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Planktonic trophic interactions in a human-impacted estuary of Argentina: a fatty acid marker approach

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Few studies have been made on planktonic food webs of temperate ecosystems, especially those from the Southern Atlantic Ocean, using molecular biomarkers. The fatty acid compositions of suspended particulate matter (SPM), microplankton and mesozooplankton were studied during summer and winter at a sewage-impacted and a control site in the Bahía Blanca Estuary (Argentina). The aim was to identify trophic relationships on a spatial and seasonal scale and to detect allochthonous inputs to the food web. Fatty acid trends were consistent with the seasonal succession of the plankton community structure supporting our underlying hypothesis that regional seasonality is mostly responsible for changes in fatty acid composition. Sewage had no clear impact on the fatty acids and may not be a significant source of SPM in the estuary. However, at the sewage site the composition of the SPM was more related to terrestrially derived compounds, diatoms and bacteria, and mesozooplankton fatty acids suggested grazing on terrestrial components and on diatoms over flagellates. Saltmarshes likely have a crucial role as the main contributors to the organic fraction of SPM followed by plankton. The seasonal fatty acid pattern of the mesozooplankton indicated different feeding strategies suggesting an active feeding mode during summer and a more terrestrially associated diet in winter. The fatty acid trophic marker approach provided relevant information to clarify planktonic

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trophic interactions and to trace the origin of organic matter in this highly dynamic temperate coastal system.

KEYWORDS: predator–prey relationships; molecular biomarkers; plankton; sewage; coastal environment; South America

INTRODUCTION

Estuaries have very complex trophic dynamics. Grazing and detrital food webs coexist in a highly physically variable environment commonly impacted by human activities with temporal and spatial changes occurring at different time scales (McLusky and Elliot, 2004). Significant and diverse inputs of organic matter from a variety of marine, terrestrial and anthropogenic sources enter estuaries and constitute relevant energy sources to the ecosystem (Bodineau *et al.*, 1998; Zimmerman and Canuel, 2001).

The study of anthropogenic impacts on trophic dynamics is fundamental for the understanding of coastal systems' responsiveness and functioning. Molecular markers are one of the most appropriate tools currently in use in aquatic environments to elucidate allochthonous and autochthonous contributions and trace these substances through the food web (e.g. Seguel *et al.*, 2001; Cloern *et al.*, 2002; Copeman and Parrish, 2003). Lipids and fatty acids comprise one of the most promising approaches for studying food web dynamics (Iverson, 2009). Trophic relationships concerning dietary preferences, feeding behaviour and ecological niche traits have been successfully inferred from the study of fatty acid profiles in different marine ecosystems (Dalsgaard *et al.*, 2003; Kelly and Scheibling, 2012). The seasonality of food supply is a very important factor influencing lipid storage in animals (Hagen and Auel, 2001). Thus, much knowledge of marine plankton lipids has been obtained chiefly from high-latitude regions where primary production only occurs during the short summer season (Kattner *et al.*, 2007). Planktonic food webs of temperate coastal habitats, especially those from the Southern Atlantic Ocean, have been little studied using fatty acid analysis (e.g. Napolitano *et al.*, 1997; Santos *et al.*, 2008). Information on planktonic fatty acids from this region is needed to perform latitudinal comparisons which provide a general field-oriented overview on this topic (Kattner and Hagen, 2009).

Analysing food web relationships with fatty acid trophic markers (FATM) in a highly turbid and human-impacted estuary will provide better knowledge of the energetic pathways of the system, and how environmental factors and pollution sources can alter lipid metabolism in a temperate ecosystem. Although planktonic organisms of temperate regions do not store large amounts of lipids compared with those from high latitudes (Lee *et al.*, 2006; Kattner *et al.*, 2007), we hypothesize that environmental factors such as regional seasonality are strong enough to induce variations in the fatty acid composition of the main planktonic groups. We also hypothesize that a local source of pollution, a domestic-waste discharge, is relevant in influencing the fatty acid composition within the estuarine food web and that sewage-derived particles can be traced by fatty acid markers in plankton fractions and particulate matter. The aims of this study were to characterize suspended particulate matter (SPM), microplankton and mesozooplankton in terms of fatty acid composition in summer and winter at a sewage-impacted and a control site, to detect seasonal and spatial variation in the fatty acid compositions and to identify allochthonous compounds entering the estuarine trophic web.

METHOD

Study area

The Bahía Blanca Estuary (38°45′–39°40′S, 61°45′–62°30′W) is a coastal plain environment located in the Southwestern Atlantic Ocean in a semi-arid region of Argentina. It is a shallow funnel-shaped estuary (mean depth of 10 m) formed by a series of NW–SE channels separated by interconnected tidal channels, islands, extensive tidal flats and low marshes (Perillo *et al.*, 2001). Its drainage area covers urban and industrial settlements and agricultural lands. The main zooplankton species in the estuary are the calanoid copepods *Acartia tonsa* and *Eurytemora americana*, zoeae of the crab *Neohelice granulata* and larvae of the cirripede *Balanus glandula* (Hoffmeyer, 2004). The two sampling sites are located in the inner estuary. Canal Vieja (CV) is a channel that receives non-treated sewage from the Bahía Blanca City (~300 000 inhabitants) and inputs from the Napostá Grande Stream during ebb tide. It is situated on the northern coast 4.5 km downstream from a port and industrial complex. Bahía del Medio (BM) is a small tidal channel not affected by domestic or industrial waste (Andrade *et al.*, 1996; Baldini *et al.*, 1999) and associated with a system of interconnected channels surrounded by saltmarshes of *Spartina alterniflora*.

Further description of the study sites and environmental data are reported in detail by Dutto *et al.* (Dutto *et al.*, 2012).

Sampling and storage

Sampling campaigns were carried out in July and December 2008 and in February, March, July and August of 2009 and 2010 at CV and BM in the Bahía Blanca Estuary. Water samples (1 L) were collected with a Van Dorn bottle at 0−2-m depth. Plankton samples were collected with plankton nets: 20-μm mesh for microplankton (which includes phytoplankton and microzooplankton) and 200-μm mesh for mesozooplankton. Large components which did not belong to the samples (e.g. plant debris) were removed using a 1.7-mm sieve. The net tows were performed horizontally at *ca.* 2 knots. At each site in both seasons, four replicates of each planktonic fraction were taken in the upper layer (0−2 m) during mid-ebb tide to favour sampling the sewage plume and the highest zooplankton abundance (Menéndez *et al.*, 2012). Additionally, dry leaves of *S. alterniflora* were collected on 7 October 2011 from the inner estuary (38°44′07″S, 62°19′05″W) to complement the results and acquire information about the saltmarshes' input to the detritus composition. Samples were vacuum filtered using muffled GF/F filters and subsequently lyophilized for 48 h to finally be weighed with an analytical balance. Following the approaches used by Albers *et al.* (Albers *et al.*, 1996), samples were then immediately transferred into glass vials with a Teflon cap containing dichloromethane:methanol (2 : 1 by volume). All samples were stored under nitrogen atmosphere at −20°C until analysis.

Lipid analysis

All samples were homogenized and a 23:0 fatty acid standard was added. The homogenate was filtered through a pre-washed (dichloromethane:methanol) Whatman® paper filter and total lipids were extracted using dichloromethane:methanol (2:1 by volume) essentially after following Folch *et al.*'s method (Folch *et al.*, 1957). Samples were evaporated with nitrogen to dryness, and lipid mass was determined gravimetrically. Lipids were hydrolysed and the fatty acids converted to their methyl ester derivatives (FAME) by transesterification in methanol-containing 3% concentrated sulphuric acid at 80°C for 4 h. After extraction with hexane, FAME were analysed with a gas chromatograph (Hewlett-Packard 6890 series), equipped with a DB-FFAP fused silica capillary column (30 m × 0.25 mm inner diameter; 0.25-μm film thickness) using temperature programming and helium as carrier gas (Kattner and Fricke, 1986). FAME were detected by flame ionization and identified by comparing retention times with those calculated from commercial and natural standard mixtures. If necessary identification was confirmed by gas chromatography-mass spectrometry. Class-VP software (4.3) (Shimadzu) was used for recording and integration.

Statistical analysis

The composition of fatty acids (≥1% of total fatty acids) was plotted to characterize each planktonic fraction. Major fatty acids (≥10%) and the total content of saturated fatty acids (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) were compared among fractions. To determine variations in the fatty acid composition between sites and periods within each planktonic fraction, ANOVAs were performed considering most fatty acids (≥0.1% of total fatty acids) including bacteria biomarkers. Summer and winter encompassed December, February and March, and July and August, respectively. The interaction between factors (site × period) was analysed and those fatty acids that showed a significant interaction ($P < 0.05$) were compared among all factor levels. The fatty acids that showed no significant interaction were compared separately between each factor level (CV vs. BM and summer vs. winter). In all cases, mean comparisons were performed using ANOVA followed by Fisher's least significant difference (LSD) after checking the assumptions of normality and homoscedasticity. If assumptions were not met for ANOVA, data were arcsine transformed (Sokal and Rohlf, 1999). The significance level was 0.05 and the statistical software used was INFOSTAT® (free version http://www.infostat.com.ar). Multivariate analysis was performed on the percentage of fatty acid composition of all samples (≥1% of total fatty acids detected in each sample) using PRIMER-E® 5. Following the approaches used by Cook *et al.* (Cook *et al.*, 2010), data were not transformed to prevent an excessive weighting to fatty acids with a low contribution to the signature. This data set was converted into a similarity matrix using the Bray−Curtis index. Non-metric multidimensional scalling (nMDS) analyses were carried out to visualize patterns according to the type of sample, sites and periods. Similarity percentage routines (SIMPER) were performed to detect the fatty acids which contributed most to each planktonic fraction, site or period. Analysis of similarity (ANOSIM) was applied to detect statistical differences in the fatty acid compositions between fractions, sites and seasons (Clarke and Warwick, 1994).

RESULTS

Fatty acid composition of the planktonic fractions and *Spartina alterniflora*

Major fatty acids of SPM were the saturates 16:0 and 18:0, and the monounsaturate 18:1(*n*-9). These fatty acids comprised 63% of total fatty acids (28, 24 and 11%, respectively) (Fig. 1) and contributed 73.8% of the average similarity (SIMPER) within the fraction (Supplementary data, Table SI). The total proportions of 16:0 and 18:0 were larger in SPM than in the planktonic fractions (Fig. 1, ANOVA and LSD test, $P < 0.05$). In SPM, total SFAs were significantly higher and PUFAs lower in comparison with microplankton and mesozooplankton during the whole period (Fig. 2).

The main fatty acids of the microplankton were the 16:0, 18:0, 16:1(*n*-7), 18:1(n-9) and 20:5(*n*-3). These fatty acids accounted for 59% of total fatty acids (Fig. 1) and, except 18:1(*n*-9), were indicated by SIMPER as the main contributors to the average of similarity within the fraction (Supplementary data, Table SI). The proportion of 16:1(*n*-7) was higher in this planktonic fraction than in other fractions (Fig. 1, ANOVA and LSD test, $P < 0.05$). Mesozooplankton and microplankton had a similar fatty acid composition, except the 22:6(*n*-3) which was significantly higher (>10%) in mesozooplankton (Supplementary data, Table SI, Figs 1 and 2, ANOVA and LSD test, $P < 0.05$). The PUFA content and particularly 20:5(*n*-3) was significantly higher in the microplankton and mesozooplankton compared with the SPM (Figs 1 and 2 ANOVA and LSD test, $P < 0.05$). SPM, microplankton and mesozooplankton were statistically different in the overall fatty acid composition, although the *R* values were low (Supplementary data, Table SII).

The dry leaves of *S. alterniflora* were mainly composed of 16:0 (20%), 18:0 (20%) and 18:2(*n*-6) (23%) and the less dominant 18:3(*n*-3) (8%) and 18:1(*n*-9) (7%).

Spatio-temporal variations

nMDS revealed a limited differentiation in the spatial ordination of the groups with a moderate goodness of fit (stress value of 0.12; Fig. 3). The mesozooplankton was ordered as two subgroups corresponding to summer (upper right ordination) and winter (bottom left ordination) (Fig. 3a). It was placed within the other two planktonic fractions (SPM and microplankton). The spatial arrangements considering sites and periods as factors showed a poorly defined pattern, although a grouping trend of CV and summer samples was indicated (Fig. 3b and c). *Spartina alterniflora* samples were arranged close to the winter mesozooplankton (Fig. 3a and c) and the CV group (Fig. 3b). The 16:0 and 18:0 were the major contributors to the formation of site and period groups (CV and BM; summer and winter) followed by the 18:1(*n*-9) in the case of CV. The 20:5(*n*-3) in BM and in winter had a contribution close to 10% for both groups (Supplementary data, Table SI). Sites were not significantly different in terms of fatty acid composition, whereas differences were found for the periods (ANOSIM, $P < 0.010$; Supplementary data, Table SII).

Significant interactions between sites and periods were revealed in some SPM and mesozooplankton fatty acids (ANOVA, $P < 0.05$). Because the effect of the site on these fatty acids was not the same in both periods, means were compared between factor levels (ANOVA and LSD test; Table I). Fatty acids which did

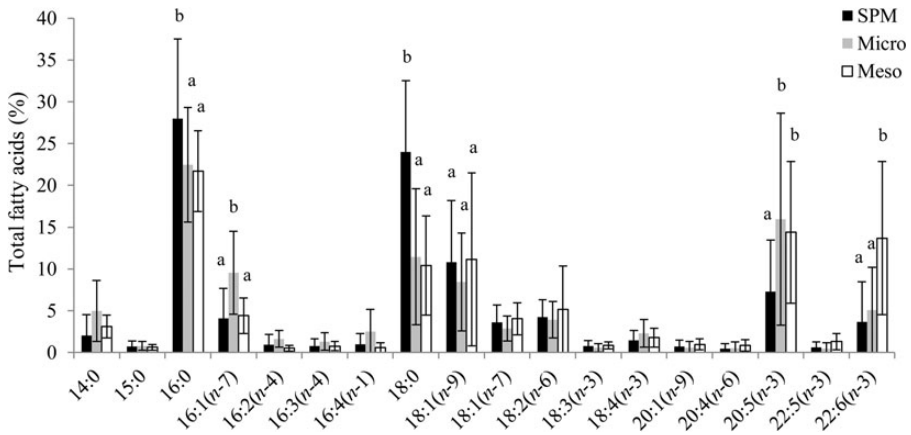

Fig. 1. Composition of fatty acids (mean and standard deviation in mass%) of the SPM, the microplankton (Micro) and the mesozooplankton (Meso). Plotted fatty acids contribute ≥1% to the total fatty acid. Main fatty acids (≥ 10%) were compared between the fractions by ANOVA and LSD test. Letters alphabetically assigned from low to high mean. Means sharing a letter do not differ at 5%. SPM: $n = 17$, Micro: $n = 19$, Meso: $n = 17$.

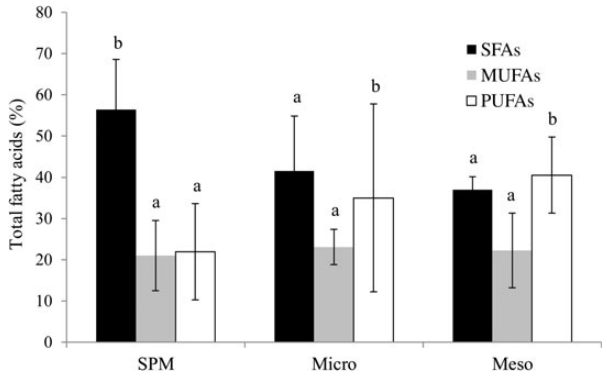

Fig. 2. SFAs, MUFAs and PUFAs in the SPM, the microplankton (Micro) and the mesozooplankton (Meso). Fatty acids were compared between the planktonic fractions by ANOVA and LSD test. Letters alphabetically assigned from low to high mean. Means sharing a letter do not differ at 5%.

not show a significant interaction between factors (site and period) were compared between sites using the average of both periods and *vice versa* (Tables II–IV). In SPM, the 20:5(*n*-3)/22:6(*n*-3) ratio showed a different pattern between the periods at each site. This ratio was not significantly different between summer and winter at CV, but at BM the winter value was significantly higher than in summer (Table I). In the mesozooplankton, the 20:4(*n*-6) decreased at CV in winter and remained constant in the rest of the cases (Table I). The proportions of the bacterial markers, i15:0 and a15:0, in the mesozooplankton were relatively constant. The i15:0 varied at BM being significantly higher in summer. The a15:0 was different in CV with a higher proportion in winter (Table I). In this planktonic fraction, the terrestrial marker, 18:2(*n*-6), the sum of 18:2(*n*-6) and 18:3(*n*-3), as well as the ratio 20:5(*n*-3)/22:6(*n*-3) were significantly higher at CV in winter (Table I).

In SPM, the 18:2(*n*-6), the sum of 18:2(*n*-6) and 18:3(*n*-3) and the ratio i+a15:0/15:0 were significantly different between sites and also higher at CV. However, none of the SPM fatty acids varied significantly between the periods (Table II). In the microplankton, the 18:1(*n*-9), 18:2(*n*-6) and 18:2(*n*-6) + 18:3(*n*-3) revealed significantly higher proportions at CV, whereas 16:1(*n*-7) and the 18:1(*n*-7)/18:1(*n*-9) ratio showed significantly higher levels at BM (Table III). Comparing the periods within the microplankton, 18:3(*n*-3) and 22:5(*n*-3) were significantly higher in summer, whereas 16:4(*n*-1) and the 20:5(*n*-3)/22:6(*n*-3) ratio were higher in winter (Table III).

Variation in the fatty acid levels of mesozooplankton was less evident between sites than between periods (Table IV). The 18:1(*n*-9) was the only fatty acid whose proportion was significantly higher at CV. 16:1(*n*-7), 18:4(*n*-3) and the ratio 18:1(*n*-7)/18:1(*n*-9) had significantly higher proportions at BM (Table IV). Comparing the two periods within the mesozooplankton, total PUFAs, 22:6(*n*-3), 20:5(*n*-3), 16:1(*n*-7), 14:0, 18:4(*n*-3), 18:3(*n*-3), the ratio 18:1(*n*-7)/18:1(*n*-9) and 15:0 showed significantly higher values in summer (Table IV), whereas total MUFAs, 18:1(*n*-9), 18:0, 16:0/16:1(*n*-7), i+a15:0/15:0, 16:4(*n*-1), 18:1(*n*-7) and 20:1(*n*-9) were significantly higher in winter (Table IV).

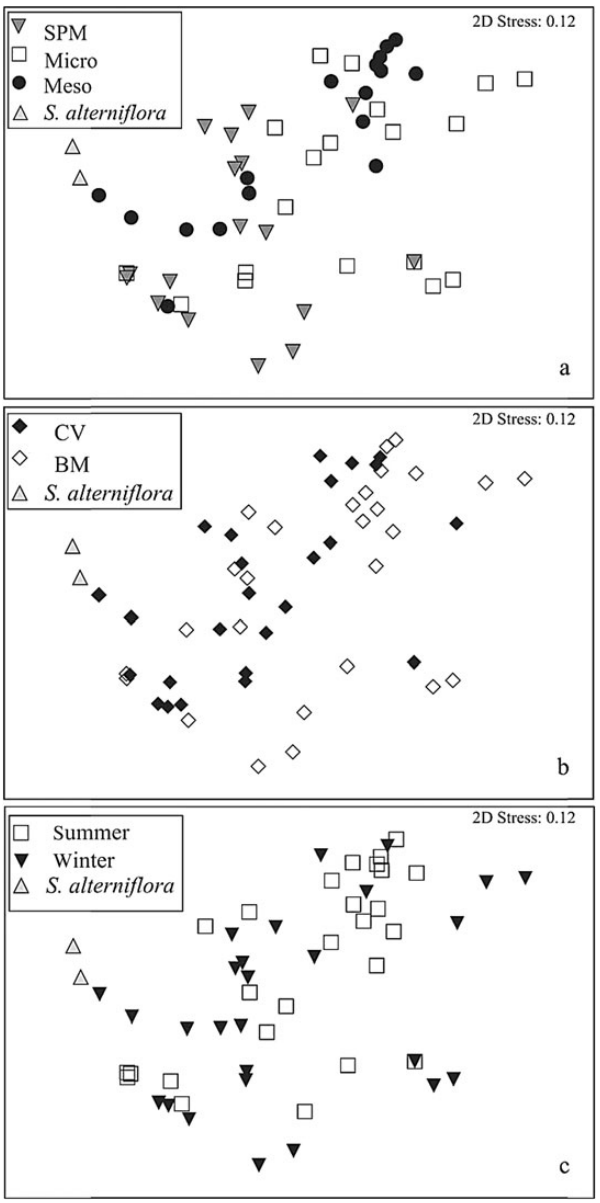

Fig. 3. nMDS plots considering (**a**) planktonic fractions, (**b**) sites and (**c**) periods studied. Numbers of samples: SPM = 17, Micro = 19, Meso = 17, *Spartina alterniflora* *n* = 2, Canal Vieja sewage-impacted site (CV) *n* = 25, Bahía del Medio control site (BM) *n* = 28, summer *n* = 26, winter *n* = 27.

Table I: Fatty acids and ratios that showed interaction between sites and periods

Fatty acid or ratio	CV		BM	
	Summer	Winter	Summer	Winter
SPM				
20:5(*n*-3)/22:6(*n*-3)	2.29 ± 0.66^{ab}	3.11 ± 1.67^{bc}	0.81 ± 0.41^{a}	3.88 ± 0.62^{c}
Mesozooplankton				
i15:0	0.17 ± 0.06^{ab}	0.23 ± 0.13^{ab}	0.26 ± 0.08^{b}	0.10 ± 0.07^{a}
a15:0	0.11 ± 0.04^{a}	0.28 ± 0.16^{b}	0.20 ± 0.09^{ab}	tr^{a}
18:2(*n*-6)	3.92 ± 2.25^{a}	13.21 ± 4.46^{b}	1.41 ± 0.29^{a}	2.99 ± 1.26^{a}
20:4(*n*-6)	1.40 ± 0.38^{b}	tr^{a}	1.05 ± 0.52^{b}	0.84 ± 0.73^{b}
18:2(*n*-6) + 18:3(*n*-3)	4.88 ± 1.88^{a}	13.76 ± 4.51^{b}	2.68 ± 0.30^{a}	3.54 ± 1.33^{a}
20:5(*n*-3)/22:6(*n*-3)	1.01 ± 0.31^{a}	2.49 ± 0.94^{b}	0.99 ± 0.13^{a}	1.26 ± 0.35^{a}

Results are expressed as percentage relative to the total fatty acids (mean ± SD). All averages were compared by ANOVA and LSD test, $P < 0.05$. Letters alphabetically assigned from low to high values. Means sharing a letter do not differ at 5%, tr, trace value (<0.01% of total fatty acids).

Table II: Fatty acid compositions (mean ± SD in mass%) of the SPM at the sites and periods studied

Fatty acid or ratio	SPM			
	CV	BM	Summer	Winter
14:0	2.49 ± 3.42	1.58 ± 1.41	1.67 ± 1.08	2.30 ± 3.39
i15:0	0.32 ± 0.38	0.21 ± 0.22	0.25 ± 0.25	0.27 ± 0.36
a15:0	0.31 ± 0.27	0.20 ± 0.22	0.25 ± 0.26	0.26 ± 0.25
15:0	0.62 ± 0.61	0.78 ± 0.70	0.58 ± 0.64	0.81 ± 0.67
16:0	24.19 ± 6.54	31.33 ± 10.84	24.59 ± 6.11	30.98 ± 11.30
16:1(*n*-7)	5.05 ± 4.88	3.20 ± 1.77	3.64 ± 2.76	4.50 ± 4.30
16:2(*n*-4)	0.73 ± 0.80	1.14 ± 1.55	1.08 ± 1.70	0.83 ± 0.69
16:3(*n*-4)	0.94 ± 1.26	0.57 ± 0.32	0.49 ± 0.50	0.97 ± 1.10
16:4(*n*-1)	0.70 ± 0.80	1.22 ± 1.62	0.99 ± 1.82	0.96 ± 0.70
i17:0	0.28 ± 0.80	0.60 ± 0.94	0.17 ± 0.48	0.71 ± 1.07
18:0	25.14 ± 9.37	22.96 ± 8.15	26.36 ± 9.63	21.87 ± 7.35
18:1(*n*-9)	12.37 ± 5.77	9.44 ± 8.61	10.68 ± 6.06	10.95 ± 8.70
18:1(*n*-7)	4.05 ± 1.88	3.22 ± 2.25	3.50 ± 2.19	3.71 ± 2.07
18:2(*n*-6)	**5.41 ± 1.70**	3.21 ± 1.82	4.58 ± 2.48	3.95 ± 1.68
18:3(*n*-3)	0.77 ± 0.70	0.79 ± 0.66	0.94 ± 0.49	0.64 ± 0.77
18:4(*n*-3)	1.30 ± 0.96	1.63 ± 1.39	1.73 ± 1.39	1.24 ± 0.99
20:1(*n*-9)	0.48 ± 0.57	0.98 ± 0.80	0.74 ± 0.64	0.75 ± 0.84
20:4(*n*-6)	0.24 ± 0.44	0.66 ± 0.65	0.51 ± 0.55	0.42 ± 0.64
20:5(*n*-3)	6.94 ± 6.04	7.58 ± 6.67	6.86 ± 6.94	7.65 ± 5.84
22:5(*n*-3)	0.54 ± 0.64	0.64 ± 0.71	0.63 ± 0.61	0.56 ± 0.74
22:6(*n*-3)	2.34 ± 1.93	4.80 ± 6.31	5.46 ± 6.51	2.03 ± 1.75
i+a15:0/15:0	**0.98 ± 0.28**	0.54 ± 0.26	0.91 ± 0.34	0.61 ± 0.31
16:0/16:1(*n*-7)	9.46 ± 8.41	12.72 ± 7.21	11.71 ± 9.44	10.72 ± 6.40
18:1(*n*-7)/18:1(*n*-9)	0.40 ± 0.23	0.47 ± 0.27	0.45 ± 0.29	0.43 ± 0.22
18:2(*n*-6) + 18:3(*n*-3)	**6.19 ± 1.63**	4.00 ± 1.93	5.53 ± 2.93	4.58 ± 1.78
20:5(*n*-3)/22:6(*n*-3)	–	–	–	–
∑SFAs	54.29 ± 8.71	58.27 ± 14.89	54.56 ± 10.74	58.03 ± 13.75
∑MUFAs	24.13 ± 4.51	18.19 ± 10.46	20.67 ± 6.34	21.27 ± 10.52
∑PUFAs	20.67 ± 9.46	23.03 ± 13.86	24.78 ± 12.92	19.38 ± 10.58

Fatty acids compared between sites or period which were significantly higher are in bold (ANOVA and LSD test, $P < 0.05$). See Table I for fatty acids which showed interaction (–). SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; CV, Canal Vieja, sewage-impacted site; BM, Bahía del Medio, control site.

DISCUSSION

Fatty acid compositions are frequently applied to determine trophic relationships and food preferences (Dalsgaard *et al.*, 2003; Iverson, 2009). However, less is known about fatty acid trends in complex estuarine environments, which are exposed to many autochthonous and allochthonous influences.

Fatty acid composition of SPM, microplankton and mesozooplankton

The main fatty acid in the planktonic fractions was 16:0, which is the most common fatty acid in marine organisms and predominates in membrane lipids (Lee *et al.*, 2006). Although the 16:0 fatty acid was a significant component of each planktonic fraction, it was significantly

Table III: Fatty acid compositions (mean ± SD in mass%) of the microplankton at the sites and periods studied

Fatty acid or ratio	Microplankton			
	CV	BM	Summer	Winter
14:0	3.88 ± 1.86	5.95 ± 4.60	4.38 ± 2.95	5.50 ± 4.25
i15:0	0.28 ± 0.24	0.41 ± 0.45	0.42 ± 0.46	0.28 ± 0.25
a15:0	0.31 ± 0.24	0.25 ± 0.31	0.33 ± 0.35	0.23 ± 0.19
15:0	0.72 ± 0.26	0.84 ± 0.68	0.97 ± 0.63	0.61 ± 0.34
16:0	23.24 ± 8.24	21.76 ± 5.71	24.51 ± 6.43	20.61 ± 7.02
16:1(*n*-7)	7.22 ± 1.99	**11.62 ± 5.95**	8.21 ± 4.61	10.73 ± 5.18
16:2(*n*-4)	1.25 ± 0.63	1.97 ± 1.15	1.32 ± 0.92	1.90 ± 1.01
16:3(*n*-4)	1.10 ± 0.99	1.52 ± 1.09	1.42 ± 1.46	1.23 ± 0.50
16:4(*n*-1)	2.48 ± 3.15	2.49 ± 2.34	0.61 ± 0.58	**4.17 ± 2.70**
i17:0	0.19 ± 0.57	1.11 ± 2.63	0.31 ± 0.75	1.00 ± 2.62
18:0	12.95 ± 5.80	10.12 ± 9.93	14.49 ± 10.24	8.73 ± 4.71
18:1(*n*-9)	**11.38 ± 5.30**	5.81 ± 5.27	7.79 ± 5.57	9.04 ± 6.37
18:1(*n*-7)	3.24 ± 0.99	2.54 ± 1.85	3.07 ± 1.13	2.69 ± 1.83
18:2(*n*-6)	**5.46 ± 1.06**	2.55 ± 2.00	4.05 ± 2.13	3.82 ± 2.32
18:3(*n*-3)	0.53 ± 0.33	0.59 ± 0.64	**0.85 ± 0.55**	0.30 ± 0.30
18:4(*n*-3)	2.19 ± 1.58	2.41 ± 1.80	2.30 ± 1.50	2.32 ± 1.87
20:1(*n*-9)	0.32 ± 0.50	0.78 ± 0.87	0.76 ± 0.53	0.38 ± 0.88
20:4(*n*-6)	0.39 ± 0.62	0.65 ± 0.81	0.77 ± 0.66	0.31 ± 0.74
20:5(*n*-3)	15.30 ± 11.84	16.52 ± 14.00	11.54 ± 9.43	19.90 ± 14.34
22:5(*n*-3)	0.42 ± 0.61	0.47 ± 0.86	**0.87 ± 0.88**	0.06 ± 0.20
22:6(*n*-3)	5.12 ± 5.88	5.09 ± 4.61	6.83 ± 6.60	3.55 ± 2.75
i+a15:0/15:0	0.91 ± 0.63	0.66 ± 0.48	0.60 ± 0.50	0.97 ± 0.59
16:0/16:1(*n*-7)	3.72 ± 2.83	3.61 ± 5.61	5.30 ± 6.06	2.18 ± 0.94
18:1(*n*-7)/18:1(*n*-9)	0.32 ± 0.11	**0.61 ± 0.36**	0.59 ± 0.37	0.37 ± 0.19
18:2(*n*-6) + 18:3(*n*-3)	**5.99 ± 1.16**	3.14 ± 2.29	4.89 ± 2.06	4.12 ± 2.57
20:5(*n*-3)/22:6(*n*-3)	6.11 ± 5.86	3.98 ± 2.39	2.31 ± 1.39	**7.40 ± 4.82**
∑SFAs	41.66 ± 14.23	41.32 ± 14.84	46.31 ± 14.32	37.13 ± 13.17
∑MUFAs	23.04 ± 6.20	23.10 ± 7.00	21.71 ± 4.20	24.30 ± 8.00
∑PUFAs	35.33 ± 19.58	34.69 ± 19.48	31.40 ± 17.79	38.22 ± 20.36

For further details refer to Table II.

higher in the SPM, together with 18:0. Both SFAs defined the SPM of the estuary in accordance with the major components of lipids reported in seston from marine environments worldwide (Canuel *et al.*, 1997; Hama, 1999; Tiselius *et al.*, 2012). These saturates have been typically associated with non-living particles (Kattner *et al.*, 1983; Fahl and Kattner, 1993; Hayakawa *et al.*, 1997) and may reflect the general biochemical nature of the particulate matter of the Bahía Blanca Estuary, suggesting a greater contribution of detrital matter than of living cells. This is supported by the high turbidity in the estuary [annual mean of SPM of 77.6 mg L^{-1} (Guinder *et al.*, 2009)] enhanced by continental runoff and high resuspension processes (Freije *et al.*, 2008). In addition, SFAs were two of the three major components of dry leaves of *S. alterniflora*, which indicates the contribution of saltmarshes to the SPM in the water column. Natural supply of detritus coming from saltmarshes and tidal flats is promoted by twice a day flooding and exacerbated by the action of dominant winds and strong tides (Negrin *et al.*, 2011). The typical dynamic of this estuary governed by the influence of tides and winds induces resuspension and mixing processes (Perillo *et al.*, 2001) and may facilitate the incorporation, recirculation and retention of detritus in the water column. Although of only moderate importance to the signature, 18:1(*n*-9) was found to be quite relevant in the SPM of the estuary, suggesting a contribution from hard-to-identify detrital material (e.g. Fahl and Kattner, 1993). 18:1(*n*-9) is an important fatty acid in organisms from higher trophic levels (Graeve *et al.*, 1994, 1997; Kattner *et al.*, 2003) and may derive from zooplankton materials in the estuary. PUFAs quickly disappear from the water column due to their susceptibility to degradation and their high assimilation efficiency by marine crustaceans (Hama, 1999). However, the moderate proportions of 20:5(*n*-3) in combination with 16:1(*n*-7) in the SPM clearly showed that phytoplankton, especially diatoms (Dalsgaard *et al.*, 2003), contributed definitely to the SPM, although it is difficult to estimate its absolute input. Thus, as shown by the dominance of SFAs and to a lesser extent by fatty acids of zooplankton origin as well as by small contributions of phytoplankton, the SPM in the Bahía Blanca Estuary seemed to be composed in a descending order by detrital

Table IV: Fatty acid compositions (mean ± SD in mass%) of the mesozooplankton at the sites and periods studied

Fatty acid or ratio	Mesozooplankton			
	CV	BM	Summer	Winter
14:0	2.72 ± 0.64	3.45 ± 1.74	**3.88 ± 1.33**	2.24 ± 0.73
i15:0	–	–	–	–
a15:0	–	–	–	–
15:0	0.54 ± 0.19	0.70 ± 0.42	**0.87 ± 0.26**	0.35 ± 0.11
16:0	22.76 ± 5.47	20.77 ± 4.28	20.56 ± 3.84	22.99 ± 5.73
16:1(*n*-7)	3.26 ± 1.34	**5.42 ± 2.21**	**5.46 ± 1.67**	3.22 ± 2.00
16:2(*n*-4)	0.43 ± 0.31	0.59 ± 0.36	0.59 ± 0.35	0.43 ± 0.33
16:3(*n*-4)	0.56 ± 0.41	0.96 ± 0.57	0.64 ± 0.49	0.91 ± 0.56
16:4(*n*-1)	0.39 ± 0.39	0.78 ± 0.65	0.29 ± 0.14	**0.95 ± 0.67**
i17:0	tr	tr	tr	tr
18:0	10.92 ± 4.52	9.99 ± 7.22	7.33 ± 2.21	**13.91 ± 6.98**
18:1(*n*-9)	**16.63 ± 10.55**	6.29 ± 7.77	5.14 ± 5.07	**17.93 ± 10.80**
18:1(*n*-7)	4.61 ± 2.27	3.52 ± 1.49	2.95 ± 0.58	**5.25 ± 2.20**
18:2(*n*-6)	–	–	–	–
18:3(*n*-3)	0.76 ± 0.35	0.95 ± 0.47	**1.13 ± 0.33**	0.55 ± 0.25
18:4(*n*-3)	1.24 ± 0.92	**2.26 ± 1.14**	**2.52 ± 0.97**	0.94 ± 0.61
20:1(*n*-9)	0.83 ± 0.40	1.09 ± 0.84	0.58 ± 0.25	**1.40 ± 0.72**
20:4(*n*-6)	–	–	–	–
20:5(*n*-3)	10.75 ± 9.56	17.60 ± 6.27	**18.81 ± 4.59**	9.39 ± 9.33
22:5(*n*-3)	1.73 ± 0.94	0.92 ± 0.85	1.51 ± 1.06	1.06 ± 0.84
22:6(*n*-3)	10.24 ± 10.33	16.72 ± 7.24	**19.46 ± 5.43**	7.16 ± 8.18
i+a15:0/15:0	0.88 ± 0.70	0.52 ± 0.31	0.43 ± 0.09	**0.98 ± 0.70**
16:0/16:1(*n*-7)	8.50 ± 4.95	5.52 ± 5.76	4.15 ± 1.81	**10.04 ± 6.56**
18:1(*n*-7)/18:1(*n*-9)	0.37 ± 0.22	**0.92 ± 0.44**	**0.90 ± 0.46**	0.40 ± 0.24
18:2(*n*-6) + 18:3(*n*-3)	–	–	–	–
20:5(*n*-3)/22:6(*n*-3)	–	–	–	–
∑ SFAs	37.91 ± 8.74	36.16 ± 9.34	33.80 ± 6.51	40.57 ± 10.08
∑ MUFAs	26.35 ± 10.73	18.63 ± 8.09	16.17 ± 4.40	**29.13 ± 10.11**
∑ PUFAs	35.73 ± 17.10	44.73 ± 14.78	**49.57 ± 10.23**	30.30 ± 15.72

For further details refer to Table II. See Table III for fatty acids which showed interaction (–).

material from bordering habitats as saltmarshes, and detrital contributions from estuarine sources (e.g. plankton) and living planktonic cells. This result confirms the contribution of multiple sources of organic matter to the SPM of the Bahía Blanca Estuary.

In microplankton and mesozooplankton, PUFAs dominated over SFAs showing a higher level than in the SPM samples, and thus indicating a clear dominance of living particles (Hama, 1999). Even so, the proportion of saturates found in the planktonic fractions different from SPM, was probably overestimated, particularly in microplankton samples, due to the collection of considerable amounts of inorganic particles and organic compounds linked to them, which cannot be separated from living organisms. The typical diatom markers 20:5(*n*-3) and 16:1(*n*-7) (Kattner and Hagen, 1995; Parrish *et al.*, 1995) predominated in the microplankton fraction. These fatty acids did not vary significantly between summer and winter. This trend is consistent with recent findings in the Bahía Blanca Estuary. Guinder *et al.* (Guinder *et al.*, 2010) reported the modification of the typical phytoplankton seasonal pattern by hydroclimatic changes, from an isolated winter bloom to the occurrence of several phytoplankton growth episodes throughout the year especially in summer. Our biochemical characterization of microplankton indicates the greater contribution of phytoplankton than microzooplankton to this fraction and supports the important contribution of diatoms in the microplankton community of the estuary (Guinder *et al.*, 2010).

As mentioned before, mesozooplankton showed a strong contribution of PUFAs. Besides denoting typical structural membrane fatty acids of marine organism (Albers *et al.*, 1996; Graeve *et al.*, 2001), 20:5(*n*-3) and 22:6(*n*-3) indicate the important input of diatoms and flagellates to the mesozooplankton diet (Zhukova and Kharlamenko, 1999; Kopprio *et al.*, 2012). The fatty acid composition reflects an opportunistic feeding behaviour with high metabolic rates and a limited lipid accumulation as is known for zooplankton from temperate regions (Kattner and Hagen, 2009). These species are suggested to be also less dependent on bloom events as reflected by the low contribution of the typical diatom marker 16:1(*n*-7) and the corresponding ratios in our study. The

key mesozooplankton species in the estuary, *A. tonsa*, *N. granulata* zoeae and *B. glandula* larvae, are thought to be omnivorous and opportunistic (Hoffmeyer and Prado Figueroa, 1997; Diodato and Hoffmeyer, 2008). The only species recognized as chiefly herbivorous and consequently dependent on phytoplankton is the invader copepod *E. americana*, which is commonly abundant in the inner estuary during the cold season (Berasategui *et al.*, 2009). However, during our study, *E. americana* was not found possibly due to the unusually high winter temperatures and thus herbivory was not reflected by the fatty acid composition. Although the fatty acid composition of *A. tonsa* has not been determined yet in this system, the analysis of summer mesozooplankton would be mainly attributable to *A. tonsa* composition due to its dominance [~90% of total abundance (Hoffmeyer, 2004; Dutto *et al.*, 2012)]. Similar to our results, its congener *A. clausi* from the temperate North Sea showed a fatty acid composition dominated by the structural fatty acids with little contribution from dietary storage lipids (Kattner and Hagen, 2009). The 18:1(*n*-9) fatty acid, also important in the mesozooplankton fraction, is associated with an omnivorous or carnivorous feeding mode (Falk-Petersen *et al.*, 1990; Kattner *et al.*, 2003) and the high proportion of 22:6(*n*-3) may be accumulated by feeding on flagellates suggesting protozoa as important part of the mesozooplankton diet.

Fatty acid spatio-temporal variations in the plankton

The composition of SPM at the sewage site was probably associated with the influence of continental runoff. The contribution of the terrestrial marker 18:2(*n*-6) (Dalsgaard *et al.*, 2003) and values >2.5% of 18:2(*n*-6)+18:3(*n*-3) are interpreted as considerable terrestrial influence on aquatic environments (Budge *et al.*, 2001). This is in accordance with the findings of Napolitano *et al.* (Napolitano *et al.*, 1997) who detected continental inputs in the CV area and attributed 18:2(*n*-6) to agricultural product derivatives in the estuary. Port and industrial activities, which promote agricultural products, export sporadically into the estuary, and the discharge of the port complex close to our sewage sampling site may contribute to these fatty acids. Furthermore, the bacterial marker ratio i+a15:0/15:0 (White *et al.*, 1980) was also more relevant in the SPM of CV. Bacterial markers at CV are possibly associated with the sewage discharge and the surrounding environmental contributions (e.g. inputs from Napostá Grande Stream). This agrees with high abundances of *Escherichia coli* and coliphages, denoting recent faecal contamination, found in sediments and water in the sewage area of the estuary (Cabezalí and Baldini, 1990; Brezina and Baldini, 2008). Nevertheless, at both sites bacterial markers were denoted and therefore a clear relation of fatty acids to sewage impact could not be deduced. Bacterioplankton analyses of both sampling sites are therefore required to identify physiological groups which help to elucidate the origin of these bacterial markers.

Different terrestrial and planktonic components were potential contributors to the SPM of the sites according to the complex mixing of abundant and diverse organic matter sources estuaries exhibit (Bodineau *et al.*, 1998; Zimmerman and Canuel, 2001). Diatoms occur throughout the year (Guinder *et al.*, 2010) and their contribution to the SPM was noticeable at both sites. Other microplankton taxa are important in the estuary (e.g. aloricate ciliates, tintinnids, rotifers, dinoflagellates; Pettigrosso and Popovich, 2009; Barría de Cao *et al.*, 2011) also being relevant components of the particular matter, particularly at BM in summer in contrast to the sewage site. There, terrestrial-derived compounds, diatoms and bacteria probably contributed more to the particulate matter. Nevertheless, none of these components were exclusively found at the sewage site. Thus, the sewage contribution could not be well distinguished within the particulate matter of the estuarine area. Sewage seemed not to be a significant contributor to the particulate matter because we did not find significant amounts of bacterial fatty acids in the SPM of the sewage site, although we had expected to find some signal of sewage contribution at least to the particulate matter.

In the microplankton from winter, the higher levels of the diatom marker 16:4(*n*-1) (Kattner *et al.*, 1983; Mayzaud *et al.*, 1990), the 20:5(*n*-3)/22:6(*n*-3) ratio as well as 16:1(*n*-7) showed the important contribution of diatoms to the estuarine plankton community. Similarly to the SPM findings, a lower level of 20:5(*n*-3)/22:6(*n*-3) was observed in summer pointing to a more favourable development of microzooplankton. Besides the terrestrial input by SPM to the microplankton fraction indicated by 18:2(*n*-6) and 18:3(*n*-3) (Budge *et al.*, 2001), the proportion of 18:1(*n*-9) was also quite variable between the sites and significantly higher at the sewage-impacted site. This MUFA is scarce in phytoplankton but common in protozoa (Falk-Petersen *et al.*, 1990; Kattner *et al.*, 2003). At CV some protozoa species belonging to the genera *Lohmanniella* and *Strombidinopsis* may occur (Barría de Cao *et al.*, 2003). In contrast, the levels of 16:1(*n*-7) in the microplankton at the control site indicated a higher diatom contribution in the microplankton community.

It is well known that zooplankton can indicate changes in the trophic transfer, reflecting shifts in prey dominance and availability at the base of the food web and/or in feeding preferences (David *et al.*, 2006; Gonçalves *et al.*, 2012). Among the planktonic fractions, mesozooplankton

was likely to be more sensitive to the effect of the spatial and the seasonal scales. Major changes in the fatty acid composition between seasons revealed that the seasonal influence was stronger than the spatial one in modulating trophic features in the Bahía Blanca Estuary. However, some fatty acids in the mesozooplankton fraction indicating terrestrial and detrital sources [unsaturated C_{18} fatty acids with 1–3 double bonds (Parrish *et al.*, 1995; Canuel *et al.*, 1997)] and phytoplankton over flagellates [balance >1 towards 20:5(*n*-3) in relation to 22:6(*n*-3) (Dalsgaard *et al.*, 2003)] showed changes at the sewage-impacted site. Consequently, and taking into account the fact that fatty acids represent an integration of the dietary intake over a longer period of time (Kelly and Scheibling, 2012 and references therein), mesozooplankton seemed to indicate a specific sewage-related trophic behaviour, particularly during winter, revealing grazing on terrestrial components and on diatoms over flagellates. Nevertheless, as mentioned above, a seasonal trophic pattern was more obvious. Thus, mesozooplankton was likely to show a very opportunistic and flexible feeding strategy: a carnivorous and herbivorous feeding mode during summer suggested by the PUFAs content [e.g. 22:6(*n*-3), 20:5(*n*-3)] and a more terrestrially associated diet in winter as shown by high levels of the 16:0/16:1(*n*-9) ratio and the prevalence of C_{18} fatty acids (Dalsgaard *et al.*, 2003 and references therein). This result indicates mesozooplankton trophic preference since microplankton prey availability seemed to be broadly constant during both periods and seston-derived compounds were higher than the diatom and zooplankton components.

Seasonal variations in mesozooplankton feeding seemed not to be related to changes in the particulate matter and microplankton community since their fatty acid compositions were similar during both periods. Physical fluctuations in the water column such as turbidity may promote shifts in zooplankton trophic behaviour via changes in trophic preference (Gasparini and Castel, 1997) and/or changes in community structure (Gonçalves *et al.*, 2012). Further research on environmental conditions acting directly or indirectly in modulating the mesozooplankton community structure is still needed in this estuarine system. Even so, the seasonal fatty acid pattern of the mesozooplankton of the estuary underlines its trophic behaviour. During summer when *A. tonsa* is dominant (Hoffmeyer, 2004), biomarkers of microalgae and protozoa were relevant in consistent with an omnivorous diet and the opportunistic trophic behaviour of this copepod (Ederington *et al.*, 1995; Kiørboe *et al.*, 1996). Furthermore, a shift in the mesozooplankton community structure towards the dominance of a cirripede larvae during winter (Dutto *et al.*, 2012) may be the main cause of detrital feeding, pointing out that the fatty acid approach can indicate the feeding strategy of a particular zooplankton assemblage (Gonçalves *et al.*, 2012).

CONCLUSIONS

The seasonality of the temperate region where the Bahía Blanca Estuary is located is strong enough to induce changes in the fatty acid composition of plankton, thus reflecting trophic dynamic variations related to seasonal succession of biomass and plankton community structure. Mesozooplankton showed the clearest seasonal influence revealing grazing preferentially on living planktonic cells (e.g. diatoms and microzooplankton) in summer and a change to a more terrestrially derived diet in winter.

The non-treated domestic sewage had no clear impact on the fatty acid composition of the plankton; however, mesozooplankton fatty acids suggested a dietary preference for diatoms over flagellates growing in the sewage-impacted region where the SPM was mainly composed of terrestrially derived particles, diatoms and bacteria. Nevertheless, none of these components were exclusively found at the sewage site, hence sewage was not a significant contribution to the particulate matter of the estuarine area influenced by it. Various sources constitute the organic fraction of the particulate matter of the estuary. Bordering habitat inputs, saltmarshes in particular, are likely to have a crucial role as one of the main contributors to SPM followed by plankton.

Our results emphasize the use of the FATM approach as a valuable tool to trace predator–prey relationships inferring trophic preferences and to detect allochthonous inputs even in turbid and eutrophic coastal systems.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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