Aquat. Living Resour. 21, 63–73 (2008)

DOI: 10.1051/alr:2008013
www.alr-journal.org

Aquatic Living Resources

Coupling changes in fatty acid and protein composition of *Artemia salina* with environmental factors in the Sfax solar saltern (Tunisia)

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Received 28 April 2007; Accepted 7 December 2007

Abstract – The biochemical composition and biometry of different *Artemia salina* stages were determined in four ponds of increasing salinity (M1, M2, M3 and B1) in the Sfax solar saltern (Tunisia). Results showed the dominance of saturated fatty acids, which made up 48 to 57% of total fatty acids (FAs). Polyunsaturated fatty acids (PUFAs) 22:6(n-3) docosahexaenoic acid (DHA) and 20:5(n-3) eicosapentaenoic acid (EPA) represented on average only 3.1 and 4.0% of total FAs respectively. *A. salina* nauplii, cysts and metanauplii, in ponds M1, M2 and B1 respectively, were found to have optimal DHA/EPA ratios (>2) for use as live feed for invertebrate and fish larvae. Significant inter-pond variation in DHA/EPA levels was also recorded. The predominant FAs in *Artemia* were negatively correlated with both temperature and salinity. FA and protein contents were strongly affected by high temperatures (>30 °C) and probably by food sources (e.g., *Dunaliella salina*) ($r = 0.9$, $n = 27$). The density of *Artemia salina* was positively correlated with protein content in pond B1. The high DHA/EPA ratios (1 to 3.3) found in this study indicate that Sfax *Artemia* could be a valuable food source for larvae in large marine hatcheries and also for some aquarium species.

Key words: Fatty acids / Proteins / Quality / Nutrition / Biometry / Saltern / brine shrimp / *Artemia salina* / Mediterranean Sea

Résumé – Variations des profils en acide gras et en protéines chez *Artemia salina* couplées aux facteurs environnementaux de la saline de Sfax (Tunisie). La composition en acides gras et en protéines, couplée à la biométrie des différents stades de développement de *Artemia salina,* a été étudiée dans quatre bassins de salinité croissante M1, M2, M3 et B1 de la saline de Sfax (Tunisie). Les résultats indiquent une dominance des acides gras saturés (48 à 57 % des acides gras totaux), les acides docosahéxanoïque (DHA) et écosapentanoïque (EPA) ne représentant que 3,1 et 4 % des acides gras totaux. Les larves nauplii, les cystes et les métanauplii observés respectivement dans les bassins M1, M2 et M3 présentent des rapports DHA/EPA (>2) compatibles avec une croissance optimale de larves de poissons et d'invertébrés. Ces rapports varient significativement d'un bassin à l'autre. Les acides gras dominants sont corrélés négativement à la température et la salinité. Les profils en acides gras et les contenus en protéines semblent être affectés non seulement par les températures élevées (>30 °C) mais aussi par les acides gras issus de l'ingestion de *Dunaliella salina* (Chlorophycée) par *Artemia salina* ($r = 0,9$, $n = 27$). Les teneurs en protéines évoluent parallèlement à la densité de *A. salina* dans le bassin B1. Les rapports DHA/EPA enregistrés au cours de cette étude sont élevés (1 à 3,3), ainsi ces *Artemia* de la saline de Sfax pourraient être considérés comme une nourriture potentielle pour les larves d'écloseries et les espèces d'aquariophilie.

1 Introduction

In both marine and freshwater ecosystems, invertebrates are known to transfer fatty acids (FAs) and especially highly unsaturated FAs from phytoplankton and/or the microbial loop to higher trophic levels (Cho et al. 1999; Kainz et al. 2004). These essential FAs make a major contribution to optimal growth and development in animals and humans (Arts et al. 2001; Mostofsky et al. 2001; Hamazaki et al. 2002). The brine shrimp *Artemia salina* (Crustacea, Anostraca, Branchiopoda), which has a world-wide distribution in coastal and inland ecosystems (Perez et al. 1994; Triantaphyllidis et al. 1998), has frequently been the subject of studies focussing on its

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FAs profiles. However, because *Artemia* is commonly used in aquaculture as live feed for invertebrates and fish larvae (Wouters et al. 1998; Wickins and Lee 2002), most of these studies were concerned with a strictly commercial aspect. Supplementing a diet of *Artemia* with essential polyunsaturated FAs has been found to improve weight gain and animal reproduction (Lavens et al. 1995; Dhont and Sorgeloos, 2002). Furthermore, both *Artemia* nauplii and adults have the great advantage of satisfying the nutritional requirements of a variety of different organisms (Espinosa-Fuentes et al. 1997). Nevertheless, field investigations of the FAs profiles of *Artemia* are very scarce (Navarro et al. 1991; Abatzopoulos et al. 2006; Moraiti-Ioannidou et al. 2007), and those carried out in extreme ecosystems like brine pools or solar salterns have concentrated on the lipid composition of halophilic microbial populations (Litchfield et al. 2000). *Artemia* is one of the most important components of these ecosystems, while high salinity excludes other less halotolerant invertebrates (Pedrós-Alió et al. 2000; Elloumi et al. 2006). In the Sfax solar saltern (Tunisia), *Artemia salina* was found to tolerate extreme salinity (330 g L^{-1}) and adapted easily to the arid environmental constraints (Mura and Brecciaroli 2004; Elloumi et al. 2008). We therefore undertook an investigation on its FA profiles and protein content at five developmental stages in this saltern. To examine the impact of salinity and temperature on the biochemical composition of these developmental stages, we selected four ponds of increasing salinity (from 157 to 312 g L^{-1}). Environmental factors in such conditions are exacerbated by a drastic negative hydric budget in the area (Ayadi et al. 2004). To evaluate the significance of the *Artemia* larvae as potential essential PUFA sources for fish hatcheries (Sorgeloos et al. 2001; Malpica Sanchez et al. 2004) we estimated the ratio of docosahexaenoic acid DHA 22:6(n-3) to eicosapentaenoic acid EPA 20:5(n-3) (DHA/EPA), which has been shown to be critical during the early larval stages of marine fish as it affects their growth and survival (Furuita et al. 2000; Estevez et al. 2001). To date, experiments on pure cultures have revealed very small amounts of DHA in *Artemia* nauplii (Webster and Lovell 1991; Kara et al. 2004). The importance of DHA is to facilitate the normal development of larval fish (Ostrowski and Divakaran 1990; Ozkizilcik and Chu 1994). In this context, we hypothesized that the extreme environmental conditions that prevail in the Sfax solar saltern could influence FA profiles in early *Artemia* larval development with respect to salinity gradients.

2 Materials and methods

2.1 Study site

This study was performed in the Sfax solar saltern (central-eastern coast of Tunisia, about 34° 39'N and 10° 42'E). This is an artificial system consisting of interconnecting ponds (reservoir, evaporation, concentration and crystallization), which extend over 1500 ha and are separated from the sea by a red silt artificial seawall (Fig. 1). The ponds are shallow (20–70 cm deep), with salinity ranging from 40 to 400 g L^{-1} due to hypersaline conditions (Ayadi et al. 2004). The composition of bottom sediments shows a longitudinal succession of seven rock types (Table 1).

Table 1. Main characteristics of the interconnected ponds (M1, M2, M3 and B1: evaporation ponds) of the Sfax solar saltern.

Ponds	Sediment characteristics
First ponds	Substrata with rock, carbonates, mud and high level of organic matter
M1	Substrata: gypso-carbonate *Artemia salina*, *Dunaliella salina*
M2-M3	Substrata with gypsum deposits *A. salina*, Chlorophyceae, *D. salina*
B1	Substrata: gypso-halitic *A. salina*, *D. salina*
Crystallization ponds	Halite deposits salt mixture: halite, sylvite and magnesium sulphate

2.2 Sampling

Four evaporation ponds, M1, M2, M3 and B1 of increasing salinity, 157, 186, 236 and 312 g L^{-1} respectively, were sampled once a month from June 3 until September 22, 2003. A Van Dorn bottle was used, 20–30 cm below the surface in the central part of the saltern located between the first ponds and crystallization ponds (Fig. 1).

Physico-chemical analyses

Temperature was measured with a mercury glass thermometer (±0.1 °C). Salinity was estimated by the dry residue method, which consists of evaporating a 50 ml sample (24 h, 120 °C) in a crystallizing dish, and calculating the salt content from the difference in weight before and after evaporation. The suspended matter (total dissolved salts and particulate matter) concentrations were determined by measuring the dry weight of the residue after water filtration through a Whatman GF/C membrane.

2.3 Biometry and weight of *Artemia salina*

Artemia samples were collected from each pond in the early morning (Sorgeloos et al. 1986). They were collected on a 110 μm mesh screen, and fixed with a formaldehyde (5%) solution. The phytoplankton caught by the screen was filtered onto precombusted (550 °C) Whatman GF/C filters (0.45 μm). These filters were then stored at –20 °C until phytoplankton lipid extraction was performed. *Artemia* were sorted into five demographic classes (Sorgeloos et al. 1986): (i) cysts; (ii) nauplii corresponding to the first four stages; (iii) metanauplii, all immature individuals possessing some thoracopods; (iv) males and females, identified on the basis of the presence of the brood pouch (ovisac). From each sample collected from ponds M1, M2, M3 and B1, a random number of cysts (n = 84,

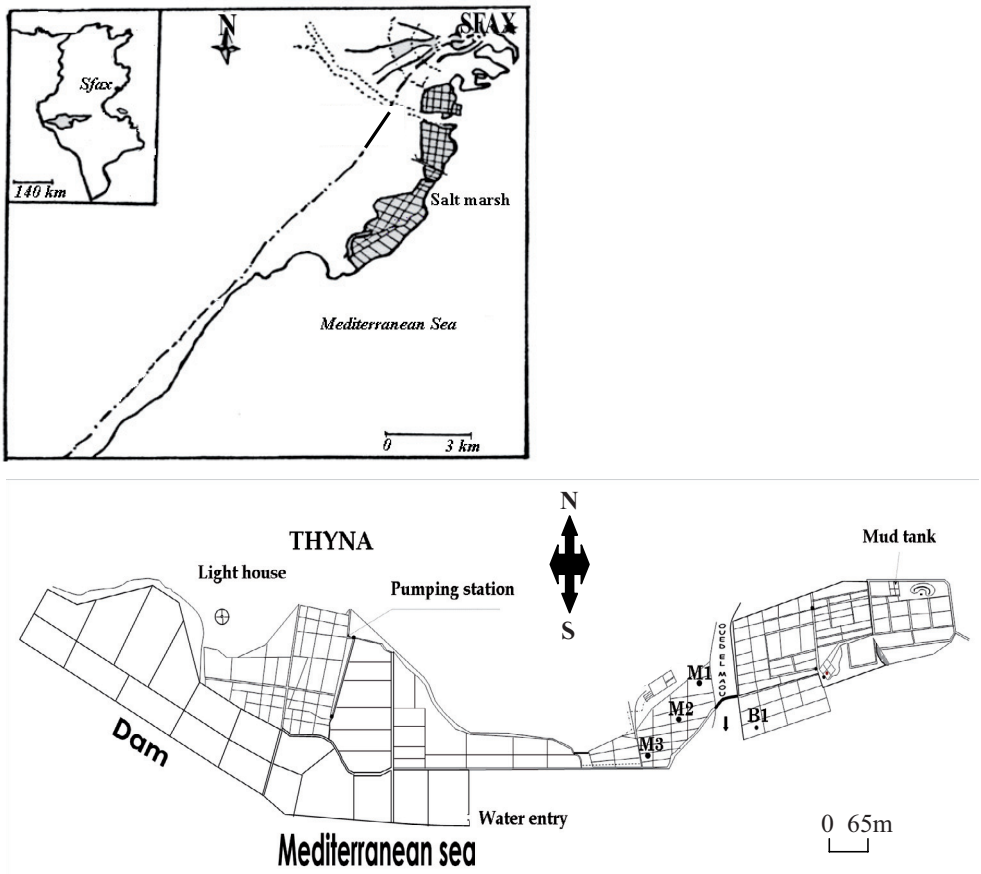

Fig. 1. Location of the evaporation ponds sampled in the Sfax solar salterns: M1, M2, M3 and B1.

66, 69 and 79 for M1, M2, M3 and B1 respectively), naupliі (n = 12, 30, 26 and 44), males (n = 10, 21, 3 and 12) and females (n = 8, 15, 6 and 11) were measured under a vertically mounted deep-focus dissecting microscope (Wild). Measurements were taken of the diameter of cysts and total length of each stage. Standard length of *Artemia* was measured as the distance from the head of to the base of the caudal furca (Amat 1979). The weight of adults (dry weight, *DW* in μg) was calculated using the equation: $DW = 0.00082\ TL^{3.14}$ and $DW = 0.0018\ TL^{2.73}$ for males and females, respectively (Barata et al. 1996) where TL (mm): adult body length including antenna.

2.4 Fatty acid and protein analyses

Fatty acid and protein analyses were performed on the five developmental stages of *Artemia* mentioned above. Five individuals from each stage, were collected at random from the samples and starved for 12 hours before lipid extraction.

Total lipids in *Artemia* and phytoplankton were extracted with a mixture of chloroform-methanol (2:1, v/v) according to the method of Folch et al. (1957). An internal standard 17:0 was added prior to the reaction. All lipids were extracted with nitrogen by cold evaporation and concentrated with hexane. Fatty acid methyl esters (FAME) were prepared from the lipid extract by transesterification using a direct transmethylation method according to Lepage and Roy (1984). Then, the FAMEs were extracted with hexane and determined quantitatively by capillary gas chromatography. We used a Chromopack, CP 9001 gas chromatograph, HPS 5890 series II chromatograph, equipped with a polar 25-m capillary column CP wax 58 (Varian SA, Les Ulis, France) (0.32 mm diameter and a layer thickness of 0.52 μm) and a flame detector (FID). We used a split-splitless injection system with nitrogen as carrier gas. The oven was programmed to rise from an initial temperature of 180 °C to 250 °C at a rate of 10 °C min^{-1} (from 180 to 220), 2 °C min^{-1} (from 220 to 240) and 5 °C min^{-1} (from 240 to 250) with the FFAC column. Individual FAMEs were identified by comparing retention times with those obtained from Supelco and laboratory standards. Protein contents of each *Artemia* stage were determined according to Lowry et al. (1951) with an albumin (BSA) standard.

2.5 Statistical analyses

The data recorded in this study were examined with a normalized principal component analysis (PCA) (Chessel and Dolédec 1992). Physical, chemical (temperature, salinity and suspended matter) and biological parameters (proteins and fatty acids of the five *Artemia* developmental stages) assessed

Table 2. Environmental parameters (mean ± SD) in the four ponds sampled in 2003 (see also details in Fig. 1), biometrics cyst, nauplii and individual dry weight of *A. salina* males and females found in the Sfax solar saltern.

	Ponds				*F (df)*
	M1	M2	M3	B1	
T (°C)	29.1 ± 6.0	29.3 ± 3.3	28.2 ± 6.3	31.5 ± 6.2	*0.471 (28)*
pH	7.1 ± 0.7	7.2 ± 0.5	7.2 ± 0.2	7 ± 0.7	*0.156 (28)*
Salinity (g L^{-1})	157 ± 11**a**	186 ± 12**a**	236 ± 15**a**	312 ± 13**a**	*230.6(28)*
Suspended matter (mg L^{-1})	837 ± 247	866 ± 632	1207 ± 953	1670 ± 1302	*1.571(28)*
Artemia salina					
Cyst diameter (μm)	229 ± 28**a**	220 ± 29**b**	222 ± 38**a**	185 ± 52**ab**	*21.5 (294)*
Nauplii length (μm)	551 ± 0.1	538 ± 0.1	532 ± 0.1	516 ± 0.2	*0.37 (108)*
Male length (mm)	5 ± 0.8	5 ± 1.2	6 ± 1.7	5 ± 0.8	*0.99 (43)*
Female length (mm)	6 ± 1.2	6 ± 1.1	6 ± 0.7	6 ± 1.2	*0.08 (35)*
Male weight (μg)	198 ± 46	207 ± 119	227 ± 92	192 ± 87	*0.93 (43)*
Female weight (μg)	356 ± 81	348 ± 118	327 ± 78	352 ± 102	*0.12 (30)*

F-value: between-groups mean square/within-groups mean square.
Values in the same row showing the same letters are significantly different as tested with one-way ANOVA and paired comparisons using Tukey's test ($p < 0.05$).

over 23 observations were considered. The discrimination between months was assessed by examining the projection of plots of the extracted factors on a factorial plane consisting of the statistically significant axes of the PCA analysis. Simple log $(x + 1)$ transformation was applied to data in order to correctly stabilize the variance (Frontier 1973). Our interpretation will concentrate on the first two eigenvectors since they account for 76.7% of total variance.

Mean and standard deviation (SD), are reported when appropriate. The potential relationships between variables were tested by Pearson's correlation coefficient. One-way ANOVA was applied to identify significant differences ($p < 0.05$) between study ponds for physico-chemical variables, total length andweig ht of *Artemia* stages. Two-way ANOVA was used to test the differences ($p < 0.05$) in the FA composition and protein contents for i) *Artemia* stages within the same pond and ii) between ponds for the same developmental stage. ANOVA tests were made using XL stat software.

3 Results

3.1 Physico-chemical analyses and morphometry of *A. salina*

Water temperatures averaged 28.2 ± 6.3 °C and 31.5 ± 6.2 °C in M3 and B1, respectively (Table 2). Such high values are usual in arid to semi arid zones. The highest salinity was recorded in salt pond B1, with values increasing from early summer to late autumn. This temporal pattern also appeared in ponds M1, M2 and M3, with significant inter-pond variation (Table 2). High temperatures (June and July) coincided with the lowest salinity, while the highest temperature was recorded at the beginning of the wet season (December). This discrepancy may be explained by the opening of a water gate to supply the ponds with seawater during the dry season. Suspended matter levels did not differ significantly among the four ponds (Table 2), but was correlated positively with salinity in M1 ($r = 0.4$, $df = 6$, $p < 0.05$) and temperature in M2 ($r = 0.35$, $df = 6$, $p < 0.05$). Suspended matter was high in B1 (1670±1302 mg L^{-1}), negatively correlated with salinity in M3 ($r = -0.4$, $df = 6$, $p < 0.05$) but positively in B1 ($r = 0.7$, $df = 6$, $p < 0.05$).

Furthermore, the average size of cysts and nauplii decreased as salinity rose (from M1 to B1 pond, Table 2). The diameter of cysts was negatively correlated with both salinity and temperature ($r = -0.4$, $df = 296$, $p < 0.01$), and varied significantly between ponds ($F = 21.53$, $df = 294$, $p < 0.001$). The impact of increasing salinity on the size of males and females was similar among ponds (5 and 6 mm, respectively). However, the individual dry weight of adult *Artemia* stages changed slightly from one pond to another (Table 2).

3.2 Fatty acid and protein analyses

Fatty acid and protein composition of the different stages of *A. salina* harvested from Sfax solar saltern in the four ponds M1, M2, M3 and B1 are summarized (see Appendix). Fatty acid composition was the same in all ponds and for all *A. salina* stages. The saturated FAs were composed of 14:0, 16:0, 18:0, 20:0, 22:0 and 24:0; but mostly dominated by 16:0 and 18:0. The level of 16:0 ranged from 20.4 to 24.8% in cysts, 24.4 to 29.5% in nauplii, 20 to 29% in males and 22.3 to 34.9% in females. FA 18:0 level was lower and ranged from 15.4 to 19.7% of total FAs in cysts, 15.2 to 23.9% in nauplii, 15.1 to 20.6% in males and 16.1 to 23.8% in females (Fig. 2). The monounsaturated FAs were composed of 14:1(n-5), 16:1(n-7), 18:1(n-9), 18:1(n-7), 20:1(n-9), 22:1(n-9) and 24:1(n-9), which were dominated by 18:1(n-9) and 18:1(n-7). FA 18:1(n-9) was more abundant than 18:1(n-7), reaching 19.1, 16.8 and 16.2% in the cysts, metanauplii and male stages respectively. FA 18:1(n-7) was recorded at its lowest abundance in these *Artemia* stages, ranging from 1.7 to 8.2% in cysts, 0.5 to 3.5% in metanauplii and 0.8 to 10.3% in males (Appendix). The pattern of polyunsaturated FAs (PUFAs) exhibited a predominance of 18:2(n-6), 18:3(n-3), 20:2(n-6), 20:3(n-6), 20:4(n-6) (arachidonic acid ArA), 20:5(n-3) (EPA), 22:4(n-6), 22:5(n-3)

and 22:6(n-3) (DHA) (Appendix). The 18:2(n-6) FA clearly dominated 18:3(n-3) in all ponds and ranged from 2.6 to 6.4% in cysts, 3.2 to 6.6% in nauplii, 1.2 to 4.4% in metanauplii, 0.7 to 5.7 in males and 2.8 to 5.3% in females, while the level of 18:3(n-3) did not generally exceed 2% in any *Artemia* stage (Fig. 2). The maximum ArA, EPA and DHA abundance was low, not exceeding 5% in the cysts, nauplii, males and females; while in the metanauplii, the abundance reached 10% (M1 pond) especially the PUFAs (EPA) (Fig. 2C). The predominant FAs in *Artemia* (all stages and all ponds combined) were negatively correlated with abiotic variables, mainly temperature ($p < 0.001$, $df = 52$) and salinity ($p < 0.05$, $df = 52$).

FAs exhibited the lowest values in the cysts, where they did not exceed 25% (Fig. 2A), with means of major FAs showing no significant variation between ponds (Appendix). Adult *A. salina* exhibited the highest FA content. FAs of males and females were dominated by 16:0, which reached 30 and 35% respectively, though the means for males and females were not significantly different (Appendix). Total saturated FAs extracted from the five stages in all ponds showed similar relative contributions (50% of total FAs). The contribution of total monounsaturated FAs ranged between 12.2 and 43.0% of total FAs while that of PUFAs was lower (12.5 to 35.7%). Acids of the (n-6) family represented the major polyunsaturated fatty acids of the different *Artemia* stages in all ponds. The highest levels were recorded in M1 and M2, where they ranged, respectively, from 6.9 ± 9.7 to 19.1 ± 19.1% and from 7.8 ± 0.6 to 18.7 ± 5.6% (Appendix). We recorded the lowest levels of (n-6)PUFAs in M3 and B1, where they did not exceed 13% (Appendix). The males and metanauplii exhibited the highest level of (n-3) PUFAs in M1 and M2 (Males, 16.5 ± 3.2% and 6.8±2.2%) and (Metanauplii, 15.4±2.7% and 21.4±6.8%), respectively (Appendix). For the other *Artemia* stages, the highest levels of (n-3) PUFAs were recorded in B1 with values ranging from 9.1 ± 8.2 to 12.9 ± 9.7%.

The major FAs which showed significant differences ($p < 0.05$) (two way ANOVA) in all *Artemia* stages and in all ponds are summarized in Appendix.

Table 3 shows that the FA composition of the lipid fraction of *Artemia* is similar to that of phytoplankton which was entirely composed of the Chlorophyceae *Dunaliella salina* and cyanobacteria. The dominant FAs were 16:0, 18:0 and 18:1(n-9) contributing 30.5±2.5, 10.2±2.1 and 9.7±1.8% of total FAs, respectively. It is worth noting that the high level of DHA/EPA ratio recorded in phytoplankton cells reached 4.8 ± 2.8. The amount of total protein in the cysts decreased slightly from M1 (5.5 ± 1.9 μg ind^{-1}) to B1 (3.4 ± 1.3 μg ind^{-1}), and changed significantly from one pond to another (Appendix). Moreover, the protein content of cysts was negatively correlated ($p < 0.05$, $df = 6$) with temperature in M2 ($r = 0.54$) and B1 ($r = 0.34$) and was mainly affected by salinity in M3 ($r = 0.74$, $df = 5$). The high protein content in larval *Artemia* stages was recorded in metanauplii in M1 (6.2 ± 2 μg ind^{-1}, Appendix). The nauplii exhibited the lowest protein contents (4.2 ± 1.5, 1.4 ± 1.2, 1.0±0.7 and 2.3±2.6 μg ind^{-1}) in M1, M2, M3 and B1, respectively (Appendix). *Artemia* males and females displayed the highest amount of protein especially in pond B1 (24.0 ± 6.5 and 44.1 ± 13.3 μg ind^{-1}, respectively) (Appendix). No significant correlation was found between the protein in *Artemia* and either temperature or salinity during the study ($p < 0.05$, $df = 56$). In contrast, the patterns of total protein roughly followed those of *Artemia* density throughout the study (with all *Artemia* stages were confounded for these two components).

Table 3. Mean composition of fatty acids (% of total fatty acids) and total protein concentrations (μg individual^{-1}) in phytoplankton and *Artemia* harvested from the Sfax solar saltern (over all developmental stages and sample dates in 2003). nd : not detected.

Biochemical components	*Artemia salina*	Phytoplankton
14:0	0.6 ± 0.1	3.3 ± 0.3
16:0	24.9 ± 2.6	30.5 ± 2.5
18:0	18.2 ± 1.0	10.2 ± 2.1
20:0	1.7 ± 1.0	0.4 ± 0.0
22:0	3.3 ± 2.3	nd
24:0	2.0 ± 1.4	0.5 ± 0.4
Total saturated FAs	49.5 ± 1.2	49.1 ± 4.4
14:1(n-5)	3.3 ± 1.1	4.8 ± 0.8
16:1(n-7)	2.5 ± 0.6	3.9 ± 0.8
18:1(n-9)	9.9 ± 3.2	9.7 ± 1.8
18:1(n-7)	6.8 ± 4.3	2.3 ± 0.7
20:1(n-9)	4.5 ± 2.4	nd
22:1(n-9)	4.2 ± 1.1	0.7 ± 0.1
24:1(n-9)	3.6 ± 5.4	nd
Total MUFAs	31.4 ± 4.4	24.8 ± 3.5
18:2 (n-6)	3.9 ± 0.8	1.8 ± 0.3
18:3 (n-3)	0.9 ± 0.4	0.3 ± 0.5
20:2 (n-6)	1.5 ± 0.8	nd
20:3 (n-6)	0.6 ± 0.2	0.5 ± 0.5
20:4 (n-6) (ArA)	2.8 ± 0.6	1.3 ± 0.3
20:5 (n-3) (EPA)	4 ± 2.3	0.3 ± 0.3
22:4 (n-6)	4.4 ± 2.0	nd
22:5 (n-3)	2.5 ± 0.7	1.6 ± 0.7
22:6 (n-3) (DHA)	3.1 ± 1.1	1.7 ± 0.9
Total (n-6) PUFA	11.7 ± 1.7	5.3 ± 0.4
Total (n-3) PUFA	9.8 ± 2.6	3.6 ± 0.6
DHA/EPA	1.3 ± 0.4	4.8 ± 2.8
Total proteins	9.9 ± 8.9	6.9 ± 4.0

3.3 Principal components analysis

The principal components analysis showed 49.4% (axis-I) and 27.4% (axis-II) of total variance in biochemical and environmental variables, respectively (Fig. 3). The negative end of the first and the second axes accounted for the major part of the biochemical components of *Artemia* (G1), while temperature, salinity and suspended matter exhibited high positive loadings on PCA I (G2). The plot of field observations showed a clear segregation between observations made in the hypersaline pond B1 and the less saline ponds M1, M2 and M3. The observations made in these latter three ponds were grouped in the negative part of the first axis together with the biochemical components, in contrast to the observations made in pond B1 which tended to group in the positive part of this axis, together with temperature, suspended matter, salinity and the protein contents of male and female *Artemia.*

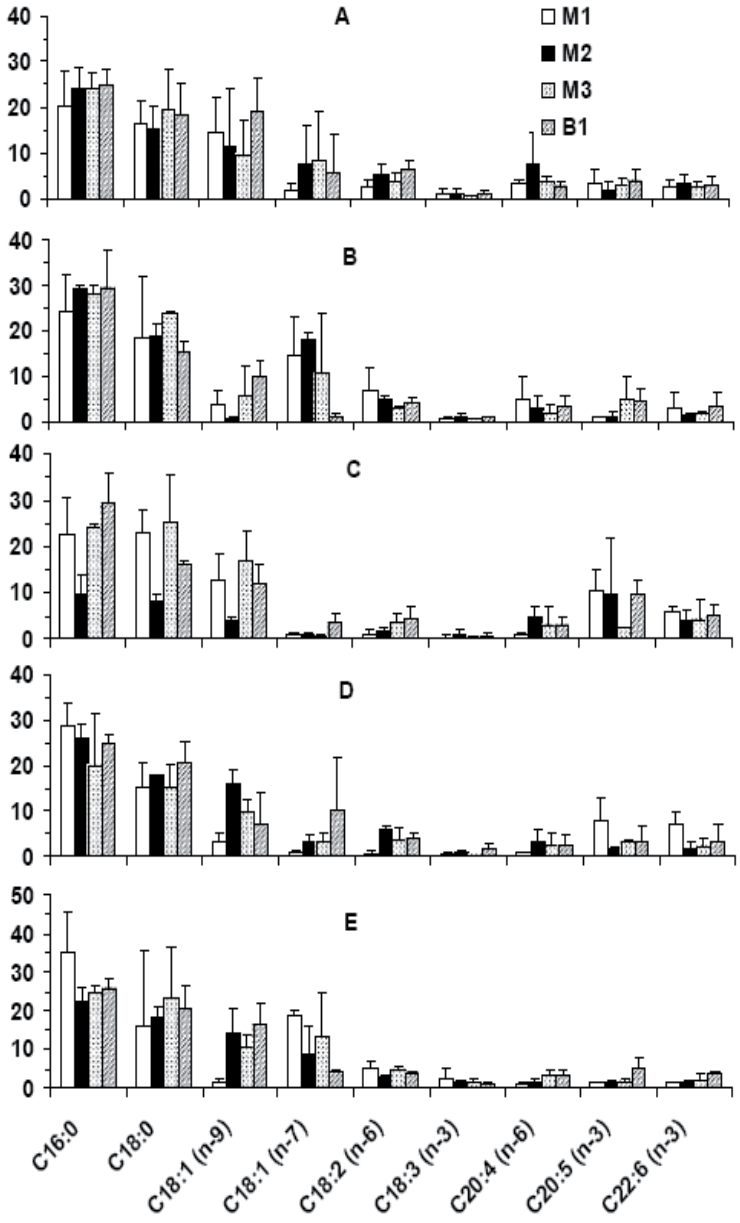

Fig. 2. Distribution of dominant fatty acid mean percentages per pond in the five different *Artemia* stages harvested from the four ponds in 2003. Vertical bars represent standard deviation. A: cysts; B: nauplii; C: metanauplii; D: males and E: females. EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, ArA: Arachidonic acid.

4 Discussion

Our results indicate that the five *Artemia* developmental stages harvested from the four ponds in the Sfax saltern contained high amounts of saturated FAs (32.5 to 58.9% of total FAs), which largely exceed those previously found in *A. franciscana* and *A. sinica* (20% of total FAs) (Evjemo et al. 1997). These saturated FAs were clearly dominated by 16:0 and 18:0, which is consistent with data reported in other studies on the lipid composition of various *Artemia* species (Ando et al. 2002; Abatzopoulos et al. 2006). However, this contrasts with findings from the hypersaline Urmia Lake (Iran) and the Great Salt Lake (USA), for which the lipid composition of the harvested nauplii of *A. urmiana* and *A. franciscana* was characterized by the dominance of PUFAs (52.1% and 34.5–43%, respectively) and mainly 18:3(n-3) (Han et al. 2001; Abatzopoulos et al. 2006). In the Sfax saltern, the percentage of 18:3(n-3) never exceeded 3% of total FAs, which is similar to levels found in unfed nauplii obtained from *A. salina* cysts (Hobby Dohse Aquaristik, Germany) (Vismara et al. 2003). The major monounsaturated fatty acids (MUFAs) extracted from the different *A. salina* stages were 18:1(n-9) and

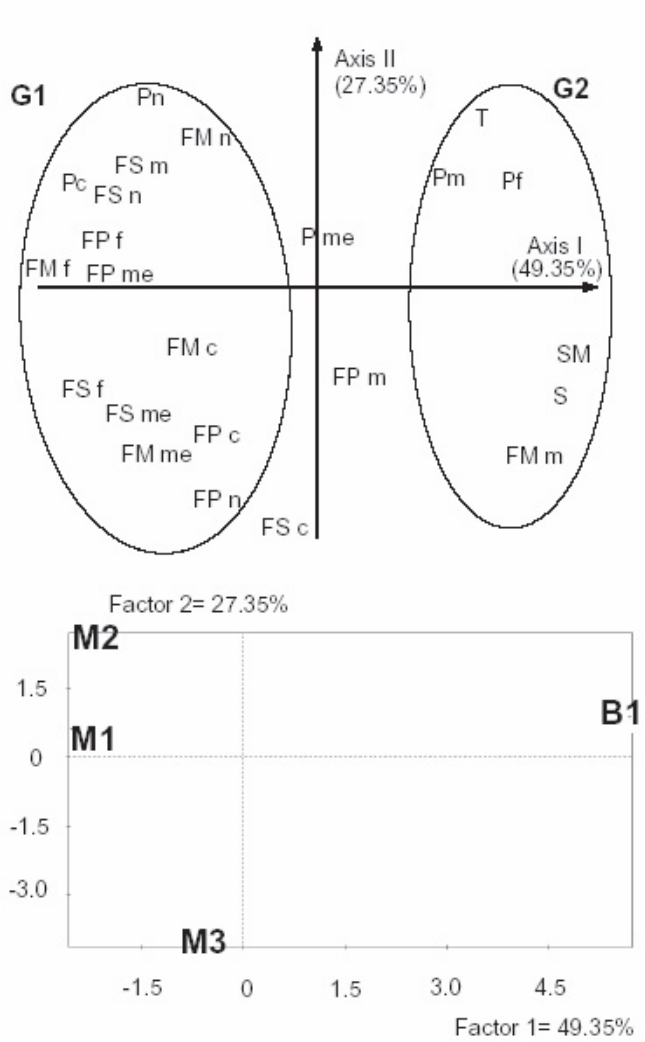

Fig. 3. Principal component analysis: (T): temperature, (S): salinity, (SM): suspended matter, (P): protein, (FS): saturated fatty acids, (FM): monounsaturated fatty acids, (FP): polyunsaturated fatty acids, (c): cysts, (n): nauplii, (me): metanauplii, (f): females, (m): males. All dates included. M1, M2, M3 and B1: ponds.

18:1(n-7). Several studies have already reported such dominance (Ando et al. 2002; Abatzopoulos et al. 2006), but the concentrations they reported were lower than those found in the Sfax adult *Artemia* stages.

One of the outstanding results in this study is the high levels of DHA and EPA, which are higher than those previously reported in several *Artemia* species (never exceeding 0.5%) (Kara et al. 2004; Camargo et al. 2005). For instance, we recorded high DHA/EPA ratios (>2) in *A. salina* nauplii, cysts and metanauplii sampled from ponds M1, M2 and B1, respectively, and inter-pond variations in DHA/EPA levels were significant (Appendix). According to the nutritional needs of fish described by Sargent et al. (1999), these high DHA/EPA levels indicate that nauplii and cysts from the Sfax saltern could be of high nutritional value for hatcheries. The lowest level (<1), detected in the hypersaline pond B1, was even higher than that reported for five *Artemia franciscana* strains (<0.5%) (Camargo et al. 2005), which confirms the variation in the amount of EPA and DHA with species taxonomic variability (Triantaphyllidis et al. 1995; Han et al. 2000).

The high amount of essential PUFAs recorded in *A. salina* from Sfax solar saltern could be due to the presence of the Chlorophyceae *Dunaliella salina*, which is the most abundant phytoplankton species in the saltern (Ayadi et al. 2004) and known to be the most suitable food source for adult *Artemia* in extreme saline environments (Lavens and Sorgeloos 1996). In fact, we found no obvious differences in the FA profiles between *Artemia* and phytoplankton (Table 3), which were strongly positively correlated (r = 0.9, n = 27, p = 0.001). The high DHA/EPA level (4.8) and the low amount of 18:3(n-3) (0.33% of total FAs) recorded in the phytoplankton clearly reflect the lipid composition of these *Artemia*. The proportion of FAs in *A. salina* appears to vary with those of phytoplankton, as reported by Williams et al. (1990) and Zhukova et al. (1998). Data from the literature clearly demonstrate that FAs, especially DHA, EPA and ArA, can be incorporated into *Artemia* (Triantaphyllidis et al. 1995; Han et al. 2000).

However, there is also data suggesting that synthesis of (n-3) PUFAs occurs in *Artemia* from 18:2(n-3), but at a lower rate (Waldock and Holland 1984; Ito and Simpson 1996).

The principal component analysis (PCA) confirmed the deleterious effect of both temperature and salinity (G2) on the biochemical components of cysts ($r = 0.64$, $r = 0.51$, respectively) and larval stages ($r = 0.7$, $r = 0.6$, respectively) (G1) (Fig. 3). Total protein content and *Artemia* density were correlated positively in both ponds M2 ($r = 0.7$) and M3 ($r = 0.56$). Dry weights of each *Artemia* stage were also affected by abiotic variables, especially salinity and temperature. A highly negative correlation was recorded between temperature and individual *Artemia* weight ($r = -0.509$, $df = 30$, $p < 0.001$ for cysts). The negative impact of salinity on several *Artemia* species has been extensively reported (Browne and Wanigasekara 2000; Baxevanis et al. 2004). The PCA also confirmed that in pond B1, abundant protein content was found in *Artemia* males (24 μg ind^{-1}) and females (44 μg ind^{-1}) (G2) (Fig. 3).

Several studies have been undertaken on the morphometry of various *Artemia* populations (Baxevanis et al. 2004; El-Bermawi et al. 2004). The biometrical results of *A. salina* from Sfax solar saltern showed high variability among the four ponds. There were significant differences in cyst diameters ($p = 0.0001$), when grouped in small (216 μm) and large (243 μm) sized-cysts. The latter were similar to those observed in Tarquinia salterns, Italy (Amat et al. 2005) and Manaure salterns, Colombian Caribbean (Camargo et al. 2005). The largest cysts were recorded in the Urmia Lake (286 μm) (Abatzopoulos et al. 2006). However, Vanhaecke and Sorgeloos (1980) found small cysts (224 μm) in the San Francisco Bay strain (USA). The same authors stated that mean cyst size varied by up to 10 μm from one batch to another owing to varying environmental conditions.

5 Conclusion

Our study performed in the Sfax solar saltern illustrates that salinity, temperature and food availability (mainly phytoplankton) affect the biochemical composition of *A. salina*. Furthermore, the relatively large nauplii encountered in the saltern with a high DHA/EPA (>2) might be a valuable food source for hatcheries of some marine and aquarium fish species. Obviously the suitability for this application needs to be examined through estimates of other biochemical parameters such as carbohydrate content.

Acknowledgements. We gratefully acknowledge support from the staff of the Sfax Saltern Company. This study was supported by the University of Franche-Comté (France) and the Tunisian Ministry of Scientific Research and Technology.

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Appendix. Mean fatty acid composition (% of total fatty acids) and protein content (μg ind^{-1}) of the different *Artemia salina* stages harvested from Sfax solar saltern in ponds M1, M2, M3 and B1 (all sample dates for 2003). MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, ArA: Arachidonic acid.

Biochemical	M1					M1				
Components	Cysts	Nauplii	Metanauplii	Males	Females	Cysts	Nauplii	Metanauplii	Males	Females
14:0	0.4 ± 0.5**ab**	0.2 ± 1.2**a**	0.1 ± 0.0**bA**	0.2 ± 0.1	0.1 ± 0.0	0.9 ± 1.2	0.9 ± 1.0	0.5 ± 0.6	0.5 ± 0.5	0.2 ± 0.1
16:0	20.4 ± 7.4**abcd**	24.4 ± 7.7**abA**	22.4 ± 7.9**cBC**	29.0 ± 4.8	34.9 ± 10.7**d**	24.3 ± 4.5**a**	29.4 ± 0.7**a**	9.4 ± 4.4	26.1 ± 3.1	22.3 ± 3.8
18:0	16.1 ± 5.3**a**	18.2 ± 13.7**b**	22.8 ± 5.0	15.2 ± 5.2	16.1 ± 19.5**abA**	15.4 ± 4.7	18.8 ± 2.7	8.3 ± 1.6	17.7 ± 0.1	18.2 ± 2.6
20:0	10.5 ± 21.9**a**	1.6 ± 0.1	1.9 ± 1.0**A**	0.7 ± 0.4**B**	1.4 ± 0.4**aC**	1.1 ± 1.0	0.8 ± 0.8	0.9 ± 0.6	1.5 ± 0.4	1.9 ± 0.3D
22:0	3.6 ± 2.2	1.4 ± 1.1	10.6 ± 3.2**aAB**	9.5 ± 3.1	0.6 ± 0.0**a**	2.6 ± 2.2	0.9 ± 0.7	8.2 ± 3.7	1.4 ± 0.7	0.9 ± 0.3
24:0	1.5 ± 1.3	2.0 ± 2.3	3.4 ± 1.4**A**	4.2 ± 2.7	0.5 ± 0.2	0.5 ± 0.2**a**	0.2 ± 0.0	10.7 ± 3.8**a**	0.9 ± 0.8	1.6 ± 0.1
Total saturated FA	52.2 ± 19.3**a**	47.9 ± 18.1**b**	53.4 ± 1.6	58.9 ± 7.7	53.8 ± 8.6**abA**	44.6 ± 7.7	50.9 ± 1.0	32.5 ± 5.2	48.0 ± 2.2	44.5 ± 0.1
14:1(n-5)	2.6 ± 2.3	2.4 ± 1.4	2.8 ± 1.3	1.0 ± 0.3	1.0 ± 0.9	2.4 ± 1.4	2.0 ± 0.7	2.8 ± 3.1	3.8 ± 3.6	9.1 ± 9.6
16:1(n-7)	2.6 ± 1.4	1.4 ± 0.9	0.9 ± 0.0	0.9 ± 0.0	4.9 ± 3.0	2.7 ± 0.6	3.9 ± 1.8	1.1 ± 0.4	5.2 ± 2.4	1.4 ± 0.2
18:1(n-9)	14.3 ± 7.9	3.7 ± 3.2	12.7 ± 5.8	3.0 ± 2.0	1.3 ± 1.0	11.6 ± 12.3	0.5 ± 0.1	4.1 ± 0.5	16.2 ± 2.9	14.2 ± 6.2
18:1(n-7)	1.7 ± 1.5	14.4 ± 8.7	0.8 ± 0.2	0.8 ± 0.3	19.0 ± 1.3	7.9 ± 8.2	18.0 ± 1.3	0.7 ± 0.1	3.2 ± 1.5	8.8 ± 7.4
20:1(n-9)	0.4 ± 0.1	3.9 ± 5.2**aA**	0.3 ± 0.2	0.3 ± 0.1**a**	0.8 ± 0.4	1.6 ± 1.5	3.8 ± 4.8	19.2 ± 25.9	1.3 ± 0.9	16.9 ± 5.4
22:1(n-9)	3.2 ± 2.0	6.6 ± 5.7	3.3 ± 2.2	3.5 ± 2.9	3.3 ± 1.3	1.7 ± 0.9**abc**	4.7 ± 2.3**a**	3.9 ± 2.5**b**	5.0 ± 4.4	8.0 ± 1.7**c**
24:1(n-9)	2.9 ± 4.5	0.9 ± 1.1**ab**	3.8 ± 1.5**acAB**	2.7 ± 1.2**dCDE**	0.4 ± 0.0**bcd**	1.0 ± 0.5**abc**	0.8 ± 0.1**a**	0.4 ± 0.2**bdAC**	0.6 ± 0.5**d**	0.2 ± 0.0**c**
Total MUFA	26.3 ± 9.0	26.4 ± 5.8**a**	24.6 ± 4.6	12.2 ± 3.2	30.8 ± 5.1**aA**	27.3 ± 8.9**a**	33.1 ± 7.5	31.7 ± 20.4	33.1 ± 9.7	43.0 ± 1.2**a**
18:2(n-6)	2.6 ± 1.3**a**	6.6 ± 5.2	1.2 ± 1.0	0.7 ± 0.5	5.3 ± 2.0**a**	5.3 ± 2.2	4.7 ± 1.3	1.6 ± 0.9	5.8 ± 1.0	2.8 ± 0.3
18:3(n-3)	1.0 ± 1.1**aA**	0.5 ± 0.4**a**	0.4 ± 0.3**BC**	0.6 ± 0.2**D**	2.8 ± 2.5**a**	1.1 ± 1.0**a**	1.0 ± 0.0**a**	1.2 ± 0.9	0.9 ± 0.2**D**	1.2 ± 0.8
20:2(n-6)	7.5 ± 13.3	4.9 ± 6.2	0.6 ± 0.1	0.9 ± 0.1	1.4 ± 0.5	1.3 ± 1.6	0.5 ± 0.1	1.5 ± 0.3	0.7 ± 0.2	1.5 ± 0.9
20:3(n-6)	0.3 ± 0.1	nd	nd	nd	nd	0.6 ± 0.3	nd	nd	nd	nd
20:4(n-6) (ArA)	3.3 ± 0.8	4.6 ± 5.4	0.7 ± 1.1**a**	0.8 ± 0.0**b**	1.1 ± 0.2**abA**	4.6 ± 1.2**a**	2.9 ± 2.7**a**	4.7 ± 2.0	2.9 ± 3.1	1.3 ± 1.0**A**
20:5(n-3) (EPA)	3.4 ± 2.8	0.9 ± 0.1**a**	10.4 ± 4.3**ab**	7.8 ± 5.2**cB**	1.2 ± 0.4**bc**	3.0 ± 1.7**A**	1.1 ± 1.0	9.5 ± 12.2	1.3 ± 0.6	1.6 ± 0.5
22:4(n-6)	3.6 ± 0.2	2.9 ± 2.3	11.2 ± 7.3	9.9 ± 1.2	1.5 ± 1.6	7.5 ± 7.3	2.7 ± 1.0	7.3 ± 6.1	2.8 ± 1.4	2.1 ± 0.1
22:5(n-3)	2.7 ± 2.0	2.2 ± 1.7	3.8 ± 1.2	1.1 ± 1.1**A**	1.0 ± 0.7	2.0 ± 1.8	3.3 ± 0.5	6.6 ± 8.3	2.5 ± 1.5	1.1 ± 0.5
22:6(n-3) (DHA)	2.5 ± 1.5	3.1 ± 3.3	5.8 ± 4.1**a**	7.0 ± 2.81**bAB**	1.2 ± 0.0**abD**	3.4 ± 1.8	1.4 ± 0.7	4.2 ± 2.1	1.7 ± 1.4**AC**	1.2 ± 0.8
Total (n-6) PUFA	13.8 ± 13.1	19.1 ± 19.1	6.9 ± 9.7	12.3 ± 4.1	9.3 ± 0.7	18.7 ± 5.6	10.5 ± 5.3	14.4 ± 8.3	12.2 ± 5.3	7.8 ± 0.6
Total (n-3) PUFA	7.5 ± 7.2	6.5 ± 4.8	15.4 ± 2.7	16.5 ± 3.2	6.1 ± 2.7	9.4 ± 2.8	5.6 ± 1.2	21.4 ± 6.8	6.8 ± 2.2	4.8 ± 0.5
DHA/EPA	1.2 ± 0.4	2.8 ± 2.8	0.8 ± 0.1	1.0 ± 1.0	1.3 ± 0.1**C**	2.0 ± 0.8**AB**	0.9 ± 0.0	1.6 ± 1.9	1.2 ± 1.2	1.3 ± 1.3
Total proteins	5.5 ± 1.9**aA**	4.2 ± 1.5	6.2 ± 2.0	28.6 ± 11.4**aD**	nd	5.0 ± 2.0**BC**	1.4 ± 1.2	1.6 ± 1.1	9.0 ± 2.7**E**	10.3 ± 0.7

Appendix. Continued.

Biochemical	M3					B1				
Components	Cysts	Nauplii	Metanauplii	Males	Females	Cysts	Nauplii	Metanauplii	Males	Females
14:0	1.2 ± 1.1	0.9 ± 1.3	0.2 ± 0.2**aA**	0.4 ± 0.4	1.0 ± 0.9**a**	0.4 ± 0.3**a**	0.6 ± 0.2	0.9 ± 0.0	1.4 ± 1.4**a**	0.8 ± 0.1
16:0	23.9 ± 3.7	28.1 ± 1.9	24.3 ± 0.7**B**	20.0 ± 11.3	24.6 ± 2.0	24.8 ± 3.3	29.5 ± 8.2**A**	29.4 ± 6.6**C**	24.6 ± 2.4	25.7 ± 2.6
18:0	19.7 ± 8.7	23.9 ± 0.2	25.2 ± 10.2	15.1 ± 5.2	23.3 ± 13.3	18.2 ± 6.9	15.2 ± 2.7	16.0 ± 0.9	20.6 ± 4.6	20.6 ± 5.8**A**
20:0	1.6 ± 0.8	0.6 ± 0.0	1.2 ± 0.4**A**	1.4 ± 0.5**B**	0.6 ± 0.1**E**	0.8 ± 0.4	1.2 ± 0.3	1.4 ± 1.0	1.1 ± 0.5	1.2 ± 0.6**CDE**
22:0	4.0 ± 5.7	1.3 ± 1.1	1.6 ± 0.4**A**	3.9 ± 4.7	1.2 ± 0.4	1.6 ± 1.7	1.9 ± 0.6**a**	7.0 ± 4.9**aB**	1.4 ± 1.3	2.1 ± 0.9
24:0	1.2 ± 1.3	2.4 ± 2.7	1.4 ± 1.3**A**	1.8 ± 2.0	nd	0.6 ± 0.1	1.1 ± 0.2	2.2 ± 1.1	0.7 ± 0.7	2.0 ± 1.1
Total saturated FA	50.8 ± 11	57.3 ± 3.0	53.4 ± 9.3	41.9 ± 18.8	50.9 ± 15.9	45.9 ± 10.3	49.4 ± 5.4	54.7 ± 7.5	49.9 ± 2.1	49.9 ± 5.5**A**
14:1(n-5)	2.2 ± 0.8**a**	0.6 ± 0.3**abc**	1.1 ± 1.3	1.1 ± 0.8**b**	2.2 ± 1.6**c**	3.5 ± 1.5	5.0 ± 1.8	5.1 ± 2.1	3.6 ± 0.7	6.4 ± 2.1
16:1(n-7)	2.1 ± 1.3	1.7 ± 0.5	2.3 ± 1.3	2.2 ± 1.2	2.5 ± 1.0	3.3 ± 2.6	2.4 ± 0.8	1.5 ± 1.1	3.0 ± 2.6	3.2 ± 0.1
18:1(n-9)	9.5 ± 7.9	5.4 ± 6.8	16.8 ± 6.4	9.7 ± 2.9	10.4 ± 3.2	19.1 ± 7.4	10.1 ± 3.6	11.9 ± 4.1	6.8 ± 7.1	16.2 ± 5.8
18:1(n-7)	8.2 ± 10.7	10.5 ± 13.3	0.5 ± 0.2	3.0 ± 2.0	13.5 ± 11.4	5.6 ± 8.4	0.9 ± 0.2	3.5 ± 2.1	10.3 ± 11.3	4.3 ± 0.3
20:1(n-9)	3.0 ± 4.9**a**	0.7 ± 0.9**A**	0.3 ± 0.4	24.8 ± 34.6	2.7 ± 1.0**a**	0.5 ± 0.2	1.9 ± 1.4	2.4 ± 1.1**a**	3.3 ± 3.3	0.9 ± 0.6**a**
22:1(n-9)	5.4 ± 2.5	6.5 ± 3.0	6.2 ± 1.7	2.6 ± 1.5	2.5 ± 0.1	1.8 ± 1.1**ab**	5.8 ± 3.5**ac**	1.4 ± 0.1**cd**	3.7 ± 3.9	5.0 ± 5.9**bd**
24:1(n-9)	0.8 ± 0.8	0.3 ± 0.2	0.6 ± 0.5**B**	0.3 ± 0.0**D**	4.6 ± 1.2	0.9 ± 0.7	1.4 ± 0.1	1.1 ± 0.2	0.9 ± 0.1**E**	0.3 ± 0.1
Total MUFA	29.5 ± 12.8	25.6 ± 10.9	28.0 ± 1.5	42.1 ± 28.9	28.3 ± 4.5	30.5 ± 7.9	25.0 ± 5.1	19.4 ± 6.5	31.3 ± 12.8	30.4 ± 3.4**A**
18:2(n-6)	3.7 ± 2.2	3.2 ± 0.2	3.6 ± 1.9	3.7 ± 2.7	4.8 ± 0.9	6.4 ± 1.9	4.1 ± 1.3	4.4 ± 1.0	3.8 ± 1.3	3.7 ± 0.5
18:3(n-3)	0.5 ± 0.4**A**	0.5 ± 0.1	0.3 ± 0.2**B**	0.3 ± 0.1	1.3 ± 0.1	1.0 ± 0.5	0.7 ± 0.4	0.4 ± 0.0**C**	1.8 ± 0.9	0.8 ± 0.4
20:2(n-6)	0.1 ± 0.5	0.4 ± 0.3	0.2 ± 0.2	0.7 ± 0.1	1.0 ± 0.3	1.1 ± 0.5	1.4 ± 0.1	1.1 ± 0.1	0.4 ± 0.1	0.8 ± 0.5
20:3(n-6)	0.5 ± 0.3**a**	0.9 ± 0.3	0.4 ± 0.0**b**	0.1 ± 0.0**ab**	0.5 ± 0.1	0.8 ± 0.7	0.8 ± 0.2	0.7 ± 0.0	0.6 ± 0.1	0.9 ± 0.1
20:4(n-6) (ArA)	3.4 ± 1.9	2.0 ± 1.4**a**	3.1 ± 3.9	2.4 ± 2.6**a**	3.2 ± 1.8	2.4 ± 1.2	3.4 ± 2.1	2.8 ± 1.1	2.5 ± 2.2	3.3 ± 1.1
20:5(n-3) (EPA)	3.2 ± 1.8**A**	4.6 ± 5.4	2.4 ± 0.0	3.0 ± 0.5	1.5 ± 1.2	3.1 ± 1.8	4.4 ± 2.9	9.6 ± 3.3	3.0 ± 3.7**B**	5.3 ± 2.5
22:4(n-6)	3.7 ± 1.2	1.9 ± 0.4	3.1 ± 1.9	3.1 ± 2.9	3.8 ± 3.0	2.7 ± 0.9	3.9 ± 2.1	8.6 ± 6.6	2.7 ± 2.5	2.5 ± 0.3
22:5(n-3)	3.0 ± 1.5	2.3 ± 0.1	1.1 ± 0.4	0.6 ± 0.3	2.8 ± 0.1	3.7 ± 2.6	4.6 ± 4.4	0.7 ± 0.1	1.2 ± 1.5**A**	3.3 ± 1.1
22:6(n-3) (DHA)	2.5 ± 1.2	1.5 ± 0.6	4.0 ± 4.7	2.2 ± 1.9	1.9 ± 1.7	3.1 ± 1.5	3.5 ± 3.0**a**	5.3 ± 1.2	3.1 ± 3.8**aBC**	3.4 ± 0.8**D**
Total (n-6) PUFA	11.2 ± 5.3	8.0 ± 1.5	10.2 ± 8.2	9.7 ± 8.8	13.1 ± 6.5	12.6 ± 2.2	12.6 ± 4.7	13.1 ± 0.2	9.7 ± 6.7	8.8 ± 4.4
Total (n-3) PUFA	8.6 ± 4.3	8.9 ± 6.3	7.3 ± 4.1	6.2 ± 1.2	7.6 ± 4.9	10.9 ± 5.3	12.9 ± 9.7**a**	12.7 ± 1.8**a**	9.1 ± 8.2	10.8 ± 4.5
DHA/EPA	0.9 ± 0.2**aA**	1.1 ± 0.6	1.8 ± 2.1	0.5 ± 0.4	1.3 ± 0.3**a**	0.9 ± 0.2**B**	0.5 ± 0.3	3.3 ± 3.4	0.9 ± 0.0**a**	0.4 ± 0.1**aC**
Total proteins	4.6 ± 1.2**abAB**	1.0 ± 0.7**acd**	2.1 ± 0.3**be**	13.3 ± 3.4**cDE**	6.8 ± 0.1**deF**	3.4 ± 1.3**abC**	2.3 ± 2.6	4.6 ± 1.5	24.0 ± 6.5**a**	44.1 ± 13.3**bF**

Letters denote statistical differences as shown by two-way ANOVA:
a, b, c, d, e, f per row denote significant differences among stages within the same ponds ($p < 0.05$);
A, B, C, D, E per row denote significant differences among ponds within the same stages ($p < 0.05$);
nd: not detected.