 13-docosenoic	20:1 cis 13	0.10	0.41
11,14-eicosadienoic	20:2 n6	0.09	0.31
8,11,14-eicosatrienoic	20:3 n6	0.07	0.18
5,8,11,14-eicosatetraenoic	20:4 n6	0.09	2.64
cis 5,8,11,14, 17-eicosapentaenoic	20:5 n3	0.23	4.48
docosanoic	22:0	0.59	1.12
tetracosaenoic	24:0	0.11	0.29