Vol. 335: 155–165, 2007 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published April 16

Diversity of the fatty acid composition of *Artemia* spp. cysts from Argentinean populations

O. Ruiz[1,*], G. R. Medina[1], R. G. Cohen[2], F. Amat[1], J. C. Navarro[1]

[1]Instituto de Acuicultura de Torre de la Sal (CSIC), 12595 Ribera de Cabanes, Castellón, Spain
[2]Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C/Ciudad Universitaria, Pabellón II, Buenos Aires 1428, Argentina

ABSTRACT: Nauplii of the branchiopod crustacean *Artemia* spp. are widely used as live food in aquaculture. Their nutritional value depends essentially on the fatty acid profile of their lipids, which can be very variable. Study of this variability (often species- and/or ecology-related) proposes interesting theoretical and applied questions. In the present study, cysts (dehydrated dormant eggs) from Argentinean *Artemia* spp. populations were analysed for their fatty acid profile. Most of the cyst samples analysed (n = 16) were obtained from *Artemia persimilis* populations, but 3 belonged to *A. franciscana*. Previous research indicated that *A. persimilis* populations are confined to inland biotopes, but in this study we report the presence of *A. persimilis* populations in coastal ecosystems. Cysts from all populations exhibited fatty acid profiles typical of either marine or freshwater, irrespective of the inland or coastal origin of their biotope. This study represents the first time that a significant amount of eicosapentaenoic acid (EPA, 20:5n-3) was detected in *A. persimilis* cyst samples. We hypothesize on some factors that presumably influence the fatty acid profiles of *Artemia* spp. cysts: (1) food resources from different habitats, which in turn can be influenced by environmental parameters, (2) the genetic make-up of each population, and/or (3) mechanisms of selective feeding.

KEY WORDS: *Artemia* · Fatty acids · Inland waters · Salt lakes · Ionic composition · Brines · Marine type · Freshwater type

INTRODUCTION

The biochemical composition of *Artemia* spp., in particular the fatty acid profiles of cysts (dehydrant dormant eggs) and nauplii, has been extensively studied because of the use of nauplii as live food in aquaculture (Bengtson et al. 1991). Yet scarce information is available on the factors that determine fatty acid composition and thus the nutritional value of diverse *Artemia* spp. as live food.

There are 2 limiting factors that constrain the use of *Artemia* nauplii in marine aquaculture. The first is related to the shortage of *Artemia* spp. resources, evidenced by the *Artemia* cyst crisis of the Great Salt Lake of Utah, GSL (Van Stappen 1997), which was exacerbated by a massive increase in cyst demand corresponding with increases in aquaculture world-wide. The second is fatty acid composition, which determines the nutritional value of different stocks as food for marine fish larvae.

At the Kyoto FAO technical conference on Aquaculture in 1976, the supply of *Artemia* spp. cysts was already foreseen as a restrictive factor in the development of world aquaculture. Since the mid 1980s, 90% of cysts consumed in world-wide aquaculture have been sourced from the GSL; consequently, Bengtson et al. (1991) strongly recommended that alternative cyst sources be prospected. Confounding this problem was the rapid spread of enrichment techniques (i.e. supplementation of essential nutrients by bioencapsulation) that allowed the use of nauplii of poor nutritional value in the larviculture of marine species and led to even more intensive harvesting of the cysts from the GSL and concomitant overexploitation of this ecosystem. Unsustainable practices, together with climatic phenomena like the El Niño Southern Oscillation (ENSO)

*Email: olga@iats.csic.es

and anthropogenic impacts, initiated a decrease in cyst harvesting, which then led to market fluctuations in cyst availability and quality and to increasing prices (Lavens & Sorgeloos 2000).

Presently, the maximum sustainable volume of harvestable cysts in the GSL is restricted to 6000 to 7000 t (wet weight) (Van Stappen 1997), and production in the GSL alone is not sufficient to supply the ever-expanding aquaculture industry. This situation has led to a more exhaustive study of *Artemia* biogeography, to the world-wide prospecting of sources of cysts, and to the exploitation of cysts from ecosystems that were previously poorly studied. Among these, the Argentinean sources deserve special attention because this country contains a broad range of saline lakes, lagoons and solar saltworks. Most of these environments are suitable for the development of natural populations of brine shrimp.

Amat et al. (1994) and Cohen et al. (1999) listed 4 locations between La Pampa and Buenos Aires provinces that represented alternative sources of cysts, and provided further evidence that the species involved was *Artemia persimilis*. Based on cytogenetic proofs, Papeschi et al. (2000) reported for the first time the presence of *A. franciscana* in Argentina. Subsequent cytogenetic analyses of *Artemia* from 4 Argentinean populations (Lipko et al. 2004) confirmed the presence of *A. franciscana*, and Amat et al. (2004) reported the presence of *A. franciscana* in Mar Chiquita salt lake, Las Tunas lagoon and a shallow lake in Salinas Grandes (Córdoba province). Amat et al. (2004) went on to confirm that *A. franciscana* was distributed north of 36° S and that *A. persimilis* was distributed south of 37° 10′ S.

The validity of biomass and polyunsaturated fatty acid (PUFAs) profiles of *Artemia* nauplii as indices of nutritional value required to meet the elemental nutritional requirements of marine fish and crustacean larvae has been discussed extensively. It is known that nauplii from different cyst stocks comprise diverse and variable amounts of highly unsaturated fatty acids (HUFA) that are essential for marine organisms (Fujita et al. 1980). Watanabe et al. (1978) divided *Artemia* cyst samples into 2 categories according to their fatty acid profiles: freshwater-type *Artemia*, with a high concentration of linolenic acid (LNA, 18:3n-3) and low concentration of eicosapentaenoic acid (EPA, 20:5n-3), which are only suitable for feeding freshwater animals; and marine-type *Artemia*, with a higher EPA content and generally lower LNA, and which are more suitable for culturing marine species. It is widely acknowledged that some HUFA, i.e. EPA and docosahexaenoic acid (DHA, 22:6n-3), are essential requirements of marine species if adequate larval growth and survival is to be obtained (Sargent et al. 1997). Essential fatty acids in general, and DHA in particular, are ubiquitous in natural marine food resources (i.e. copepods), but larvae are not capable of their synthesis — hence use of the term 'essential dietary requirement'. Of importance are reports demonstrating that DHA is either absent or present only in trace amounts in *Artemia* cysts and nauplii (Navarro et al. 1992a, Léger et al. 1986).

The generalization that *Artemia* populations from coastal hypersaline biotopes are associated with high percentages of EPA, and those from inland ecosystems with increased amounts of LNA (Vos et al. 1984) is not true in all cases. Navarro et al. (1992a) reported on *Artemia* populations from inland that produced cysts with quantities of EPA as well as high levels of LNA. This 'intermediate' type of *Artemia* was also reported by Watanabe et al. (1978).

At present, information on the fatty acid composition of *Artemia* focuses mainly on the presence/absence of some fatty acids in cysts and nauplii of different populations: Amat et al. (2004) studied *A. franciscana* populations at Las Tunas and Mar Chiquita, and Sato et al. (2004) studied an *A. persimilis* population from Salinas Chicas de Buenos Aires, and an *A. franciscana* population from Salinas Grandes de Córdoba. The latter was presumably mistakenly reported (by Sato et al. 2004) as *A. persimilis* (Amat et al. 2004). The specific underlying factors that determine particular fatty acid profiles remain unclear.

Between 1992 and 2003, Argentina was surveyed for hypersaline biotopes and the presence of brine shrimp populations. A total of 17 new sampling spots from the provinces of Santiago del Estero, Córdoba, Santa Fe, Buenos Aires, La Pampa, Río Negro, Chubut and Santa Cruz were visited. The survey was not comprehensive, because the northern and western provinces could not be included for logistic reasons; however, the survey was at least representative of the areas of *Artemia* distribution within Argentina.

This study provides detailed information on the fatty acid profiles of *Artemia* cysts sampled in Argentina. Our objectives were to (1) gain an understanding of the factors that determine the fatty acid composition of *Artemia* cysts, and (2) obtain information of interest to aquaculture.

MATERIALS AND METHODS

Location and biotope characteristics. Evidence for the presence of brine shrimp was found in most biotopes, either as live populations under conditions of low salinity, or as accumulations of cysts along shores where high salinity brines did not allow the persistence of living populations. The biotopes and populations from where cysts were sampled are summarized

in Table 1. Mar Chiquita and Las Tunas populations, described by Amat et al. (2004), are included for the sake of comparison. The biotopes were very diverse: most were located inland, but a few were coastal (El Inglés, Caleta Olivia and San Julián) (Fig. 1). Biotope size was also extremely diverse, ranging from a few hectares at Caleta Olivia, San Julián and Ruta 3 to the small inland sea of Mar Chiquita, whose variable surface area comprises between 200 000 and 500 000 ha (Amat et al. 2004). Generally, most ecosystems covered a few thousand ha; some lay in shallow depressions above sea level, whereas others enclosed deep endorheic basins, such as Gualicho (Bajo Gualicho salt mine), located 45 m below sea level.

Table 1. Summary of sites prospected for *Artemia* cyst populations in Argentina. Salitral Negro sampled in 2 areas—2S and 3S

Province	Locality (species)	Area (ha)	Region	Latitude, longitude
Santiago del Estero	El Saladillo (*A. franciscana*)	6100	Inland	28° 20′ S, 62° 70′ W
Córdoba	Mar Chiquita (*A. franciscana*)	500 000	Inland	30° 20′ S, 62° 10′ W
	Las Tunas (*A. franciscana*)	1700	Inland	33° 45′ S, 62° 32′ W
La Pampa	Colorada Chica (*A. persimilis*)	1100	Inland	38° 23′ S, 63° 36′ W
	Salinas Grandes de Hidalgo (*A. persimilis*)	3900	Inland	37° 13′ S, 63° 26′ W
	Colorada Grande (*A. persimilis*)	6740	Inland	38° 18′ S, 63° 42′ W
	Callaqueo (*A. persimilis*)	2140	Inland	38° 34′ S, 63° 32′ W
	Salitral Negro 2S (*A. persimilis*)	2400	Inland	38° 44′ S, 63° 13′ W
	Salitral Negro 3S (*A. persimilis*)	2400	Inland	38° 45′ S, 63° 27′ W
Buenos Aires	Piedras (*A. persimilis*)	2200	Inland	40° 41′ S, 62° 40′ W
	Algarrobo (*A. persimilis*)	200	Inland	40° 36′ S, 62° 56′ W
	El Inglés (*A. persimilis*)	200	Coastal	40° 43′ S, 62° 28′ W
	Palos Blancos (*A. persimilis*)	300	Inland	39° 28′ S, 62° 45′ W
	Salinas Chicas (*A. persimilis*)	3460	Inland	38° 43′ S, 62° 56′ W
	Salitral de la Vidriera (*A. persimilis*)	5000	Inland	38° 42′ S, 62° 40′ W
Río Negro	Gualicho (*A. persimilis*)	32 800	Inland	40° 24′ S, 65° 13′ W
Santa Cruz	Caleta Olivia (*A. persimilis*)	30	Coastal	46° 27′ S, 67° 32′ W
	San Julián) (*A. persimilis*)	42	Coastal	49° 17′ S, 67° 46′ W
	Ruta 3 (*A. persimilis*)	12	Inland	47° 28′ S, 67° 16′ W

Collection of samples. Samples of cysts were collected from the surface of brines or the banks of salt ponds or pools and preserved in saturated brine. Once in the laboratory, they were processed as described by Navarro (1985).

When present (see Table 3), brines were evaluated *in situ*. Temperature and pH were measured with a digital thermometer and pH meter (Hanna Instruments); salinity was recorded with a Shibuya S-1 rephractometer (Optical Co.). Brine samples were collected and transported to the laboratory for ionic analysis. Carbonates and bicarbonates were determined by acid titration, chloride by $AgNO_3$ titration, and sulphate by precipitation titration with $BaCl_2$ and sodium rodizonate as an indicator in water-acetone medium. Na^+ and K^+ were analysed by atomic spectrophotometric emission, and Ca^{2+} and Mg^{2+} by atomic spectrophotometric absorption (Varian Techtron Model AA275) (Apha 1993). Analyses were performed in duplicate, and all relative errors were < 1.0%.

Total dissolved solids (TDS) were calculated as the sum of the above ion concentrations, because these accounted for most of the dissolved salts.

Fatty acid analysis. Lipid extractions and fatty acid analyses were carried out as in Navarro et al. (1992a,b). Prior to lipid extraction, cyst samples were hydrated in distilled water under strong aeration until cysts were observed to be completely spherical. They were then decapsulated with sodium hypochlorite. Total lipids were extracted and stored in chloroform/methanol (ratio 2/1 v/v) with 0.01% butylated hydroxytoluene (BHT) (Sigma Chemical) as an antioxidant. Lipid aliquots were transmethylated overnight after the addition of nonadecaenoic fatty acid (19:0) (99% pure; Sigma Chemical) as an internal standard. Fatty acid methyl esters (FAMEs) were extracted with hexane/diethyl ether (ratio 1/1 v/v) and purified by thin-layer chromatography (silica gel G 60, Merck) using hexane/diethyl ether/acetic acid (ratio 85/15/1.5 by vol.) as the solvent

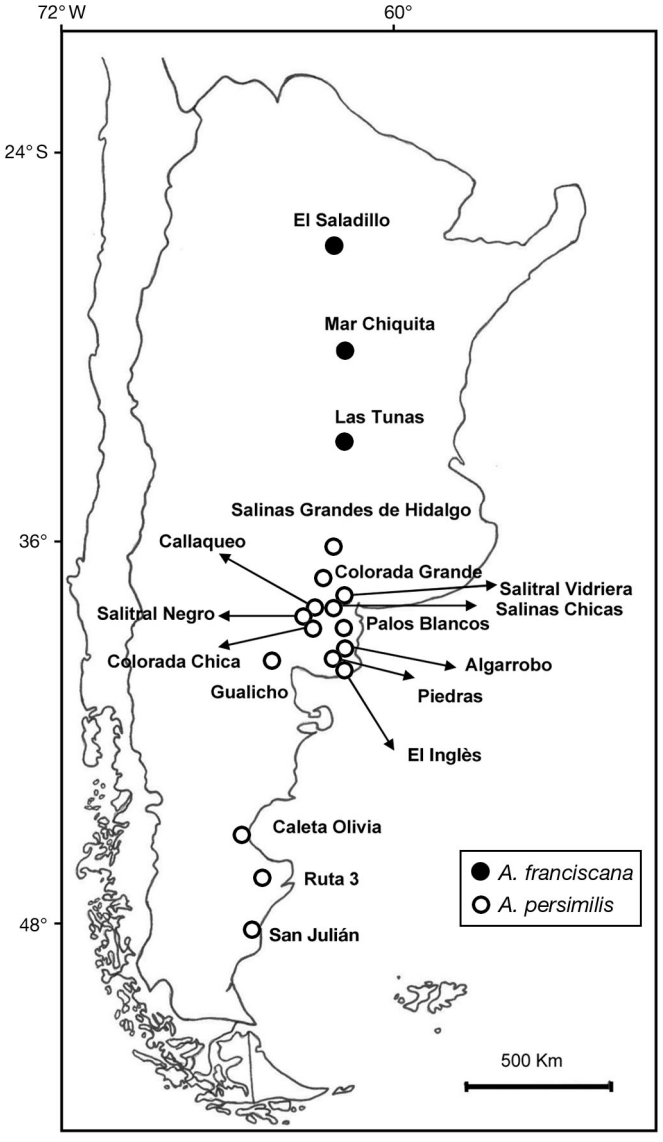

Fig. 1. Sampling sites of Argentinean *Artemia* populations

system. Analyses of FAMEs were performed with a Fisons Instruments GC 8000 (Thermo Electron) gas chromatograph equipped with a fused silica 30 m × 0.25 mm open tubular column (tracer, TR-WAX, film thickness: 0.25 µm; Teknokroma) and a cold on-column injection system, using helium as carrier and a 50 to 220°C thermal gradient. Peaks were recorded on a personal computer using the software Chrom-Card for Windows (Fisons CE Intstruments), and were identified by comparison with known standards. All solvents used in lipid extractions and fatty acid analyses were analytical grade and purchased from Merck, with the exception of glacial acetic acid that was acquired from Panreac.

Statistical analyses. Fatty acid data were analysed statistically using a chemometric method. Variables were entered into SPSS version 12.0 in order to conduct multivariate principal components analysis (PCA). In this analysis, different variables (relative proportions of selected fatty acids) are displayed on a single plane (principal axis) that accounts for maximum dispersion (variance). Principal component plots are positions of the original variables along the new axes (principal components; PC). Points in the component plot are the variables, and the coordinates of each variable are its factor loadings. Variables at the end of each axis have high loadings on only that factor. Variables near the intersection of the axes are associated with neither factor. The graphical representation (factor score plot) of scores of cases shows the relationships among populations, and is also useful for identifying outliers and unusual cases. The 9 most common fatty acids were used as variables (palmitic [16:0], estearic [18:0], palmitoleic [16:1n-7], *cis*-7-hexadecenoic [16:1n-9], *cis*-vaccenic [18:1n-7], oleic [18:1n-9], linoleic [18:2n-6], linolenic [18:3n-3], eicosapentaenoic [20:5n-3]), in addition to the 16:0/16:1 ratio.

The ionic composition of the brine samples available, together with data from literature (Angelelli et al. 1976, Amat et al. 2004), were plotted in a Piper diagram. With this method it is possible to display specific cations (Ca^{2+}, Mg^{2+}, Na^{+} + K^{+}) and specific anions (CO_3H^{-} + CO_3^{2-}, Cl^{-}, SO_4^{2-}) as percentages of total cations and anions, respectively, on separate trilinear diagrams. The 2 points obtained for every sample in both trilinear diagrams are then projected onto a central diamond-shaped plot, parallel to the upper edges of the trilinear diagrams. This method allows the graphical representation of the classification of waters by hydrochemical facies, with the added benefit of allowing hydrochemical mixing trends to be identified.

RESULTS

According to biometrical, morphometrical, cytogenetic and DNA marker studies in progress, the population from El Saladillo (province of Santiago del Estero) together with those from Laguna Mar Chiquita and Las Tunas (previously reported by Amat et al. 2004) represented *Artemia franciscana*. The remainder of the populations included in the present study represented *A. persimilis*, as determined by morphometric studies and results obtained by the Instituto de Acuicultura Torre de la Sal (CSIC) (unpubl. data).

Fatty acid profiles of cysts from the Argentinean *Artemia* populations investigated are shown in Table 2. Based on the fatty acid profile of decapsulated *Artemia franciscana* cysts, populations from Mar Chiquita and Las Tunas Lagoons could be ascribed to marine-type *Artemia* (high EPA, low LNA,

Table 2. Fatty acid composition (% of total fatty acids) of total lipids from Argentinean populations of *Artemia* cysts. Data are mean of 3 replicates. Variability <10%. LNA: linolenic acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; tr.: trace amounts; nd: not detected; PUFA: polyunsaturated fatty acids; n-3 HUFA: ≥20:3n-3; n-6 HUFA: ≥20:2n-6

	Population																			
	Freshwater-type[a]												Marine-type[a]							
Fatty acid	El Saladillo (inland)	Hidalgo (inland)	Colorada Grande (inland)	Colorada Chica (inland)	Callaqueo (inland)	Salitral Negro 2S (inland)	Salitral Negro 3S (inland)	Piedras (inland)	El Inglés (coastal)	Salinas Chicas (inland)	Gualicho (inland)	Caleta Olivia (coastal)	Las Tunas[b] (inland)	Mar Chiquita[b] (inland)	Salitral Vidriera (inland)	Algarrobo (inland)	Palos Blancos (inland)	Ruta 3 (inland)	San Julián (coastal)
14:0	nd	0.7	0.6	0.7	0.7	0.7	0.5	1.0	0.7	0.5	2.6	0.7	1.1	1.3	1.7	1.6	0.7	4.5	1.9
14:1	nd	1.8	nd	2.1	nd	nd	nd	1.4	1.9	1.4	1.3	nd	1.2	0.6	1.4	1.2	0.6	1.0	nd
15:0	0.6	0.3	0.2	0.3	0.2	0.1	0.3	0.4	0.4	0.2	0.4	0.3	0.9	0.5	0.8	0.5	0.5	0.9	1.1
15:1	1.0	1.7	0.7	1.4	0.5	0.7	0.5	nd	nd	1.3	nd	0.3	0.7	0.3	nd	nd	nd	nd	0.2
16:0	15.7	14.6	13.8	12.4	13.3	13.7	11.8	17.1	14.0	12.8	28.9	13.4	12.7	15.6	17.8	14.7	15.2	19.7	15.8
16:1n-9	0.9	0.5	0.4	0.1	0.4	0.5	0.5	0.4	0.3	0.1	0.1	1.2	nd	0.6	1.1	nd	nd	2.5	0.4
16:1n-7	6.0	5.7	4.9	7.4	5.1	4.9	5.4	6.3	7.1	4.3	7.2	6.0	10.8	10.1	15.8	20.8	26.4	13.4	16.5
16:2	nd	0.2	0.4	0.4	0.2	0.5	0.3	nd	0.2	0.1	0.4	0.3	0.6	0.3	0.7	0.6	0.5	1.1	0.5
17:0	1.9	0.4	0.8	0.1	0.8	1.0	0.8	0.5	0.6	0.1	0.6	0.7	nd	nd	1.0	0.8	0.6	1.0	1.2
16:3	1.7	1.4	1.7	1.1	1.6	1.4	1.2	2.2	1.2	1.0	2.8	0.9	4.1	1.5	1.5	0.9	0.9	1.3	2.83
18:0	4.1	4.7	4.1	4.4	4.0	4.1	3.7	4.5	4.4	3.9	5.7	4.1	3.7	3.7	4.8	4.7	4.1	5.0	4.8
18:1n-9	17.5	14.2	15.4	13.5	14.9	15.2	14.3	14.0	13.7	12.8	16.3	18.3	16.2	18.9	13.5	12.7	11.4	15.0	14.5
18:1n-7	8.1	7.8	6.6	10.9	7.1	6.2	7.3	7.8	8.6	6.4	5.2	9.3	9.4	7.1	12.5	12.3	12.5	9.3	8.4
18:2n-6	10.5	3.8	5.5	5.3	5.7	5.7	5.3	4.2	4.0	4.3	3.6	4.0	3.3	4.1	1.8	1.8	1.7	2.3	2.9
18:3n-6	nd	nd	nd	nd	nd	0.1	nd	nd	nd	nd	nd	nd	0.3	0.1	0.3	0.3	0.2	nd	0.2
18:3n-3 (LNA)	15.5	23.6	24.6	21.7	24.4	24.0	25.2	26.9	24.0	30.2	12.5	17.0	8.8	14.9	2.1	1.8	6.5	1.4	2.8
18:4n-3	2.1	5.4	5.6	4.6	5.6	5.6	5.7	5.1	5.9	7.0	2.3	4.8	0.8	3.3	1.1	1.0	1.9	1.0	1.5
20:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.3	nd	0.3	nd	nd	nd	nd	0.3	0.2
20:1n-9	0.3	0.3	0.3	0.1	0.3	0.2	0.3	0.3	0.3	0.3	0.8	0.3	0.3	0.3	0.4	0.3	0.3	0.9	0.3
20:1n-7	0.3	nd	nd	nd	nd	0.1	nd	nd	nd	nd	0.2	nd	0.1	nd	0.1	nd	0.1	nd	nd
20:2n-6	nd	0.2	0.2	0.2	0.2	0.1	0.2	nd	0.1	0.2	0.1	nd	0.1	0.2	nd	nd	nd	nd	nd
20:3n-6	nd	0.2	nd	0.1	nd	nd	nd	nd	0.2	0.2	0.1	tr	0.1	0.1	0.2	0.2	0.2	0.3	1.3
20:3n-3	nd	0.5	0.6	0.2	0.5	0.7	0.5	0.2	0.3	0.6	0.2	0.3	nd	0.2	nd	nd	nd	nd	nd
20:4n-6 (ARA)	0.5	0.1	nd	nd	nd	nd	nd	nd	0.2	0.1	0.1	0.8	1.8	1.1	1.1	1.6	0.6	1.8	1.3
20:4n-3	0.6	0.7	0.8	0.6	0.8	0.8	0.8	0.4	0.6	0.8	0.3	0.8	0.2	0.7	0.4	0.3	0.4	0.3	0.4
20:5n-3 (EPA)	3.6	0.2	0.1	0.1	0.5	0.1	0.2	0.3	1.4	0.3	1.1	10.1	11.6	8.7	14.4	16.0	11.0	9.7	14.2
22:0	0.4	nd	0.1	0.2	0.3	0.1	0.3	0.3	0.2	nd	0.4	0.2	0.2	nd	nd	nd	nd	nd	0.3
22:5n-3	nd	nd	nd	nd	nd	nd	nd	nd	0.1	nd	0.3	nd	nd	nd	0.3	nd	nd	0.6	nd
22:6n-3 (DHA)	nd	nd	nd	nd	0.2	nd	nd	nd	nd	nd	tr	tr	nd	tr	nd	nd	tr	1.5	tr
24:1n-9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	91.3	89.3	87.5	88.1	87.2	86.3	85.2	92.8	90.2	88.9	93.1	93.7	90.1	94.4	93.8	94.1	96.5	95.4	92.4
Saturates	22.7	20.7	19.5	18.0	19.3	19.6	17.4	23.5	20.3	17.5	38.4	19.4	18.4	21.2	26.0	22.3	21.0	31.4	25.2
Monoenes	34.1	32.3	28.4	36.0	28.3	27.7	28.3	30.0	31.9	26.8	31.1	35.4	39.4	37.8	44.0	47.3	51.7	42.8	40.3
PUFA	34.5	36.3	39.6	34.2	39.6	39.0	39.6	39.3	38.0	44.7	23.6	39.0	32.0	35.1	23.8	24.5	23.9	21.2	26.9
n-3	21.3	30.4	31.8	27.2	31.9	31.2	32.2	32.8	32.2	38.9	16.7	33.0	21.4	27.7	18.2	19.2	19.9	14.5	19.0
n-6	11.0	4.3	5.7	5.6	5.9	5.8	5.5	4.3	4.6	4.7	3.8	4.8	5.6	5.5	3.5	3.9	2.7	4.3	5.7
n-3 HUFA	4.2	1.5	1.6	0.8	2.0	1.6	1.6	0.8	2.3	1.7	2.0	11.2	11.9	9.5	15.0	16.4	12.0	12.0	14.8
n-6 HUFA	0.5	0.3	0.2	0.2	0.2	0.1	0.2	0.0	0.4	0.2	0.1	0.8	2.0	1.3	1.4	1.8	0.8	2.0	2.7
16:0/16:1	2.3	2.4	2.0	1.7	2.4	2.6	2.0	2.7	1.9	2.9	4.0	1.9	1.1	1.4	1.1	0.7	0.6	1.2	0.9

[a]Classification according to Watanabe et al. (1978)
[b]Populations described by Amat et al. (2004)

16:0/16:1 ratio ≤ 1; Navarro et al. 1992b), whereas the population from El Saladillo could be categorised as freshwater-type *Artemia* (high LNA, low EPA). Among the cysts of *A. persimilis* populations, those from Salitral de la Vidriera, Palos Blancos, Algarrobo, Ruta 3 and San Julián were marine-type. Cysts from all the other *A. persimilis* were freshwater-type, exhibiting 16:0/16:1 ratios close to 2 or higher, as in the case of Gualicho.

Palmitic acid (16:0) was found in relatively constant proportions (range: 11.8 to 19.7% of total fatty acids) in the cysts of all populations regardless of type, again with the exception of Gualicho where 16:0 contributed 28.9%. Proportions of palmitoleic acid (16:1n-7) were higher in marine-type populations. Arachidonic acid (ARA, 20:4n-6) was found in all marine-type cysts, but was present in only a few freshwater-type cyst samples and in lower quantities, except in those samples from Caleta Olivia.

High proportions of n-3 HUFA were found in the marine-type when compared with freshwater-type cysts, again with the exception of Caleta Olivia (Table 2). As expected, DHA was generally absent, or present only in trace amounts in some samples, for both kinds of cysts. Ruta 3 was an exception: in this population, DHA accounted for 1.5% of total fatty acids.

The component plot (Fig. 2) obtained from a PCA shows variables that were responsible for separation along the 2 PCs calculated. The 2 PCs of this analysis accounted for 71% of the variation in the data set. When the fatty acid data were projected onto the PCs generated, a factor score plot was obtained (Fig. 3) in which the populations were grouped according to variable (fatty acid) loadings. Variables defining marine- (16:1n-7, 20:5n-3, 18:1n-7) or freshwater-type (18:2n-6, 18:3n-3, 16:0/16:1 ratio) are associated with Component 1, and both sets are negatively correlated. Freshwater-type populations are located on the righthand side of Component 1, and marine-type populations on the lefthand side. Intermediate-type populations are grouped between both. Note that the population of Gualicho has the most extreme score in Factor 2, being 3.4 SD larger than the mean Factor 2 score. This population is an outlier because of its high 16:0/16:1 ratio. Populations were essentially grouped according to their profile type — marine vs. freshwater — and grouping could not be associated with coastal/inland locations, providing evidence for the presence of both freshwater- and marine-types regardless of geographical distribution.

Table 3 gives the concentrations of major ions, expressed as % of total major ions. These concentrations, displayed on the cation/anion triangles in the Piper plot (Fig. 4), represent sodium- and chloride-dominated brines, classified as Na^+-Cl^- facies, which are typical of marine and deep ancient ground waters.

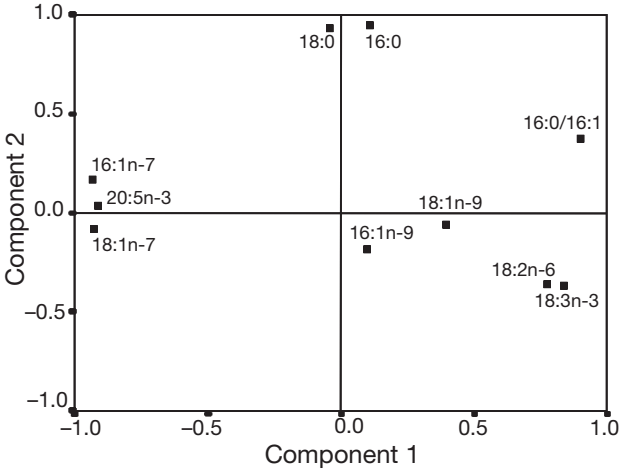

Fig. 2. Component plot of principal component analysis (PCA) of selected fatty acids from total lipids of Argentinean *Artemia* cysts

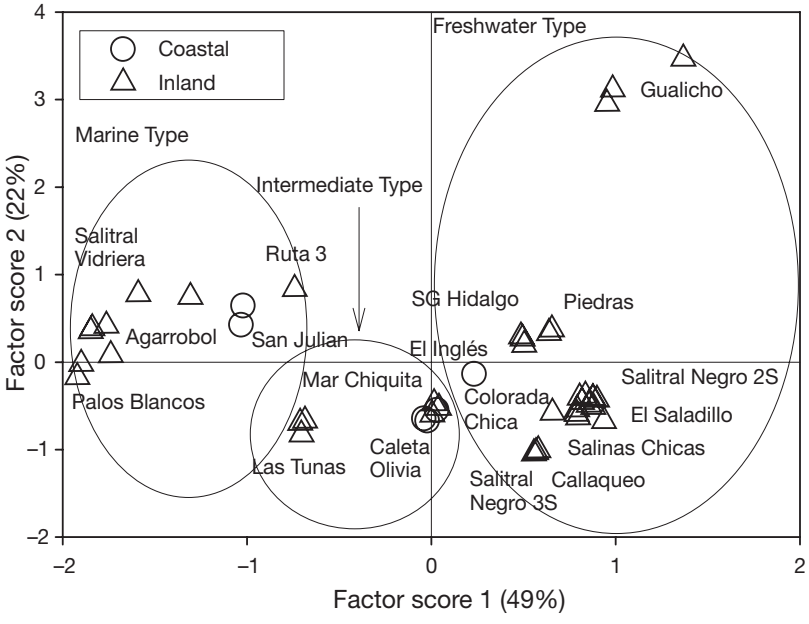

Fig. 3. Factor score plot of PCA of selected fatty acids from total lipids of Argentinean *Artemia* cysts. Grouping is based on the freshwater- (righthand side of Factor 1, *x*-axis) or marine-type (lefthand side of Factor 1) fatty acid character of *Artemia* cysts

Table 3. Physicochemical parameters and ion chemistry of brines of selected sampling sites. Values are mg l^{-1} unless otherwise stated. S: salinity

	Mar chiquita[a] (Chaco-Pampa Plain)	Las Tunas[a] (Chaco-Pampa Plain	El Saladillo[b] (Chaco-Pampa Plain)	Hidalgo[b] (Chaco-Pampa Plain)	Piedras (Patagonia Plateau)	El Inglés (Patagonia Plateau)	Salinas Chicas (Chaco-Pampa Plain)	Salitral Vidriera (Chaco-Pampa Plain)	Palos Blancos (Chaco-Pampa Plain)	Caleta Olivia (Patagonia Plateau)	Ruta 3 (Patagonia Plateau)	San Julian (Patagonia Plateau)
T (°C)	30	34	–	–	18	26	19.9	24	26	9	7.50	6
S (‰)	48	40	185	300	345	240	–	280	270	230	12	80
pH	8.1	9	7.72	–	7	7	7.77	7.1	6.7	7.68	8.80	8.40
Ca^{2+}	21100	20000	524	11900	969	798	857	826	9890	684	68	1522
Mg^{2+}	8000	172000	3.1	33000	15120	10224	2112	9168	6864	3826	10	1320
Na^{+}	317900	299400	77.4	361000	97431	110819	111600	105613	102789	84728	1904	25585
K^{+}	29600	22300	1.5	3800	3591	6949	4156	2693	765	1004	40	206
Σ cations (ppm)	376600	513700	606	409700	117111	128790	118725	118300	120308	90242	2022	28633
% Ca^{2+}	5.60	3.89	86.47	2.90	0.83	0.62	0.72	0.70	8.22	0.76	3.36	5.32
% Mg^{2+}	2.12	33.48	0.51	8.05	12.91	7.94	1.78	7.75	5.71	4.24	0.49	4.61
% Na^{+}	84.41	58.28	12.77	88.11	83.20	86.05	94.00	89.28	85.44	93.89	94.16	89.35
% K^{+}	7.86	4.34	0.25	0.93	3.07	5.40	3.50	2.28	0.64	1.11	1.98	0.72
CO_3H^{-}	700	3400	444	100	461	282	193.4	461	179	824	95	293
SO_4^{2-}	171000	246000	56.6	62200	1	23040	11500	19200	960	24960	768	13440
Cl^{-}	444300	386400	87.2	556900	190000	188000	173597	174000	193000	123380	2587	35820
Σ anions (ppm)	616000	635800	587.8	619200	190462	211322	185290.4	193661	194139	149164	3450	49553
% CO_3H^{-}	0.11	0.53	75.54	0.02	0.24	0.13	0.10	0.24	0.09	0.55	2.75	0.59
% SO_4^{2-}	27.76	38.69	9.63	10.05	0.00	10.90	6.21	9.91	0.49	16.73	22.26	27.12
% Cl^{-}	72.13	60.77	14.83	89.94	99.76	88.96	93.69	89.85	99.41	82.71	74.99	72.29

[a]From Amat et al. (2004)
[b]From Angelelli et al. (1976)

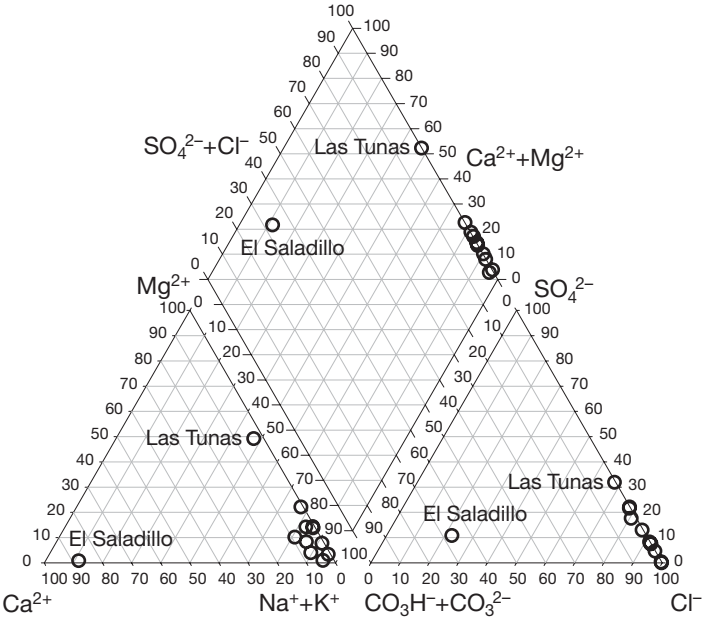

Fig. 4. Piper diagram of the ions (%) of brines of some Argentinean *Artemia* populations: El Saladillo, Mar Chiquita, Las Tunas, Hidalgo, Piedras, Salinas Chicas (Buenos Aires), El Inglés, Salitral de la Vidriera, Palos Blancos, Caleta Olivia, Ruta 3 and San Julián (only outliers named on diagram)

Only 1 brine sample (from El Saladillo) was characterised by Ca^{2+}-CO_3H^- dominance, typical of shallow and fresh ground waters, and was consequently separated from all other brines.

DISCUSSION

Several authors reported that the fatty acid profile of *Artemia* and other zooplankton reflects the fatty acid profile of their food (Léger et al. 1986, Navarro & Amat 1992). Vos et al. (1984), Léger et al. (1986) and Lavens et al. (1989) have suggested that variations in HUFA content among cyst batches might result from variations in the composition of the microalgae available as food for the parental *Artemia* populations in the natural habitat. Consequently, the fatty acid profile of *Artemia* cysts could be considered as a biomarker for the composition of algal food consumed by the brine shrimp parental population.

Sargent et al. (1988) established that biomarkers are chemical components of organisms that can be analyzed directly from the environment and, ideally, can be interpreted both quantitatively and qualitatively in terms of *in situ* biomass. Some fatty acids, or groups of them, are preferentially associated with certain classes of algae, and can therefore be useful as biomarkers for these algal classes whose presence (diversity) and growth may in turn vary depending on the characteris-

tics of the medium in which they live. Mesocosm-type studies conducted by Fraser et al. (1989) revealed the presence of 18:4n-3 and 18:5n-3 in marine phytoplankton samples (Loch Thurnaig, Scotland) at the beginning of experimentation, suggesting the proliferation of Chrysophiceae, Haptophyceae and Dinoflagellata. The phytoplankton profiles then exhibited a decrease in the proportions of these fatty acids, and increases in proportions of 16:4 and 20:5n-3 — major PUFA of diatoms — were instead observed. If *Artemia* reflect the fatty acid profile of their diet, then some fatty acids could be used as indicators of the food composition within each ecosystem.

Fatty acids can be used as biomarkers not only in terms of *in situ* biomass but also on a larger scale, characterizing for example inland and/or coastal biotopes, as reported in the study of Navarro et al. (1992b). These authors pointed to LNA as a phenotypic indicator of *Artemia* cysts from inland biotopes, supporting the results of Léger et al. (1986) on the fatty acid composition of cysts from 2 inland *A. franciscana* populations (from Canada and GSL). LNA as a biomarker of inland habitat is also evident in Amat et al.'s (2004) research on the fatty acid profiles of cysts from the inland populations included in this study: Mar Chiquita and Las Tunas.

In the present study we reported that inland populations of *Artemia persimilis* lack high levels of LNA (Salitral de la Vidriera, Algarrobo and Ruta 3), or exhibit a moderate proportion of LNA (6.5%) as in the case of cysts from Palos Blancos. However, it should be noted that although Palos Blancos Lagoon is not very close to the sea (50 to 60 km), it was connected with it in the past. Nowadays the brine composition of the lagoon is typical of sea water, even though it is altered by the inflow of the Rio Colorado local irrigation systems. In contrast, high LNA levels were found in cysts from coastal biotopes (Caleta Olivia and El Inglés). To the best of our knowledge, this is the first time that freshwater-type cysts have been reported in coastal *Artemia* populations. Thus, although the difference in LNA content between both general types of population — inland and coastal — is indicative of their freshwater (high LNA, low EPA) or marine (low LNA, high EPA) character, it does not determine their ascription to these groups.

Watanabe (1987) reported that ARA was associated with marine-type *Artemia*. All marine-type populations described here exhibited higher values of ARA than did freshwater types, in contrast with the results of Navarro et al. (1992b) whereby this fatty acid was present regardless of the inland (freshwater-type) or coastal (marine-type) origin of populations.

Another issue that deserves close attention is the ratio of 16:0/16:1. Navarro et al. (1993) stated that this ratio was lower in marine-type than in freshwater-type cysts. The results of the present study support this assertion, because the 16:0/16:1 ratio was lower (~0.5 to 1) in all marine-type populations than in freshwater types (Table 2). It can thus be concluded that the 16:0/16:1 ratio is a good biomarker that characterizes samples in terms of marine- vs. freshwater-type. For instance, cysts from the Caleta Olivia population exhibited high LNA (17%) and high EPA (10.1%), and hence would be classified as mixed type, but their high 16:0/16:1 ratio (1.9) would allow their classification as freshwater type.

The hypersaline biotopes studied here are characterized by variable ecological parameters that depend on local climate, altitude and the ionic composition of brines. Quirós & Drago (1999) studied Argentina according to geomorphological traits and established 6 major regions: (1) Puna, (2) Chaco-Pampa Plain, (3) Peri-Pampean Sierras, (4) Andean-Patagonia, (5) Patagonia Plateau and (6) Misiones Plateau. They determined that the principal mechanisms influencing the chemistry of inland waters were rock dominance and evaporation, with atmospheric contributions being less important. Mineral weathering mainly controls the chemical composition of the diluted continental water bodies, whereas the evaporation-precipitation process influences the saline and hypersaline water bodies.

The populations studied here are located in 2 of the above-mentioned regions: the Chaco-Pampa Plain and the Patagonia Plateau. The Chaco-Pampa Plain is represented by El Saladillo, Mar Chiquita, Las Tunas, Salinas Grandes de Hidalgo, Colorada Grande, Colorada Chica, Callaqueo, Salinas Chicas, Salitral Negro, Salitral de la Vidriera and Palos Blancos. This region exhibits a large degree of hydrochemical heterogeneity that ranges from the much-diluted waters of the aeolian shallow lakes (located in the central and northwestern portions of the Corrientes Province) to the highly saline waters in the provinces of Buenos Aires and La Pampa. Populations from El Inglés, Piedras, Algarrobo, Gualicho, Caleta Olivia, Ruta 3 and San Julián are located on the Patagonia Plateau region, whose large hypersaline 'salares' host medium-high salinity (10 to 100 g l^{-1}) waters.

We compared the percentages of major ions from brines analysed in this study with those reported by Quirós & Drago (1999) for Argentinean lakes; 7 lagoons are located within the Chaco-Pampa Plain (Table 3), whereas the remaining 5 lagoons are located on the Patagonia Plateau and are largely characterized by water chemistry typical of endorheic basins.

Owing to great climatic variability and a peculiar north-south orographic distribution, the Argentinean lakes system includes a broad diversity of aquatic environments. Perhaps the most updated information on

this topic is available from Quirós & Drago (1999); however, this review only gives the common characteristics of a few representative lakes from each region, and it is therefore very difficult to characterize each lake individually. Within the biotopes associated with the *Artemia* populations studied here, the anionic fraction of brines was dominated by Cl^- and the cationic fraction by Na^+. This indicates the nature of each biotope and demonstrates that all waters are thalassic solutions, with the exception of El Saladillo which was rich in CO_3H^- and Ca^{2+} and hence athalassic. In summary, the waters of the biotopes studied here were sodium chloride-dominated (only one being calcium bicarbonate-dominated), regardless of the geomorphological region to which they belong. Therefore, it was not possible to associate particular fatty acid profiles (marine- or freshwater-type) to particular ionic compositions.

Very high and variable salinity levels favour several species of blue-green algae and flagellates that are low in n-3 HUFAs (Scott & Middleton 1979). In contrast, diatoms and flagellates from low-salinity waters exhibit higher n-3 HUFA levels. This could explain the higher proportions of n-3 HUFA in the cysts of *Artemia* populations from Mar Chiquita and Las Tunas lagoons, whose waters are less saline (Table 3).

Navarro & Amat (1992) brought attention to a possible genotypic influence on the fatty acid profile of *Artemia*, given the presence and proportion of some fatty acids in cysts irrespective of dietary levels available to parental populations. Phenotype is the result of gene interaction with the environment; i.e. variability in phenotypic expression is determined by 2 components: genotypic and environmental variability. In a descriptive study such as this, it is very difficult to ascertain both components. Preliminary controlled experimental studies conducted in our laboratory (unpubl. data) point towards an important phenotypic influence on the diet that is less evident on a mesocosm scale.

The biosynthesis of lipids in algae can be markedly influenced by the physiological state of the organism and by environmental conditions (Sargent et al. 1988). Algae grown at high light intensities tend to increase concentrations of n-3 PUFAs in their total lipids (Pohl & Zurheide 1979). Decreasing temperatures tend to increase the concentration of n-3 PUFAs in cellular total lipid (Ackman & Tocher 1968). We can hypothesize that the factors that influence the nutritional value of *Artemia* cysts could be related to phytoplankton dynamics, which are responsive to variable environmental conditions such as temperature, light and nutrients. In phytoplankton, variable tracer lipid signals have been observed within and between species in the same group (Chuecas & Riley 1969). St John & Lund (1996) observed variations in the ratio of 16:0/16:1 in *Skeletonema costatum* that ranged from 0.43 to 2, and attributed these variations to different environmental conditions. In the present study, as in many others, information on environmental parameters (temperature, photoperiod, rainfall, nutrients, etc.) over extended time periods was not available. All the available hydrological and environmental data were instantaneous; thus, it becomes difficult to establish any relationships between fatty acid profiles and ecological environmental conditions.

Another potential influence on the fatty acid profile of *Artemia* could be the salinity of brines. Salinity influences biodiversity in hypersaline biotopes: biodiversity decreases as salinity increases, owing to the mere exclusion of less-tolerant organisms (Davis 1990). *Artemia* will graze on a diversity of microparticulate food (microalgae, bacteria) whose composition is determined by its salinity tolerance.

Studies conducted by Jones & Flynn (2005) indicate that, in terms of the conversion of prey carbon-nitrogen (CN) into predator CN, copepod population growth is higher when feeding on a mixed diet (diatoms and dinoflagellates) rather than on a monospecific diet. These authors emphasized the importance of selective feeding, necessary to ensure a mixed diet and to maximize growth rates. Given the selective feeding abilities of copepods, the occurrence of a near-monospecific phytoplankton bloom does not mean that the dominant organism is the only or even the main food item consumed. *Artemia* also likely exhibits preferential selective grazing under natural environmental conditions that are suboptimal in terms of microparticulate food availabilty. This is substantiated by the behaviour of *Artemia*, which grazes on algae settled on the bottom or walls of culture containers where diatom n-3 HUFA-rich (EPA-rich) plankton thrives (Savage & Knott 1998; authors' unpubl. data). These factors may help to account for the variable fatty acid profiles of *Artemia* cysts.

The lipid metabolism of *Artemia* is largely unknown. Unusual features such as the capacity for bioconversion from linoleic acid (Schauer & Simpson 1985) to EPA have been suggested. The different activity of enzymes involved in chain elongation and desaturation might be influenced by external factors. High proportions of HUFA, mainly EPA, are characteristic of *Artemia tibetiana* cysts (Van Stappen et al. 2003; authors' unpubl. data regarding 3 Tibetan samples — Ying Yu, Lagor Ko and Gaize). Van Stappen et al. (2003) pointed out at a link between the HUFA patterns of phytoplankton living at high altitudes and increasing UV radiation, and proposed that the unusually rich HUFA content of *A. tibetiana* cysts may reflect the feeding environment of the parent population.

In the relatively cold Tibetan biotopes (which exceed 4500 m above sea level and are exposed to high levels

of UV radiation), *Dunaliella salina* and *Chlamydomonas* sp. (Chlorophyta) are the dominant algae (Zheng 1997). These species are poor in HUFA when cultured, reflecting *in situ* conditions (Poerschmann et al. 2004); in contrast, the HUFA content in *Artemia tibetiana* cysts is high, reaching values up to e.g. 16 to 21% for EPA (Van Stappen et al. 2003). From this viewpoint, a direct correlation between the fatty acid profile of *A. tibetiana* cysts or nauplii and that of dominant algae remains undemonstrated. However, it is commonly suggested that certain PUFAs affect animal physiology via their impact on cell membrane fluidity. PUFAs can act as a membrane lipid antifreeze. The capacity to adjust cell membrane fluidity is an advantageous characteristic of organisms that remain active at low temperatures (Pruitt 1990). This has been well established for many aquatic invertebrates such as freshwater cladocerans and marine and freshwater copepods (Farkas 1979). It is possible that the HUFAs found in cysts of *A. tibetiana* (Van Stapen et al. 2003) have a similar lipid-antifreeze function. This same phenomenon may account for the fatty acid profile of cysts from the San Julian population in Argentina. San Julian was the most southern Argentinean *Artemia* population studied here, and therefore the coldest and the most exposed to UV radiation.

It remains unclear why inland Argentinean populations of *Artemia franciscana* (like those from Mar Chiquita and Las Tunas Lagoons) show marine-type profiles whereas most *A. persimilis* populations present in habitats of similar nature exhibit freshwater-type profiles. An explanation might be found in a combination of factors, such as (1) the variability of environmental conditions (salinity, ionic composition, UV intensity, microalgae composition etc.) in different ecosystems, (2) the genetic characteristics of each population or species, which perhaps predetermines the fatty acid profile to some extent, (3) selective feeding, dependent on the food resources available, or (4) all of the above.

Further research and genetic characterization of marine- and freshwater-type populations is needed to contribute to an understanding of factors that affect the fatty acid profile and thus the nutritional value of *Artemia persimilis* in particular, and *Artemia* in general. Research on some of these aspects is already underway in our laboratories. Argentinean *Artemia* may be ideal for such studies, given the great diversity of biotopes and the peculiar distribution of the species, and the fact that cultures remain apparently free from accidental or deliberate inoculations.

Acknowledgements. We thank M. A. Montolío and F. Fernandez for their technical assistance. Comments by O. Monroig and F. Hontoria improved the manuscript. This research was supported in part by the Spanish Ministry of Science and Technology project AGL2001-1968 and EU project ICA4-CT-2001-10020 (INCO). O.R. is a pre-doctoral fellow of the Spanish Education and Culture Ministry.

LITERATURE CITED

Ackman RG, Tocher CS (1968) Marine phytoplankter fatty acids. J Fish Res Board Can 25:1603–1620

Amat F, Hontoria F, Navarro JC, Cohen RG, Rodriguez Gil SG (1994) Aproximación preliminar a la distribución del género *Artemia* (especie *A. persimilis*) en Argentina, provincias de Buenos Aires y La Pampa. In: Hernández A, Hernández CT, Morales JR, Pereira F, Perdomo JM (eds) Actas del VIII Congreso Latinoamericano de Acuicultura, Santa Fe de Bogotá, p 73–84

Amat F, Cohen RG, Hontoria F, Navarro JC (2004) Further evidence and characterization of *Artemia franciscana* Kellogg, 1906 populations in Argentina. J Biogeogr 31: 1–15

Angelelli V, Schalamuk I, Arrospide A (1976) Los yacimientos no metalíferos y rocas de aplicación de la región Patagonia-Comahue. Anales XVII. Ministerio de Economía, Buenos Aires

APHA (American Public Health Association) (1993) Standard methods for the examination of water and wastewaters. American Public Health Association, Washington, DC

Bengtson DA, Léger P, Sorgeloos P (1991) Use of *Artemia* as a food source for aquaculture. In: Browne RA, Sorgeloos P, Trotman CAN (eds) *Artemia* biology. CRC Press, Boca Raton, FL, p 255–285

Chuecas L, Riley JP (1969) Component fatty acids of the total lipids of some marine phytoplankton. J Mar Biol Assoc UK 49:97–116

Cohen RG, Amat F, Hontoria F, Navarro JC (1999) Preliminary characterization of some Argentinean *Artemia* populations from La Pampa and Buenos Aires provinces. Int J Salt Lake Res 8:329–340

Davis S (1990) Biological management for the production of salt from seawater. In: Akatsuka I (ed) Introduction to applied phycology. SPB Academic Publishing, The Hague, p 479–488

Davis S, Giordano M (1996) Biological and physical events involved in the origin, effects, and control of organic matter in solar saltworks. Int J Salt Lake Res 4:335–347

Farkas T (1979) Adaptation of fatty acid compositions to temperature: a study on planktonic crustaceans. Comp Biochem Physiol B 64:71–76

Fraser AJ, Sargent JR, Gamble JC, Seaton DD (1989) Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Mar Chem 27:1–18

Fujita S, Watanabe T, Kitajima C (1980) Nutritional quality of *Artemia salina* from different locations, as a living feed from the viewpoint of essential fatty acids for marine fish. In: Persoone G, Sorgeloos P, Roels O, Jaspers E (eds) Proc. Int Symp brine shrimp Artemia. Universa Press, Wetteren, p 277–290

Jones RH, Flynn KJ (2005) Nutritional status and diet composition affect the value of diatoms as copepod prey. Science 307:1457–1459

Lavens P, Sorgeloos P (2000) The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. Aquaculture 181:397–403

Lavens P, Léger P, Sorgeloos P (1989) Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. In: De Paw N, Jaspers E, Acke-

fors H, Wilkins N (eds) Aquaculture: biotechnology in progress. European Aquaculture Society, Bredene, p 731–739

Léger P, Bengtson DA, Simpson PM, Sorgeloos P (1986) The use and nutritional value of *Artemia* as a food source. Oceanogr Mar Biol Annu Rev 24:521–623

Lipko P, Papeschi AG, Cohen RG, Amat F (2004) Cytogenetic analysis of four Argentinean populations of *Artemia* (Crustacea Branchiopoda: Anostraca). Caryologia 57:283–289

Navarro JC (1985) Aportaciones al conocimiento del valor nutritivo de nauplios de *Artemia* de diferentes cepas como alimento para larvas de peces y crustáceos. MS thesis, Universidad de Valencia

Navarro JC, Amat F (1992) Effect of algal diets on the fatty acid composition of brine shrimp *Artemia* sp. cysts. Aquaculture 101:223–227

Navarro JC, Amat F, Sargent JR (1992a) Fatty acid composition of coastal and inland *Artemia* sp. populations from Spain. Aquaculture 102:219–230

Navarro JC, Amat F, Sargent JR (1992b) Lipid composition of cysts of the brine shrimp *Artemia* sp. from Spanish populations. J Exp Mar Biol Ecol 155:123–131

Navarro JC, Amat F, Sargent JR (1993) The lipids of the cysts of freshwater-and marine-type *Artemia*. Aquaculture 109: 327–336

Papeschi AG, Cohen RG, Pastorino X, Amat F (2000) Cytogenetic proof that the brine shrimp *Artemia franciscana* (Crustacea, Branchiopoda) is found in Argentina. Hereditas 133:159–166

Poerschmann J, Spijkerman E, Langer U (2004) Fatty acid patterns in *Chlamydomonas* sp as a marker for nutritional regimes and temperature under extremely acidic conditions. Microb Ecol 48:78–89

Pohl P, Zurheide F (1979) Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Hoppe HA, Levring T, Tanaka Y (eds) Marine algae in pharmaceutical science. Walter de Gruyter, Berlin, p 473–523

Pruitt NL (1990) Adaptations to temperature in the cellular membranes of crustacea- membrane structure and metabolism. J Therm Biol 15:1–8

Quirós R, Drago E (1999) The environmental state of Argentinean lakes: an overview. Lakes Reserv Res Manage 4: 55–64

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1988) Lipid biomarkers in marine ecology. Microbes Sea 5: 119–138

Sargent JR, McEvoy LA, Bell JG (1997) Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155:117–127

Sato NE, Mallo JC, Fenuccii JL (2004) Calidad de los quistes de *Artemia persimilis* (Piccinelli & Prosdocimi) (Crustacea: Branchiopoda) de diferentes zonas de Argentina, como alimento en acuicultura. Rev Biol Mar Oceanogr 39:79–92

Savage A, Knott B (1998) *Artemia parthenogenetica* in Lake Hayward, Western Australia. II. Feeding biology in a shallow seasonally stratified, hypersaline lake. Int J Salt Lake Res 7:13–24

Schauer PS, Simpson KL (1985) Bioaccumulation and bioconversion of dietary labelled fatty acids in *Artemia* and winter flounder (*Pseudopleronectes americanus*). Can J Fish Aquat Sci 42:1430–1438

Scott AP, Middelton C (1979) Unicellular algae as a food for turbot (*Scophthalmus maximus*) larvae. The importance of dietary long-chain polyunsaturated fatty acids. Aquaculture 18:227–241

St John MA, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131:75–85

Van Stappen G (1997) Introduction, biology and ecology of *Artemia*. In: Lavens P, Sorgeloos P (eds) Manual on the production and use of live food for aquaculture. FAO Fish Tech Pap No. 361. FAO, Rome, p 79–106

Van Stappen G, Sui LY, Xin NH, Sorgeloos P (2003) Characterisation of high-altitude *Artemia* populations from the Qinghai-Tibet Plateau, PR China. Hydrobiologia 500:179–192

Vos J, Léger P, Vanhaecke P, Sorgeloos P (1984) Quality evaluation of brine shrimp *Artemia* cysts produced in Asian Salt ponds. Hydrobiologia 108:17–23

Watanabe T (1987) Requerimientos de ácidos grasos y nutrición lipídica en los peces. In: Espinosa de los Monteros J, Labarta U (eds) Nutrición en acuicultura, plan de formación de técnicos superiores en acuicultura. Feuga, Madrid, p 99–165

Watanabe T, Arakawa T, Kitajima C, Fukusho K, Fujita S (1978) Nutritional quality of living feed from the viewpoint of essential fatty acids for fish. Bull Jpn Soc Sci Fish 44: 1223–1227

Zheng M (1997) An introduction to saline lakes on the Quinghai-Tibet Plateau. Kluwer, Dordrecht

Editorial responsibility: Robert Feller (Contributing Editor), Columbia, South Carolina, USA

Submitted: March 30, 2006; Accepted: September 12, 2006
Proofs received from author(s): March 29, 2007