Marine Pollution Bulletin 137 (2018) 617–626

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Biogeochemical markers across a pollution gradient in a Patagonian estuary: A multidimensional approach of fatty acids and stable isotopes

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ARTICLE INFO

Keywords:
Organic matter
Sewage
Bacteria
Ammonium
18:1(n-7)
^{15}N depletion

ABSTRACT

A combined approach merging stable isotopes and fatty acids was applied to study anthropogenic pollution in the Río Negro estuary. Fatty acid markers of vegetal detritus indicated considerable allochthonous inputs at freshwater sites. Correlative evidence of diatom fatty acids, δ^{13}C, chlorophyll and particulate organic matter suggested the importance of diatoms for the autochthonous organic matter production at the river mouth. Low δ^{15}N values (~0‰) and high fatty acid 18:1(n-7) concentrations in the suspended particulate matter, in combination with the peaks of coliforms and ammonium, indicated a strong impact of untreated sewage discharge. The ^{15}N depletion was related to oxygen-limited ammonification processes and incorporation of ^{15}N depleted ammonium to microorganisms. This work demonstrates that the combined use of lipid and isotopic markers can greatly increase our understanding of biogeochemical factors and pollutants influencing estuaries, and our findings highlight the urgent need for water management actions to reduce eutrophication.

1. Introduction

Temperate estuaries are highly dynamic systems located in to rapidly changing environments. These systems are subject to ever-changing hydrology due to tides, coastal storms, winds, and seasons, in conjunction with human activities that strongly influence the cycle of nutrients and the ecology of coastal communities (Biancalana et al., 2014; Fricke et al., 2016; Bermejo et al., 2018). Estuaries are characterized by large fluxes of organic matter from diverse sources, which can fluctuate in abundance and composition. Estuarine systems are sites of production, transformation, removal, and exchange of both, dissolved as well as particulate organic matter. Although considerable biogeochemical research has focused on these systems, knowledge about the source, fate and transformation of the organic matter in estuaries is still limited (Bristow et al., 2013; Dubinenkov et al., 2015; Canuel and Hardison, 2016).

Estuaries represent hot spots for socio-economic activities; however, their water quality is being degraded globally, and in particular in developing countries, by increasing inputs of organic and inorganic nutrients derived from domestic, agricultural and industrial sources. Sewage pollution threatens human health, biodiversity and ecosystem services (Harvell et al., 2005; Schwarzenbach et al., 2010; La Colla et al., 2015). Because nitrogen is generally the principal nutrient limiting primary production in coastal systems, its increased concentration and availability causes eutrophication worldwide (Nixon, 1995; Bowen and Valiela, 2001). Furthermore, likely effects of global change are projected to be similar to effects of eutrophication (Jarvie et al., 2012; Kopprio et al., 2015a), with undesirable consequences including increases of microbial load and activity, algal blooms, hypoxia and nekton mortality.

Stable isotopes of nitrogen and carbon are valuable tools for investigating anthropogenic impacts and origins of organic matter in aquatic systems. The isotopic signature of δ^{15}N is usually heavier in polluted sites than in pristine ones (Olsen et al., 2011; Moynihan et al., 2012; Connolly et al., 2013), while the δ^{13}C values indicate mainly the origin of the organic matter (Perkins et al., 2014; Kopprio et al., 2014). Nevertheless, several environmental factors and metabolic processes influence isotopic fractionation in aquatic organisms (Carmichael et al., 2004; Reuss et al., 2013; Kopprio et al., 2015b) and consequently their signature in the organic matter. Fatty acids are complementary ecological and biogeochemical indicators, which are often used to infer the diet of consumers, and the composition and origin of organic matter. In addition, fatty acid biomarkers can be used to determine the community composition within the water and sediments of estuaries.

Diatoms are characterized by the monounsaturated fatty acid 16:1(n-7) and the polyunsaturated fatty acids (PUFA) 16:2(n-4), 16:3(n-4), 16:4(n-1) and 20:5(n-3) (reviewed by Dalsgaard et al., 2003).

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https://doi.org/10.1016/j.marpolbul.2018.10.059
Received 20 March 2018; Received in revised form 14 October 2018; Accepted 29 October 2018

Dinoflagellates and flagellates by the 18:4(n-3) and 22:6(n-3) and bacteria by the 18:1(n-7), 15:0, 17:0 and iso and anteiso branched fatty acids (Graeve et al., 2005; Bec et al., 2010; White et al., 2017). Moreover, some highly unsaturated long-chain fatty acids like the 20:4(n-6), 20:5(n-3) and 22:6(n-3) are essential for the growth, development and reproduction of higher trophic levels (Sargent et al., 1995; Parrish, 2009; Bertucci et al., 2017). As many fatty acids are useful markers of primary producers, they can also be used to trace the origin of the organic matter. Within this context, the 18:2(n-6) is a marker of terrestrial vegetal detritus and seagrasses (Alfaro et al., 2006; Signa et al., 2017), as well as for chlorophytes (Viso and Marty, 1993; Kravchuk et al., 2014). The combination of stable isotopes and fatty acids markers represents a tool of extraordinary resolution and accuracy in the study of trophic webs (Nyssen et al., 2005; Kopprio et al., 2015b; Kohlbach et al., 2016; Sushchik et al., 2017). However, this multidimensional approach has been hardly used to assess coastal pollution.

To date, a combined approach of stable isotopes and fatty acids has not been applied to study potential anthropogenic impacts on the biogeochemistry of coastal and estuarine zones of the South-western Atlantic. The aims of our study were: 1) to elucidate the origin, source and fate of organic and inorganic nutrients; 2) to survey plankton communities and study their relation to markers and organic matter; and 3) to assess sewage pollution and biogeochemical processes across the estuarine gradient of the Río Negro. A multidimensional combined approach was used to explore these three objectives. We hypothesize strong differences in the composition of markers in the suspended particular matter (SPM) and seston fractions in regards to: a) the source (marine or fresh water), b) the distribution of planktonic organisms, and c) the influence of detritus and waste water. Specifically, we expected heavier $\delta^{15}N$ signatures and bacterial fatty acid markers closer to the sewage discharges.

2. Materials and methods

2.1. Study site

The Río Negro (RN) river is the main freshwater source of the Argentinian Patagonia and its water catchment area comprises a surface of 116,000 km^2 crossing a vast semiarid steppe with annual precipitation lower than 200 mm. This river, with a mean historical discharge of ~900 $m^3 s^{-1}$, was modified by the presence of dams and irrigation channels, and now discharges in some cases only ~300 $m^3 s^{-1}$. The RN estuary (Fig. 1) is a meso-tidal system with valuable wetlands offering several ecosystem services for climate change adaptation and is vitally important for the protection of biodiversity and local fisheries. The RN system has been preliminarily classified from eutrophic to highly eutrophic (Abrameto et al., 2017). The main environmental concerns for this estuary are several organic pollutants, untreated sewage discharges, eutrophication, and the presence of *Vibrio* species with pathogenic factors (Miglioranza et al., 2013; Kopprio et al., 2015a; Kopprio et al., 2017).

Likely as consequence of the eutrophic conditions, an unidentified macroalgae has begun to spread across the estuary in recent years (Kopprio pers. obs.). Some representative species of the aquatic vegetation in the freshwater region are the plants *Potamogeton* spp. and *Myriophylum aquaticum* and the green algae *Chara contraria* (Dall Armellina et al., 1999). In this region, the terrestrial vegetation at the coastline of the estuary is characterized by introduced species of ornamental and wind protection trees (e.g., *Salix* spp., *Populus* spp.). In the marine region, the intertidal and coastal vegetation consists of grasslands composed by *Spartina* spp. and *Sarcocornia* sp.

2.2. Sampling

Ten sampling stations (Fig. 1), ranging from higher marine influence (S1) to higher freshwater influence (S10), were selected across a ~30 km transect of the estuarine gradient in the RN. Stations S7 and S8 coincided with the output of the water treatment plants of Viedma and Carmen de Patagones cities, respectively. Sampling was carried out with a motor boat against the riverine current starting in S1 at high tide. Samples were taken monthly from January (midsummer) to March (early autumn) 2014. At each sampling event, conductivity, salinity, pH, dissolved oxygen and turbidity were measured in situ with electronic probes (PCE-PHD 1 and PCE-TUM 20). At all stations, water samples for nutrient, pigment and biomarker analyses were taken at 30 cm below the surface, using 5 L plastic bottles. The particulates obtained during this procedure were denominated as suspended particulate matter (SPM). For colony forming units (CFUs) and Utermöhl counts, water was sampled at the same depth and kept in sterile 0.5 L glass bottles and in 100 mL PET bottles with Lugol's solution, respectively.

At stations S1, S3, S7 and S9, net towing was performed with nets of 60 and 200 μm pore size (Nitex) and the filtered volume calculated with a mechanical flow meter (Hydro-Bios). The filtrates from the plankton nets, were divided into two seston fractions i) 60 to 200 μm seston: containing microplankton, and ii) > 200 μm seston: containing mesoplankton; and maintained in 0.5 L clean plastic flasks. About 50 mL of each seston fraction was preserved with Lugol's solution and buffered formaldehyde (4%) for later counting. All samples were transported in insulated boxes cooled with gel refrigerant packs and processed under laboratory conditions within 6 h.

2.3. Laboratory analyses

Water samples for pigments, nutrients, stable isotopes and fatty acids in the SPM were filtered through glass fibre filters of 0.7 μm pore size (Whatman GF/F, precombusted at 500 °C for 5 h). A known volume of fractionated water from the net was filtered following the same procedure for determination of markers and particulate organic matter. Filters for stable isotope and particulate organic matter measurements were dried overnight at 50 °C and stored at room temperature in a vacuum desiccator. Filters for fatty acid analyses were kept in 4 mL vials with Teflon cups (Agilent) in a dichloromethane-methanol solution (2:1) under nitrogen atmosphere at −20 °C. For the quantification of pigments, filters were frozen (−20 °C) and kept in dark. Filtrates for dissolved inorganic nutrient and for dissolved organic carbon (DOC) determinations were preserved frozen at −20 °C in chemically clean 100 mL PE bottles and in 10 mL precombusted glass ampoules, respectively.

For determination of faecal coliforms, water and filters (nitrocellulose, Gamafil, 0.45 μm pore size) were directly spread or placed (after filtration of 1 to 50 mL) on Endo-agar (Merck) and incubated overnight at 44 °C. The abundance of plankton groups in SPM and seston from net fractionation preserved in Lugol's solution was estimated counting 40 random fields across the main diameter using the Utermöhl method in a combined plate chamber (Hydro-Bios). Zooplankton from seston > 200 μM was preserved in buffered formaldehyde and counted totally in a counting chamber for zooplankton based on the Bogorov design (Hydro-Bios).

Pigment extraction was performed with 90% acetone in water for 24 h at 4 °C and chlorophyll *a* and pheo-pigments were quantified after Lorenzen (1967). Dissolved inorganic nutrients were determined following standard methods (Grasshoff et al., 1999) and dissolved organic carbon was measured by high temperature catalytic oxidation with a Shimadzu TOC-V_{CPN} analyzer. The performance of the analyzer was checked using reference water (Hansell Lab.) and internal standards. Dried filters containing suspended particulate matter (SPM) and seston fractions for particulate organic carbon and nitrogen (POC and PON) measurements were acidified (0.1 M HCl) to remove inorganic carbon, set in tin capsules and completely oxidized at 1000 °C under pure oxygen in an elemental analyzer (EURO EA). Acetanilide (Hekatech) was used as an internal standard.

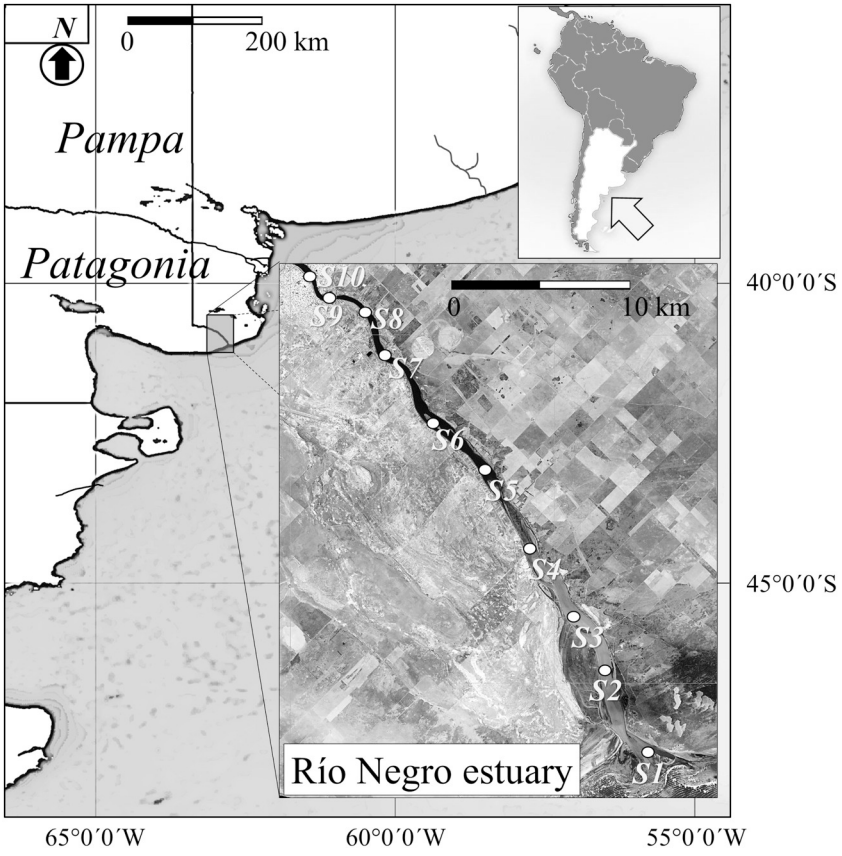

Fig. 1. Location of the Río Negro (RN) estuary and sampling stations (S) in the Argentinian Patagonia.

Stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) were determined with a Thermo Finnigan Delta Plus mass spectrometer coupled with a Flash EA 1112 elemental analyzer. Samples were analyzed in duplicate including peptone as standard every seven samples. The amount of isotope per sample was within the analytical range. The stable isotopes composition of low-mass (light) elements such as carbon and nitrogen are normally reported as delta (δ) values in parts per thousand (denoted as ‰), carbon relative to Pee Dee Belemnite and nitrogen relative to nitrogen in air according to the formula:

$R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ and

δ (‰) = $[(R_{sample}/R_{standard} - 1) \times 1000]$.

The isotope ratios were measured in accordance with reference standards of the International Atomic Energy Agency (IAEA) to normalize the $\delta^{15}N$: IAEA-N1 ($\delta^{15}N = 0.4$‰) and IAEA-N2 ($\delta^{15}N = 20.3$‰), and with those of the National Bureau of Standards (NBS) and United States Geological Survey (USGS) to normalize the $\delta^{13}C$: NBS 22 ($\delta^{13}C = -30.0$‰) and USGS-24 ($\delta^{13}C = -16.5$‰).

The internal standard 23:0 was added to the samples of SPM and seston fractions (200–60 μM and > 200 μM) in dichloromethane:methanol solution for fatty acid quantification. Subsequently, samples were ultra-sonicated for 10 min for cell disruption and content homogenization. Lipid extraction were performed essentially according to Folch et al. (1957), this protocol was modified by using the less hazardous dichloromethane with similar extraction efficiency as chloroform. Lipid extracts were transesterificated under nitrogen atmosphere with 3% concentrated sulphuric acid in methanol for 4 h at 80 °C. Fatty acid methyl esters (FAME) were extracted with hexane and analyzed by gas-liquid chromatography (Hewlett Packard 6890 GC) on a 30-m wall-coated capillary column (inner diameter 0.25 mm, film thickness 0.25 mm; liquid phase DB-FFAP) basically after Kattner and Fricke (1986). FAME were quantified with the 23:0 internal standard and identified with standard mixtures. The identity of selected FAMEs was also confirmed by mass spectrometry (GC–MS). FAME data were acquired with the software Chemstation Vers B04.01 (Agilent).

2.4. Data analysis

From the 46 fatty acids detected, those > 1% (mass % of the total fatty acids) were selected for further analysis. Differences between SPM with higher marine (from S1 to S5) and SPM with higher freshwater influence (from S6 to S10) in stable isotopes (‰), C/N ratio, total fatty acid content (μg mg C^{-1}) and main fatty acid proportions (% total) were evaluated using a non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA). For ordination analyses, the 14:0, 16:0, 18:0 and 20:0 are ubiquitous among organisms and detritus and were therefore group together as even-chain saturated fatty acids (SFA even 14–20). Terrestrial indicators, 22:0 and 24:0, were clustered as SFA even 22–24. Moreover, the bacterial markers 15:0 and 17:0 (including branched-chain iso and anteiso) were clustered as odd-chain saturated fatty acids (SFA odd), and diatom indicators 16:2(n-4), 16:3(n-4) and 16:4(n-1) as polyunsaturated fatty acids (PUFA) with 16 carbon atoms (PUFA 16C).

Relationships between and within environmental factors (water quality variables from the electronic probes, pigments and nutrients), stable isotopes (‰), fatty acid content (μg mg C^{-1}), and bacterial and plankton counts were evaluated using Spearman rank correlations. Canonical Correspondence Analysis (CCA) was performed using the environmental variables as explanatory variables of the distribution of fatty acids and stable isotopes in the SPM. CCA identifies optimal linearly coupled patterns between two multivariate data sets: one explanatory and other of response. The data were log-transformed and linearity was checked after 1000 permutations with the Monte Carlo Test. CCA has several advantages over other multivariate approaches: it performs well even with skewed species distributions, unusual sampling designs, high noise levels and highly intercorrelated environmental variables and it is robust to violations of assumptions (Palmer, 1993). Samples of SPM and seston fractions were ordinated by Principal Component Analysis (PCA) based on the Spearman rank correlation matrix of stable isotope ratios (‰) and fatty acid proportions (% total).

Table 1
Biogeochemical markers in the suspended particulate matter of marine and freshwater samples in the Río Negro estuary. Values compared with Kruskal-Wallis one-way analysis of variance. Significant differences are printed in bold, superscript letters indicate higher mean values found in marine (M) and freshwater (F), respectively.

	Marine (S1 to S5)				Freshwater (S6 to S10)					
Markers	Mean	SD	Min	Max	Mean	SD	Min	Max	K	*p*
Isotopes, ratio and content										
$\delta^{13}C$ (‰)	−22.3	1.4	−25.3	−20.5	−24.9	0.6	−25.7	−23.9	16.7	< **0.001**^{M}
$\delta^{15}N$ (‰)	9.6	1.5	6.8	12.4	7.1	2.2	0.5	8.8	12.3	< **0.001**^{M}
C/N	5.8	1.1	4.1	7.9	5.7	0.8	4.2	6.9	0.1	0.820
Fatty acids (mg g C^{-1})	57.5	20.0	15.5	92.2	50.7	30.1	26.1	147.5	2.7	0.101
Main fatty acids (% total)										
Saturated										
14:0	7.5	2.2	3.9	11.8	5.6	1.6	3.4	8.7	6.3	**0.012**^{M}
15:0[a]	3.1	1.0	1.3	4.7	4.7	1.4	1.6	7.3	10.1	**0.002**^{F}
16:0	17.3	6.3	11.3	37.4	21.5	4.0	17.7	32.1	9.0	**0.003**^{F}
17:0[a]	2.8	0.8	1.0	3.9	2.7	1.1	1.0	5.3	0.7	0.42
18:0	6.8	5.6	1.4	16.5	9.1	8.3	4.3	37.3	1.7	0.191
22:0	1.2	2.1	n.d.	8.3	1.1	0.6	n.d.	2.5	2.6	0.110
24:0	0.9	0.6	n.d.	2.0	2.0	1.0	0.7	4.2	10.3	**0.001**^{F}
Monounsaturated										
16:1(n-7)	12.6	4.5	2.7	18.0	17.1	5.4	5.5	26.6	6.3	**0.012**^{F}
18:1(n-9)	3.5	3.3	1.1	11.9	5.6	2.1	3.2	10.2	7.8	**0.005**^{F}
18:1 (n-7)	2.8	1.1	1.0	5.4	4.8	2.4	2.3	12.2	8.1	**0.005**^{F}
Polyunsaturated										
16:2(n-4)	3.4	1.9	n.d.	6.2	1.9	1.3	n.d.	4.0	4.6	**0.033**^{M}
16:3(n-4)	6.6	4.6	0.8	14.3	2.3	2.2	n.d.	8.8	9.8	**0.002**^{M}
16:4(n-1)	1.8	1.3	n.d.	4.2	n.d.	n.d.	n.d.	n.d.	20.0	< **0.001**^{M}
18:2(n-6)	2.7	2.8	0.8	9.8	3.0	1.4	1.6	6.1	4.9	**0.027**^{F}
18:3(n-3)	1.3	0.7	n.d.	3.1	3.3	2.0	0.9	8.0	10.1	**0.002**^{F}
18:4(n-3)	2.7	1.2	n.d.	4.1	1.4	0.6	0.4	2.4	9.8	**0.002**^{M}
20:4(n-6)	0.9	0.5	n.d.	1.9	1.4	1.0	n.d.	3.3	3.2	0.074
20:5(n-3)	13.3	6.3	0.5	20.3	6.3	3.3	1.5	12.0	8.1	**0.005**^{M}
22:6 (n-3)	3.8	1.6	n.d.	5.6	1.2	1.1	n.d.	3.4	13.1	< **0.001**^{M}

S: Station, SD: standard deviation, Min: minimum, Max: maximum, C: organic carbon, N: nitrogen, n.d.: not detected.
[a] Includes branched-chain iso and anteiso.

All statistical tests were considered significant at $p < 0.05$ and performed with the statistical software XLSTAT-Ecology.

3. Results

3.1. Characterization of organic matter and nutrients across the estuarine continuum

No significant differences were found between SPM C/N ratios from fresh water and marine stations (Table 1). Both mean values of (C/N = ~6) indicated a strong impact of plankton in the composition of the SPM. Marine and freshwater stations differed significantly in the composition of the observed SPM markers (isotopes and fatty acids). Considering the Kruskal-Wallis ANOVA, marine stations presented significantly higher values of $\delta^{13}C$ and $\delta^{15}N$; together with higher proportions of 14:0; the diatoms markers 16:2(n-4), 16:3(n-4), 16:4(n-1) and 20:5(n-3); and the flagellates and dinoflagellate markers 18:4(n-3) and 22:6(n-3). In contrast, the freshwater stations were characterized by significant higher values of the bacterial marker 15:0, 16:0, the terrestrial marker 24:0, the diatom marker 16:1(n-7), the bacterial marker 18:1(n-7), the 18:1(n-9), and the terrestrial vegetation or chlorophyte markers 18:2(n-6) and 18:3(n-3).

The distribution of particulate organic matter varied along the estuarine gradient, with particulate organic carbon (POC) and nitrogen (PON) being significantly correlated with salinity (considered as indicator of the estuarine continuum) at $r_s = 0.75$ ($p < 0.001$) and $r_s = 0.72$ ($p < 0.001$), respectively. Overall POC, PON, chlorophyll *a* and turbidity showed the same patterns as salinity for the following markers: $\delta^{15}N$, $\delta^{13}C$ and 22:6(n-3) (Table 2). Moreover, salinity was positively correlated with 20:5(n-3) and 18:4(n-3), while chlorophyll *a* was only correlated with the diatom markers 20:5(n-3) and PUFA 16C. The last group of fatty acids was positively correlated with POC and turbidity. The 18:3(n-3) was negatively correlated with POC and PON, and the 18:1(n-9) followed the same trend only with PON.

Considering the dissolved nutrients, DOC was weakly but significantly correlated with the bacterial marker SFA odd (Table 2) and phosphate ($r_s = 0.38$, $p = 0.037$). The distribution of phosphate and ammonium was more related to the sewage pollution and will be explained in the Section 3.3. Nitrate was positively correlated with salinity ($r_s = 0.45$, $p = 0.012$), PON ($r_s = 0.43$, $p = 0.019$) and 22:6(n-3) (Table 2). Silicate exhibited an inverse trend than salinity with the same markers. Temperature, usually higher at the freshwater stations, was also negatively correlated with both stable isotopes and 20:5(n-3), PUFA 16C and 18:4(n-3). Dissolved oxygen and pheopigments were positively related to $\delta^{13}C$ and $\delta^{15}N$, respectively.

In the seston fractions (net fractionated), the C/N ratios indicated a higher allochthonous or terrestrial input than in the SPM, particularly in the freshwater samples of seston > 200 μm and 200–20 μm (Table 3). Comparing marine and freshwater stations, the mean values of markers in both seston fractions followed generally a similar trend than SPM in the proportions of stable isotopes, monounsaturated and polyunsaturated fatty acids. The means of $\delta^{13}C$, $\delta^{15}N$, 22:6(n-3), 20:5(n-3), 18:4(n-3), 16:4(n-1), 16:3(n-4) and 16:2(n-4) were higher in the marine stations than in those of freshwater. The trend with the monounsaturated fatty acids, 18:2(n-6) and 18:3(n-3) followed the opposite pattern. The trends were generally not as clear with the saturated fatty acids, and only the terrestrial markers 22:0 and 24:0 were higher in the freshwater stations.

3.2. Plankton influence on the dynamic of markers

Diatoms and microzooplankton strongly influenced the composition

Table 2
Environmental parameters and main Spearman rank correlations with stable isotope ratios (‰) and fatty acid content (μg mg C^{-1}) in SPM.

Environmental parameters	Mean	SD	Min	Max	Markers	r_s	p
Physico-chemical							
Temperature (°C)	22.3	2.9	17.8	26.1	$\delta^{13}C$	−0.57	0.001
					$\delta^{15}N$	−0.45	0.014
					20:5(n-3)	−0.51	0.005
					18:4(n-3)	−0.43	0.018
					PUFA 16C	−0.57	0.001
Salinity (PSU)	4.9	9.3	0.1	28.3	$\delta^{13}C$	0.90	< 0.001
					$\delta^{15}N$	0.77	< 0.001
					22:6(n-3)	0.74	0.001
					20:5(n-3)	0.65	0.006
					18:4(n-3)	0.65	0.004
pH	8.3	0.2	8.1	8.6	n.s.		
Dissolved oxygen (mg L^{-1})	8.4	1.6	5.9	11.1	$\delta^{13}C$	0.44	0.016
					20:4(n-6)	−0.47	0.010
Turbidity (NTU)	18.7	14.6	5.4	82.0	$\delta^{13}C$	0.57	0.001
					$\delta^{15}N$	0.43	0.020
					22:6(n-3)	0.43	0.017
					PUFA 16C	0.43	0.020
Pigments							
Chlorophyll *a* (μg L^{-1})	7.7	7.1	1.8	32.0	$\delta^{13}C$	0.75	< 0.001
					$\delta^{15}N$	0.56	0.002
					22:6(n-3)	0.71	< 0.001
					20:5(n-3)	0.62	< 0.001
					PUFA 16C	0.71	< 0.001
Pheo-pigment (μg L^{-1})	1.0	1.3	n.d.	4.5	$\delta^{15}N$	0.47	0.010
Inorganic nutrients							
Nitrate (μM)	6.8	4.4	2.9	21.5	22:6(n-3)	0.40	0.028
Ammonium (μM)	4.3	10.8	0.2	53.7	n.s.		
Phosphate (μM)	0.8	0.9	0.1	4.8	n.s.		
Silicate (μM)	114.2	44.4	20.2	195.1	$\delta^{13}C$	−0.57	0.001
					$\delta^{15}N$	−0.66	< 0.001
					22:6(n-3)	−0.52	0.003
					20:5(n-3)	−0.40	0.028
					18:4(n-3)	−0.40	0.034
Organic nutrients							
DOC (μM)	97.1	19.9	50.6	153.0	SFA odd	0.40	0.029
POC (μM)	78.4	56.3	16.7	259.8	$\delta^{13}C$	0.72	< 0.001
					$\delta^{15}N$	0.51	0.005
					22:6(n-3)	0.60	< 0.001
					18:3(n-3)	−0.53	0.003
					PUFA 16C	0.48	0.008
PON (μM)	14.2	10.9	2.8	54.4	$\delta^{13}C$	0.66	< 0.001
					$\delta^{15}N$	0.50	0.006
					22:6(n-3)	0.59	< 0.001
					18:3(n-3)	−0.52	0.003
					18:1(n-9)	−0.44	0.015

SPM: suspended particulate matter, SD: standard deviation, Min and Max: minimum and maximum values, PUFA 16C: polyunsaturated fatty acids of 16 carbon atoms, n.s.: not significant, NTU: nephelometric turbidity units, n.d.: not detected, DOC: dissolved organic carbon, SFA odd: saturated fatty acids of odd number of carbon atoms and branched, POC: particulate organic carbon, PON: particulate organic nitrogen.

Table 3
Biogeochemical markers in seston fractions of marine and freshwater samples in the Río Negro estuary.

	Seston > 200 μm				Seston 200–20 μm			
	Marine		Freshwater		Marine		Freshwater	
Markers	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Isotopes, ratio and content								
$\delta^{13}C$ (‰)	−21.9	1.8	−25.5	0.5	−22.1	1.9	−25.1	0.3
$\delta^{15}N$ (‰)	11.1	1.9	7.1	0.8	11.1	1.0	8.4	0.4
C/N	6.9	1.5	10.9	2.6	6.6	1.1	8.2	1.1
Fatty acids (mg g C^{-1})	81.3	44.1	61.8	37.4	56.1	47.5	100.5	64.9
Main fatty acids (% total)								
Saturated								
14:0	5.3	1.2	4.7	0.5	7.7	1.8	4.8	0.7
15:0[a]	2.2	0.8	2.0	0.7	3.2	0.6	2.0	0.8
16:0	28.1	6.1	27.5	8.8	22.4	3.8	31.5	8.6
17:0[a]	2.6	0.5	2.1	0.8	3.0	0.8	2.6	0.9
18:0	10.9	4.9	10.4	5.2	3.8	2.1	12.3	7.2
22:0	1.3	0.5	1.8	0.7	1.0	1.0	1.8	0.6
24:0	0.6	0.4	2.4	1.5	1.8	1.6	2.2	1.2
Monounsaturated								
16:1(n-7)	7.8	3.4	11.1	4.7	14.9	2.0	10.5	6.4
18:1(n-9)	3.6	1.2	6.7	3.0	2.2	1.1	4.8	3.0
18:1 (n-7)	2.0	1.0	2.5	0.9	2.5	1.0	2.2	0.9
Polyunsaturated								
16:2(n-4)	1.4	0.9	1.5	0.8	2.9	1.1	1.7	1.3
16:3(n-4)	1.9	0.7	1.3	0.8	5.0	3.6	0.8	1.2
16:4(n-1)	0.4	0.5	0.3	0.4	1.3	1.2	0.3	0.5
18:2(n-6)	4.2	2.3	5.3	1.6	1.3	0.4	4.5	3.0
18:3(n-3)	1.8	1.0	2.3	1.0	0.7	0.4	1.1	0.6
18:4(n-3)	1.0	0.4	0.9	0.4	1.3	0.5	0.7	0.5
20:4(n-6)	1.1	1.0	1.8	1.0	1.3	0.3	1.2	1.0
20:5(n-3)	7.8	3.4	6.9	3.6	12.8	4.4	5.8	4.1
22:6 (n-3)	6.9	3.3	1.5	1.3	4.6	1.9	1.0	0.7

SD: standard deviation, Min: minimum, Max: maximum, C: organic carbon, N: nitrogen, n.d.: not detected.
[a] Includes branched-chain iso and anteiso.

of particulate organic matter and their markers in the SPM, particularly at the marine stations. Peaks of diatom abundance, chlorophyll *a* concentration, $\delta^{13}C$ and 20:5(n-3) content in the SPM were detected at the stations of higher and intermediate salinity, predominantly during March (Fig. 2). The ratio of carbon stable isotope increased relatively constantly from S10 (freshwater) to S1 (marine water). Diatom abundance was significantly correlated with chlorophyll *a* ($r_s = 0.74$, $p < 0.001$), $\delta^{13}C$ ($r_s = 0.69$, $p < 0.001$), 20:5(n-3) ($r_s = 0.69$, $p < 0.001$) and PUFA 16C ($r_s = 0.67$, $p < 0.001$). However, not significantly with the other diatom marker: the 16:1(n-7). Cyanobacteria and bacteria were also an important component of the SPM at S7 and S8, principally connected with sewage pollution, and their dynamic will be presented in the Section 3.3.

The abundances of microzooplankton were also elevated at the marine stations, particularly during January and February (Fig. 2). Microzooplankton in SPM were only weakly correlated with the 22:6(n-3) ($r_s = 0.39$, $p < 0.036$). Following a similar pattern and principally at marine stations, zooplankton abundance in seston > 200 μM was significantly correlated with the 22:6(n-3) ($r_s = 0.78$, $p = 0.004$), $\delta^{15}N$ ($r_s = 0.78$, $p = 0.004$) and $\delta^{13}C$ ($r_s = 0.73$, $p = 0.009$). The microzooplankton in seston 200–20 μM were significantly correlated with $\delta^{15}N$ ($r_s = 0.60$, $p = 0.041$) and $\delta^{13}C$ ($r_s = 0.67$, $p = 0.019$). Zooplankton were composed mainly by the copepod *Paracalanus parvus* in the seston > 200 μM and *nauplii* and ciliates in the seston 200–60 μM and SPM. Diatoms were an abundant component of seston 200–60 μM, however, there were no significant correlation between their abundances and markers. A considerable amount of vegetal detritus was observed in both seston fractions from the freshwater stations.

3.3. Sewage pollution

The maximum discharge of untreated sewage in February at S8 (Carmen de Patagones city) was evidenced by the maximum concentrations of ammonium and coliforms (Fig. 3). Furthermore, it coincided with a marked depletion of ^{15}N (~0‰), the highest values of cyanobacteria abundance, and the content of the bacterial marker 18:1(n-7). These trends were similar in January at S8 but with a lower magnitude. Downstream at S7, a second peak of ammonium occurred in February, coinciding with the sewage discharge of Viedma city, and an elevated abundance of coliforms but without the increases in 18:1(n-7) and cyanobacteria abundance, and without a marked depletion of ^{15}N in PON (~6‰). Phosphate was significantly correlated with ammonium ($r_s = 0.60$, $p < 0.001$) and markedly influenced by

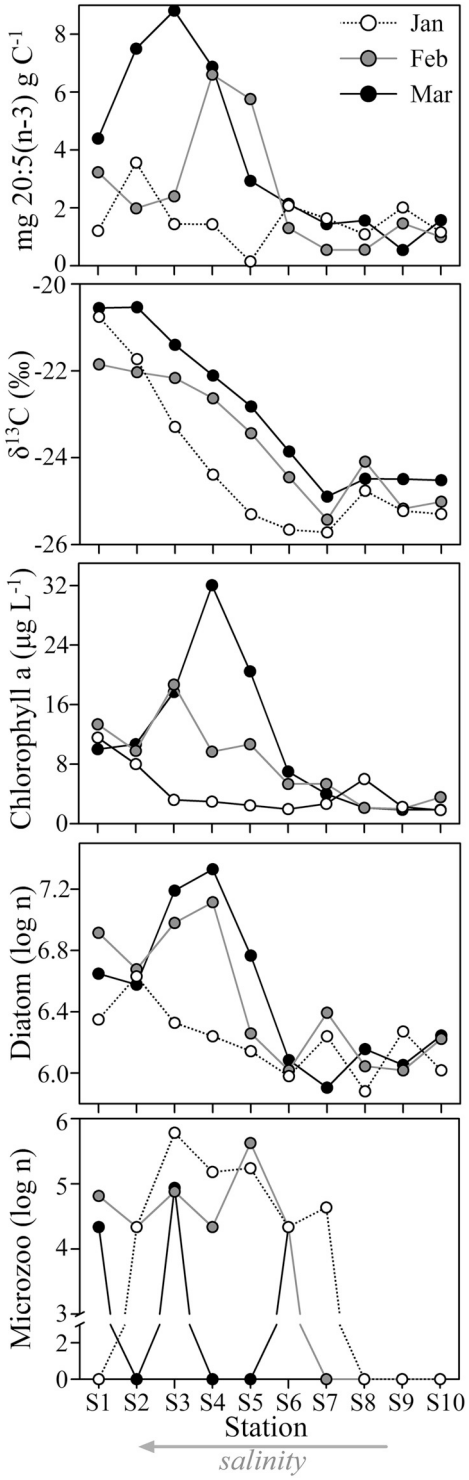

Fig. 2. Monthly dynamics of the content of 20:5(n-3) fatty acid, proportion of stable isotope of carbon, concentration of chlorophyll *a*, and abundance of diatoms and microzooplankton in the suspended particulate matter across the saline gradient of the Río Negro estuary.

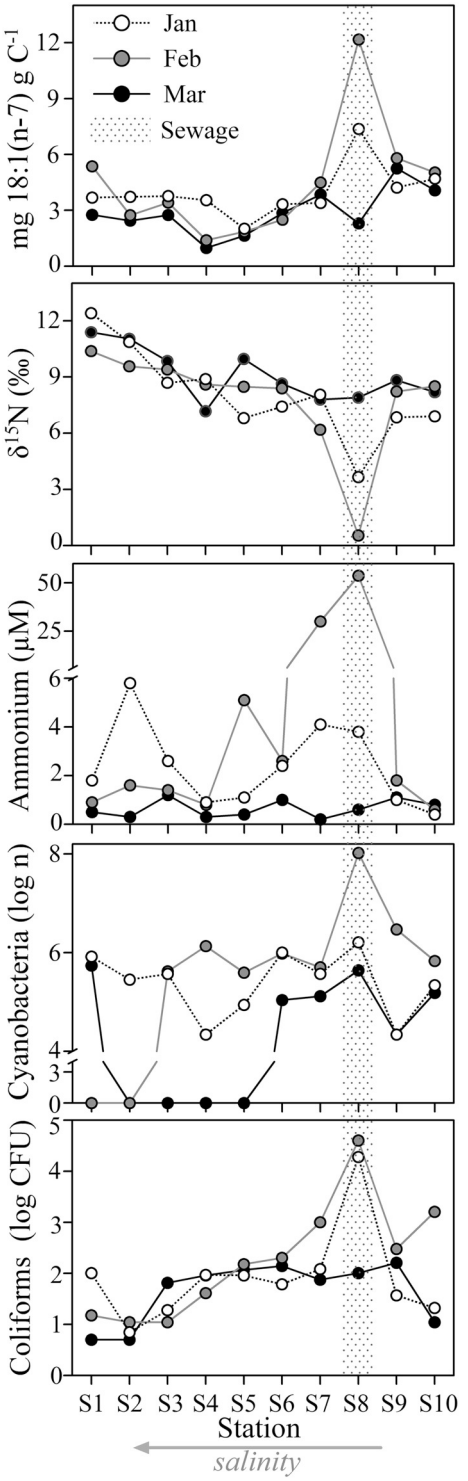

Fig. 3. Monthly dynamics of the content of 18:1(n-7) fatty acid, proportion of stable isotope of nitrogen, concentration of ammonium, and abundance of cyanobacteria and coliforms in the suspended particulate matter across the saline gradient of the Río Negro estuary.

anthropogenic pollution. Coliforms were negatively correlated with $\delta^{15}N$ ($r_s = 0.48$, $p = 0.008$), $\delta^{13}C$ ($r_s = 0.47$, $p = 0.009$) and 20:5(n-3) ($r_s = 0.56$, $p = 0.002$), though not significantly correlated with 18:1(n-7).

3.4. Multidimensional approaches: exploring all objectives

SPM samples from the stations across the estuarine gradient were ordinated by a CCA based on their biochemical marker and environmental variables (Fig. 4). The constrained inertia was 57% of the total inertia and the relations between the variables were linear after the permutation test (Pseudo F = 2.13, $p < 0.001$). Almost all freshwater stations were located at the positive side of the CC1 (which explains the 65.3% of the variation) together with most of the SFA, MUFA, 18:2(n-6), 18:3(n-3) and 20:4(n-6) biomarkers, and were associated mainly to

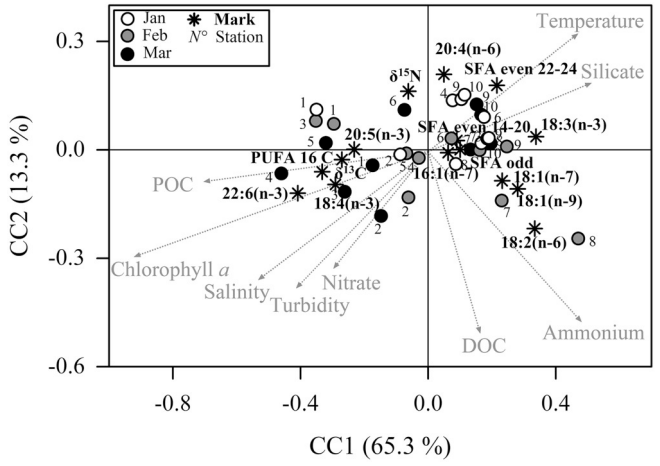

Fig. 4. Canonical correspondence analysis (CCA) using environmental factors as explanatory variables of the distribution of fatty acid and stable isotopes markers in the suspended particulate matter. SFA odd: saturated fatty acids of odd carbon number, SFA even 14-20: saturated fatty acids of even number between 14 and 20 carbon atoms, SFA even 22-24: saturated fatty acids of even number between 22 and 24 carbon atoms, PUFA 16 C: polyunsaturated fatty acids of 16 atoms of carbon and two or more double bonds, DOC: dissolved organic carbon, POC: particulate organic carbon.

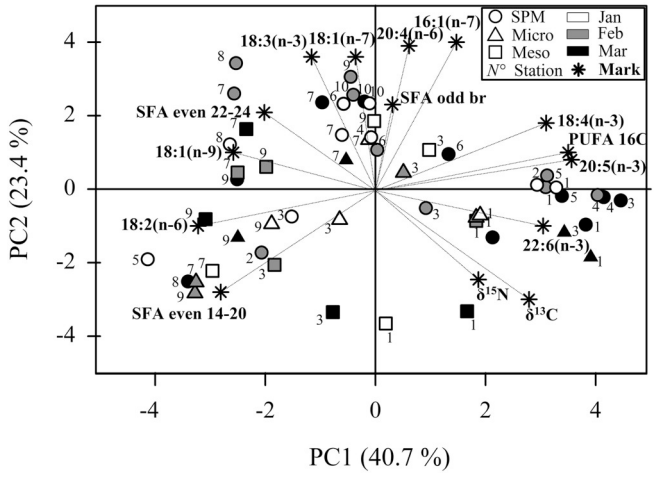

Fig. 5. Principal component analysis (PCA) of the proportions of stable isotopes (‰) and main fatty acids (%) in the suspended particulate matter (SPM), seston 200-60 μM (Micro) and seston > 200 μM (Meso). SFA even: saturated fatty acids of even carbon number, SFA odd: saturated fatty acids of odd carbon number, PUFA 16 C: polyunsaturated fatty acids of 16 atoms of carbon and two or more double bonds.

ammonium, silicate, temperature and DOC. Ammonium was closely related to the samples more influenced by the sewage discharge (e.g., S8 in February) and the 18:1(n-7), 18:1(n-9) and 18:2(n-6) biomakers. Other freshwater stations with relatively similar characteristics (e.g., S7 and S9) were ordinated closer to the last sample.

Most of the samples with a higher marine influence, together with stable isotopes and some fatty acid markers of diatoms (20:5(n-3) and PUFA 16C), flagellates and dinoflagellates (18:4(n-3) and 22:6(n-3)), and microzooplankton (22:6(n-3)), were ordinated at the negative side of the CC1 in combination with POC, chlorophyll *a*, salinity, turbidity and nitrate. The stations influenced by the phytoplankton bloom in March and some of S1 and S3 were located at the negative extreme of CC1. The CC2, which explained the 13.3% of the variation, did not evidence a clear ordination pattern of samples. Only the samples of higher temperature from January were located at its positive extreme.

The PCA showed the strong effect of the spatial and temporal variations in the proportions of markers in the seston fractions and SPM (Fig. 5). The first two components explained the 64.1% of the variation: 40.7% the first (PC1) and 23.4% the second (PC2). Most of the relations among components and variables are summarized in the PCA. For instance, the $\delta^{15}N$ was highly positively correlated with the $\delta^{13}C$ ($r_s = 0.80$, $p < 0.001$), the 20:5(n-3) positively strongly with PUFA 16C ($r_s = 0.88$, $p < 0.001$) and negatively with the 18:2(n-6) ($r_s = 0.78$, $p < 0.001$). PC1 can be associated mainly with the spatial scale across the estuary. At the negative or "freshwater" side grouped the samples with markers of terrestrial detritus, poor in PUFA and lower isotopic ratios. At the positive or "marine" side of PC1 were ordinated all the seston samples of S1 and some of S3, richer in PUFA and with higher stable isotope ratios. To some extent and with several exceptions, PC2 of the ordination could be related positively to the sewage influence and negatively to a higher trophic level. The SPM sample related to the highest impact of the sewage discharge (S8 in February) and the maximum percentage (12.2%) of 18:1(n-7) was ordinated at the positive extreme of PC2. Other SPM and seston samples influenced by the sewage discharge, freshwater and the cities (S7 and S9) were also ordinated at this side. Seston > 200 from S1 and S3 with a higher zooplankton influence were grouped at the negative extreme of PC2.

4. Discussion

4.1. Characterization of the organic matter: vegetal detritus and plankton influence

The saturated fatty acids, particularly even chains of 22–24 carbon atoms, the 18:1(n-9), 18:2(n-6) and 18:3(n-3) indicated mainly the input of vegetal detritus in the SPM and seston fractions of freshwater stations. The mentioned fatty acids are typical of terrestrial vegetation and detritus (Napolitano et al., 1997; Dalsgaard et al., 2003; Döring and Ekau, 2017). This is also supported by the C/N ratio of the seston fractions (> 200 μm and 200–20 μm) at the freshwater stations, but not in the SPM. Bacteria or heterotrophic organisms with markers of vegetal detritus were likely an important fraction of the organic matter in the SPM at the freshwater stations. Although in some cases saturated fatty acids, 18:1(n-9) and 18:2(n-6), have been attributed to the main component of domestic sewage (Jardé et al., 2005; Boëchat et al., 2014; Dutto et al., 2014) and aquaculture waste (White et al., 2017; Mayor et al., 2017), the patterns of these fatty acids were not helpful for sewage detection in the RN estuary. The 18:2(n-6) and 18:3(n-3) are also markers of chlorophytes; nevertheless, this group influenced weakly the phytoplankton in the RN estuary.

At the marine stations, diatoms sustained the autochthonous production of organic matter deduced by their relations to $\delta^{13}C$ values, 20:5(n-3), PUFA 16C, chlorophyll *a*, salinity and particulate organic nutrients. Moreover, the mean C/N ratio of SPM in comparison with the Redfield ratio indicated mainly autochthonous production by phytoplankton. Turbidity was a good estimator of the particulate organic matter and plankton at the marine stations. The silicate of the river was consumed and elevated values of nitrate at the river mouth may have contributed to the diatom abundance. Diatoms contribute to increased $\delta^{13}C$ values at the marine stations because they are characterized by higher $\delta^{13}C$ values (Fry et al., 1991).

The elevated proportions of 22:6(n-3) and 18:4(n-3) at the marine stations indicated also the influence of dinoflagellates and flagellates at the marine stations. Diatoms, flagellates and dinoflagellates are key sources of long-chain PUFA (Dalsgaard et al., 2003; Galloway and Winder, 2015). Some studies showed also an enrichment of PUFA in the

organic matter of marine origin (e.g., Ackman, 1999). Estuarine systems have increasing $\delta^{13}C$ values from freshwater to marine stations (Thornton and McManus, 1994; Gireeshkumar et al., 2015; Ke et al., 2017). Moreover, the higher $\delta^{13}C$ values typical of detritus from C_4 plant (e.g., Bristow et al., 2013) from the extensive wetland of *Spartina* spp. at the RN mouth might have been influenced the ^{13}C signature of the organic matter.

The presence of zooplankton at the marine stations may have contributed to the higher content of PUFA and elevated proportions of both stable isotopes. The 22:6(n-3) proportion was closely related to the $\delta^{15}N$, copepods are richer in 22:6(n-3) and PUFA in general increase with the trophic level (Graeve et al., 2005; Kopprio et al., 2015b). The essential fatty acid 20:4(n-6) followed a different trend than the 20:5(n-3) and 22:6(n-3) biomarkers across the estuarine gradient, with higher proportions detected at the freshwater stations. This phenomenon could be due to the abundance of macroalgae richer in 20:4(n-6) (e.g., Jaschinski et al., 2008).

The 18:1(n-7) was the most relevant bacterioplankton marker in the suspended organic matter and was directly associated with sewage pollution. Other bacterial markers, such as 15:0 or 17:0 including branched-chain iso and anteiso (grouped as SFA odd), were closely related to 18:1(n-7) in the ordination analysis. Bacteria may interact with the labile fraction of DOC as was suggested by the weak correlations of DOC with SFA odd. These weak relations may indicate that a considerable portion of the DOC remained recalcitrant. The relative close arrangement of DOC with silicate, temperature, ammonium and the freshwater stations in the CCA, indicated the river as a likely source of dissolved organic matter for the coastal region. The 16:1(n-7) was only weakly related to the 20:5(n-3) dynamic and, surprisingly, not directly correlated with the abundance of diatoms. Some bacteria are also rich in 16:1(n-7) (e.g., Teece et al., 1999) and may have contributed to the SPM.

4.2. Sewage pollution

The maximum content and proportions of 18:1(n-7) and the depletion in $\delta^{15}N$ were clear indicators of sewage pollution in the Rio Negro estuary. This evidence is supported and strengthened by the co-occurrence of the highest concentrations of ammonium and faecal coliforms at the location of a likely failure in the water treatment plant and raw discharge release at S8 (Carmen de Patagones city). These relations were strongest in February but also seen, although less markedly, in January. Although organic matter and organisms impacted by waste water are usually associated with higher $\delta^{15}N$ values (McKinney et al., 2002; Hadwen and Arthington, 2007; Moynihan et al., 2012), recent studies showed a marked depletion in ^{15}N of the particulate organic matter associated with ammonium peaks in strong polluted aquatic systems (Sato et al., 2006; Ke et al., 2017; Kopprio et al., unpublished data).

Elevated ammonium concentrations indicate generally oxygen limitation in aquatic systems and under this condition, there is a marked depletion in the $\delta^{15}N$ values because the accumulation of ^{15}N-depleted bacterial biomass in the particulate organic matter (Lehmann et al., 2002; Möbius, 2013; Bardhan et al., 2017). Negative values of $\delta^{15}N$ in the sludge before any wastewater treatment have also been reported (Toyoda et al., 2011). Ammonia released from the ammonification of untreated waste has usually very ^{15}N-depleted with $\delta^{15}N$ values from −23 to −56‰ (David Felix et al., 2013; Chang et al., 2016). Depleted-ammonia at the pH of the estuary (~8) is converted to ammonium at S8 and was likely incorporated to the biomass of bacteria.

Moreover, 18:1(n-7) is generally found at higher concentrations in bacteria under anaerobic conditions (Canuel et al., 1995; Yano et al., 1998; Ding and Sun, 2005) and the maximum value at the sewage input location supports the hypothesis of the occurrence of hypoxic conditions at station S8 in the Rio Negro estuary. However, the lowest oxygen concentration measured in the water column of this estuary was ~6 mg L^{-1}. For this reason, hypoxic conditions in the water treatment plant (previous to the waste water release into the river) or in the estuarine sediments are inferred at S8. In the case of the sewage discharge of Viedma city at S7, at least some aerobic treatment in the waste water plant is indicated by the increased $\delta^{15}N$ signature of PON and dissolved inorganic nitrogen. Under these conditions, despite the relative elevated values of ammonium and coliforms, the $\delta^{15}N$ values were several times higher (~6‰) and the peak of the 18:1(n-7), the hypoxic bacterial indicator, was not evident.

In addition, cyanobacteria may incorporate the ^{15}N-depleted ammonium into their biomass, and contribute to very low $\delta^{15}N$ values in the PON. Nitrogen-fixation by cyanobacteria produces $\delta^{15}N$ values close to zero (e.g., Fernández et al., 2014). However, this process is generally observed in areas of nitrogen limitation and likely does not occur at the concentrations of ammonium detected. The highest abundance of cyanobacteria at station S8 could be related to their tolerance to the toxicity of ammonium, which reached 13,000 μM (Collos and Harrison, 2014). Furthermore, the fatty acid 18:1(n-7) has been shown to be a marker of picocyanobacteria (Bec et al., 2010) and could indicate not only the contribution of bacteria but also of cyanobacteria.

5. Conclusions

The use of multiple markers in a multidimensional approach increases our ability to detect and tease apart the effects of pollution and biogeochemical processes in highly dynamic aquatic systems, such as in temperate estuaries of intermediate latitudes. Clear differences in the proportions of markers and content of fatty acids among sources and origins of organic matter were detected in the RN estuary. The fresh water stations were characterized by high detritus from terrestrial vegetation and bacterial markers, while the marine stations were richer in PUFA and with a higher isotopic signature. Diatoms were the main producers of autochthonous organic matter and may represent, together with flagellates and dinoflagellates, a PUFA source for secondary consumers at the marine stations of the RN estuary. The exportation of essential fatty acids from the RN estuary to the oceans may be significant contributors to the development of higher trophic levels and fisheries in coastal zones of the South-western Atlantic.

The depletion of ^{15}N and the peaks of 18:1(n-7) in SPM were specific markers of raw sewage pollution in this study, while other suggested markers of anthropogenic impacts, such as the 18:1(n-9), 18:2(n-6) and SFA, were not. The combination of several biogeochemical markers indicated strong pollution by the sewage discharge in the RN estuary, with characteristics very similar to other severely polluted eutrophic systems. Urgent waste-water management measures should be undertaken to improve water quality and reduce the eutrophic conditions in the RN estuary. These actions will help to counterbalance the water management and ecosystem health challenges expected under a changing climate.

Acknowledgements

The authors thank F. Biancalana and A. Fricke for field and laboratory assistance, M. E. Streitenberger for microbiological support, A. Martínez and Kai-Uwe Ludwichowski for inorganic nutrient analyses, C. Burau and B. P. Koch for dissolved organic carbon determinations and D. Dasbach for stable isotopes measurements. This work was financed by FONCYT (PICT-2013-1241 and PICT-2015-0426). We are grateful with anonymous reviewers for their helpful commentaries on earlier versions of the manuscript and with F. Belshe for proofreading.

References

Abrameto, M.A., Torres, M., Ruffini, G., 2017. Nutrient distributions in an estuary of the argentine coast. WIT Trans. Ecol. Environ. 216, 277–283. https://doi.org/10.2495/WS170271.

Ackman, R.G., 1999. Comparison of lipids in marine and freshwater organisms. In: Arts, M.T., Wainman, B.C. (Eds.), Lipids in Freshwater Ecosystems. Springer, New York, NY. https://doi.org/10.1007/978-1-4612-0547-0_12.

Alfaro, A.C., Thomas, F., Sergent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar. Coast. Shelf Sci. 70, 271–286. https://doi.org/10.1016/j.ecss.2006.06.017.

Bardhan, P., Naqvi, S.W.A., Karapurkar, S.G., Shenoy, D.M., Kurian, S., Naik, H., 2017. Isotopic composition of nitrate and particulate organic matter in a pristine dam reservoir of western India: implications for biogeochemical processes. Biogeosciences 14, 767–779. https://doi.org/10.5194/bg-14-767-2017.

Bec, A., Perga, M.E., Desvilettes, C., Bourdier, G., 2010. How well can the fatty acid content of lake seston be predicted from its taxonomic composition? Freshw. Biol. 55, 1958–1972. https://doi.org/10.1111/j.1365-2427.2010.02429.x.

Bermejo, P., Helbling, E.W., Durán-Romero, C., Cabrerizo, M.J., Villafañe, V.E., 2018. Abiotic control of phytoplankton blooms in temperate coastal marine ecosystems: a case study in the South Atlantic Ocean. Sci. Total Environ. 612, 894–902. https://doi.org/10.1016/j.scitotenv.2017.08.176.

Bertucci, J.I., Tovar, M.O., Unniappan, S., Navarro, J.C., Canosa, L.F., 2017. Effects of dietary sunflower oil on growth parameters, fatty acid profiles and expression of genes regulating growth and metabolism in the pejerrey (*Odontesthes bonariensis*) fry. Aquac. Nutr. 24, 748–757. https://doi.org/10.1111/anu.12603.

Biancalana, F., Dutto, M.S., Berasategui, A., Kopprio, G., Hoffmeyer, M., 2014. Mesozooplankton assemblages and their relationship with environmental variables: a study case in a disturbed bay (Beagle Channel, Argentina). Environ. Monit. Assess. 186, 1–19. https://doi.org/10.1007/s10661-014-4032-y.

Boëchat, I.G., Krüger, A., Chaves, R.C., Graeber, D., Gücker, B., 2014. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. Sci. Total Environ. 482-483, 62–70. https://doi.org/10.1016/j.scitotenv.2014.02.111.

Bowen, J.L., Valiela, I., 2001. The ecological effects of urbanization of coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. Can. J. Fish. Aquat. Sci. 58, 1489–1500. https://doi.org/10.1139/f01-094.

Bristow, L.A., Jickells, T.D., Weston, K., Marca-Bell, A., Parker, R., Andrews, J.E., 2013. Tracing estuarine organic matter sources into the southern North Sea using C and N isotopic signatures. Biogeochemistry 113, 9–22. https://doi.org/10.1007/s10533-012-9758-4.

Canuel, E.A., Cloern, J.E., Ringelberg, D.B., Guckert, J.B., Rau, G.H., 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnol. Oceanogr. 40, 67–81. https://doi.org/10.4319/lo.1995.40.1.0067.

Canuel, E.A., Hardison, A.K., 2016. Sources, ages, and alteration of organic matter in estuaries. Annu. Rev. Mar. Sci. 8, 409–434. https://doi.org/10.1146/annurev-marine-122414-034058.

Carmichael, R.H., Rutecki, D., Annett, B., Gaines, E., Valiela, I., 2004. Position of horseshoe crabs in estuarine food webs: N and C stable isotopic study of foraging ranges and diet composition. J. Exp. Mar. Biol. Ecol. 299, 231–253. https://doi.org/10.1016/j.jembe.2003.09.006.

Chang, Y., Liu, X., Deng, C., Dore, A.J., Zhuang, G., 2016. Source apportionment of atmospheric ammonia before, during, and after the 2014 APEC summit in Beijing using stable nitrogen isotope signatures. Atmos. Chem. Phys. 16, 11635–11647. https://doi.org/10.5194/acp-16-11635-2016.

Collos, Y., Harrison, P.J., 2014. Acclimation and toxicity of high ammonium concentrations to unicellular algae. Mar. Pollut. Bull. 80, 8–23. https://doi.org/10.1016/j.marpolbul.2014.01.006.

Connolly, R.M., Gorman, D., Hindell, J.S., Kildea, T.N., Schlacher, T.A., 2013. High congruence of isotope sewage signals in multiple marine taxa. Mar. Pollut. Bull. 71, 152–158. https://doi.org/10.1016/j.marpolbul.2013.03.021.

Dall Armellina, A.A., Bezic, C.R., Gajardo, O.A., 1999. Submerged macrophyte control with herbivorous fish in irrigation channels of semiarid Argentina. Hydrobiologia 415, 265–269. https://doi.org/10.1023/A:1003881302520.

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46, 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7.

David Felix, J., Elliott, E.M., Gish, T.J., McConnell, L.L., Shaw, S.L., 2013. Characterizing the isotopic composition of atmospheric ammonia emission sources using passive samplers and a combined oxidation-bacterial denitrifier approach. Rapid Commun. Mass Spectrom. 27, 2239–2246. https://doi.org/10.1002/rcm.6679.

Ding, H., Sun, M.-Y., 2005. Biochemical degradation of algal fatty acids in oxic and anoxic sediment–seawater interface systems: effects of structural association and relative roles of aerobic and anaerobic bacteria. Mar. Chem. 93, 1–19. https://doi.org/10.1016/j.marchem.2004.04.004.

Döring, J., Ekau, W., 2017. Using oocyte essential fatty acid composition to assess spawner reproductive potential under hypersaline conditions. Mar. Ecol. Prog. Ser. 584, 199–212. https://doi.org/10.3354/meps12366.

Dubinenkov, I., Flerus, R., Schmitt-Kopplin, P., Kattner, G., Koch, B.P., 2015. Origin-specific molecular signatures of dissolved organic matter in the Lena Delta. Biogeochemistry 123, 1–14. https://doi.org/10.1007/s10533-014-0049-0.

Dutto, M.S., Kopprio, G.A., Hoffmeyer, M.S., Alonso, T.S., Graeve, M., Kattner, G., 2014. Planktonic trophic interactions in a human-impacted estuary of Argentina: a fatty acid marker approach. J. Plankton Res. 36, 776–787. https://doi.org/10.1093/plankt/fbu012.

Fernández, A., Marañón, E., Bode, A., 2014. Large-scale meridional and zonal variability in the nitrogen isotopic composition of plankton in the Atlantic Ocean. J. Plankton Res. 36, 1060–1073. https://doi.org/10.1093/plankt/fbu041.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

Fricke, A., Kopprio, G.A., Alemany, D., Gastaldi, M., Narvarte, M., Parodi, E.R., Lara, R.J., Hidalgo, F., Martínez, A., Sar, E.A., Iribarne, O., Martinetto, P., 2016. Changes in coastal benthic algae succession trajectories and assemblages under contrasting nutrient and grazer loads. Estuar. Coasts 39, 462–477. https://doi.org/10.1007/s12237-015-9999-2.

Fry, B., Jannasch, H.W., Molyneaux, S.J., Wirsen, C.O., Muramoto, J.A., King, S., 1991. Stable isotope studies of the carbon, nitrogen and sulfur cycles in the Black Sea and the Cariaco Trench. Deep-Sea Res. I 38, S1003–S1019. https://doi.org/10.1016/S0198-0149(10)80021-4.

Galloway, A.W.E., Winder, M., 2015. Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. PLoS One 10, e0130053. https://doi.org/10.1371/journal.pone.0130053.

Gireeshkumar, T.R., Deepulal, P.M., Chandramohanakumar, N., 2015. Distribution and sources of aliphatic hydrocarbons and fatty acids in surface sediments of a tropical estuary south west coast of India (Cochin estuary). Environ. Monit. Assess. 187, 56. https://doi.org/10.1007/s10661-015-4308-x.

Graeve, M., Albers, C., Kattner, G., 2005. Assimilation and biosynthesis of lipids in Arctic Calanus species based on feeding experiments with a ^{13}C labelled diatom. J. Exp. Mar. Biol. Ecol. 317, 109–125. https://doi.org/10.1016/j.jembe.2004.11.016.

Grasshof, K., Ehrhardt, M., Kremling, K., 1999. Methods of seawater analysis, 3rd ed. Wiley-VCH Verlag, Germany, pp. 600.

Hadwen, W.L., Arthington, A.H., 2007. Food webs of two intermittently open estuaries receiving ^{15}N-enriched sewage effluent. Estuar. Coast. Shelf Sci. 71, 347–358. https://doi.org/10.1016/j.ecss.2006.08.017.

Harvell, D., Aronson, R., Baron, N., Connell, J., Dobson, A., Ellner, S., Gerber, L., Kim, K., Kuris, A., McCallum, H., Lafferty, K., McKay, B., Porter, J., Pascual, M., Smith, G., Jardé, E., Mansuy, L., Faure, P., 2005. Organic markers in the lipidic fraction of sewage sludges. Water Res. 39, 1215–1232. https://doi.org/10.1016/j.watres.2004.12.024.

Jardé, E., Mansuy, L., Faure, P., 2005. Organic markers in the lipidic fraction of sewage sludges. Water Res. 39, 1215–1232. https://doi.org/10.1016/j.watres.2004.12.024.

Jarvie, H.P., Jickells, T.D., Skeffington, R.A., Withers, P.J.A., 2012. Climate change and coupling of macronutrient cycles along the atmospheric, terrestrial, freshwater and estuarine continuum. Sci. Total Environ. 434, 252–258. https://doi.org/10.1016/j.scitotenv.2012.07.051.

Jaschinski, S., Brepohl, D.C., Sommer, U., 2008. Carbon sources and trophic structure in an eelgrass *Zostera marina* bed, based on stable isotope and fatty acid analyses. Mar. Ecol. Prog. Ser. 358, 103–114. https://doi.org/10.3354/meps07327.

Kattner, G., Fricke, H.S.G., 1986. Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J. Chromatogr. A 361, 263–268. https://doi.org/10.1016/S0021-9673(01)86914-4.

Ke, Z., Tan, Y., Huang, L., Zhao, C., Jiang, X., 2017. Spatial distributions of $\delta^{13}C$, $\delta^{15}N$ and C/N ratios in suspended particulate organic matter of a bay under serious anthropogenic influences: Daya Bay, China. Mar. Pollut. Bull. 114, 183–191. https://doi.org/10.1016/j.marpolbul.2016.08.078.

Kohlbach, D., Graeve, M., Lange, B., David, C., Peeken, I., Flores, H., 2016. The importance of ice algae-produced carbon in the central Arctic Ocean ecosystem: food web relationships revealed by lipid and stable isotope analyses. Limnol. Oceanogr. 61, 2027–2044. https://doi.org/10.1002/lno.10351.

Kopprio, G.A., Biancalana, F., Fricke, A., Garzón Cardona, J.E., Martínez, A., Lara, R.J., 2015a. Global change effects on biogeochemical processes of Argentinian estuaries: an overview of vulnerabilities and ecohydrological adaptive outlooks. Mar. Pollut. Bull. 91, 554–562. https://doi.org/10.1016/j.marpolbul.2014.08.021.

Kopprio, G.A., Kattner, G., Freije, R.H., José de Paggi, S., Lara, R.J., 2014. Seasonal baseline of nutrients and stable isotopes in a saline lake of Argentina: biogeochemical processes and river runoff effects. Environ. Monit. Assess. 186, 3139–3148. https://doi.org/10.1007/s10661-013-3606-4.

Kopprio, G.A., Lara, R.J., Martínez, A., Fricke, A., Graeve, M., Kattner, G., 2015b. Stable isotope and fatty acid markers in plankton assemblages of a saline lake: seasonal trends and future scenario. J. Plankton Res. 37, 584–595. https://doi.org/10.1093/plankt/fbv023.

Kopprio, G.A., Streitenberger, M.E., Okuno, K., Baldini, M., Biancalana, F., Fricke, A., Martínez, A., Neogi, S.B., Koch, B.P., Yamasaki, S., Lara, R.J., 2017. Biogeochemical and hydrological drivers of the dynamics of *Vibrio* species in two Patagonian estuaries. Sci. Total Environ. 579, 646–656. https://doi.org/10.1016/j.scitotenv.2016.11.045.

Kravchuk, E.S., Sushchik, N.N., Makhutova, O.N., Trusova, M.Y., Kalacheva, G.S., Gladyshev, M.I., 2014. Differences in carbon isotope signatures of polyunsaturated fatty acids of two microalgal species. Dokl. Biochem. Biophys. 459, 183–185. https://doi.org/10.1134/S1607672914060015.

La Colla, N.S., Negrin, V.L., Marcovecchio, J.E., Botté, S.E., 2015. Dissolved and particulate metals dynamics in a human impacted estuary from the SW Atlantic. Estuar. Coast. Shelf Sci. 166, 45–55. https://doi.org/10.1016/j.ecss.2015.05.009.

Lehmann, M.F., Bernasconi, S.M., Barbieri, A., McKenzie, J.A., 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. Geochim. Cosmochim. Acta 66, 3573–3584. https://doi.org/10.1016/S0016-7037(02)00968-7.

Lorenzen, C.J., 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346. https://doi.org/10.4319/lo.1967.12.2.0343.

Mayor, D.J., Gray, N.B., Hattich, G.S.I., Thornton, B., 2017. Detecting the presence of fish farm-derived organic matter at the seafloor using stable isotope analysis of phospholipid fatty acids. Sci. Rep. 7, 5146. https://doi.org/10.1038/s41598-017-05252-w.

McKinney, R.A., Lake, J.L., Charpentier, M.A., Ryba, S., 2002. Using mussel isotope ratios to assess anthropogenic nitrogen inputs to freshwater ecosystems. Environ. Monit. Assess. 74, 167–192. https://doi.org/10.1023/A:1013824220299.

Miglioranza, K.S.B., Gonzalez, M., Ondarza, P.M., Shimabukuro, V.M., Isla, F.I., Fillmann, G., Aizpún, J.E., Moreno, V.J., 2013. Assessment of Argentinean Patagonia pollution: PBDEs, OCPs and PCBs in different matrices from the Río Negro basin. Sci. Total Environ. 452–453, 275–285. https://doi.org/10.1016/j.scitotenv.2013.02.055.

Möbius, J., 2013. Isotope fractionation during nitrogen remineralization (ammonification): implications for nitrogen isotope biogeochemistry. Geochim. Cosmochim. Acta 105, 422–432. https://doi.org/10.1016/j.gca.2012.11.048.

Moynihan, M.A., Baker, D.M., Mmochi, A.J., 2012. Isotopic and microbial indicators of sewage pollution from Stone Town, Zanzibar, Tanzania. Mar. Pollut. Bull. 64, 1348–1355. https://doi.org/10.1016/j.marpolbul.2012.05.001.

Napolitano, G.E., Pollero, R.J., Gayoso, A.M., MacDonald, B.A., Thompson, R.J., 1997. Fatty acids as trophic markers of phytoplankton blooms in the Bahía Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). Biochem. Syst. Ecol. 25, 739–755. https://doi.org/10.1016/S0305-1978(97)00053-7.

Nixon, S.W., 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41, 199–219. https://doi.org/10.1080/00785236.1995.10422044.

Nyssen, F., Brey, T., Dauby, P., Graeve, M., 2005. Trophic position of Antarctic amphipods – enhanced analysis by a 2-dimensional biomarker assay. Mar. Ecol. Prog. Ser. 300, 135–145. https://doi.org/10.3354/meps300135.

Olsen, Y.S., Fox, S.E., Teichberg, M., Otter, M., Valiela, I., 2011. $\delta^{15}N$ and $\delta^{13}C$ reveal differences in carbon flow through estuarine benthic food webs in response to the relative availability of macroalgae and eelgrass. Mar. Ecol. Prog. Ser. 421, 83–96. https://doi.org/10.3354/meps08900.

Palmer, M.W., 1993. Putting things in even better order: the advantages of canonical correspondence analysis. Ecology 74, 2215–2230. https://doi.org/10.2307/1939575.

Parrish, C., 2009. Essential fatty acids in aquatic food webs. In: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), Lipids in Aquatic Ecosystems. Springer New York, pp. 309–326. https://doi.org/10.1007/978-0-387-89366-2_13.

Perkins, M.J., McDonald, R.A., van Veen, F.J.F., Kelly, S.D., Rees, G., Bearhop, S., 2014. Application of nitrogen and carbon stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) to quantify food chain length and trophic structure. PLoS One 9, e93281. https://doi.org/10.1371/journal.pone.0093281.

Reuss, N.S., Hamerlík, L., Velle, G., Michelsen, A., Pedersen, O., Brodersen, K.P., 2013. Stable isotopes reveal that chironomids occupy several trophic levels within West Greenland lakes: implications for food web studies. Limnol. Oceanogr. 58, 1023–1034. https://doi.org/10.4319/lo.2013.58.3.1023.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. J. Appl. Ichthyol. 11, 183–198. https://doi.org/10.1111/j.1439-0426.1995.tb00018.x.

Sato, T., Miyajima, T., Ogawa, H., Umezawa, Y., Koike, I., 2006. Temporal variability of stable carbon and nitrogen isotopic composition of size-fractionated particulate organic matter in the hypertrophic Sumida River Estuary of Tokyo Bay, Japan. Estuar. Coast. Shelf Sci. 68, 245–258. https://doi.org/10.1016/j.ecss.2006.02.007.

Schwarzenbach, R.P., Egli, T., Hofstetter, T.B., Gunten, U.v., Wehrli, B., 2010. Global water pollution and human health. Annu. Rev. Environ. Resour. 35, 109–136. http://www.annualreviews.org/doi/abs/10.1146/annurev-environ-100809-125342.

Signa, G., Mazzola, A., Kairo, J., Vizzini, S., 2017. Small-scale variability in geomorphological settings influences mangrove-derived organic matter export in a tropical bay. Biogeosciences 14, 617–629. https://doi.org/10.5194/bg-14-617-2017.

Sushchik, N.N., Rudchenko, A.E., Gladyshev, M.I., 2017. Effect of season and trophic level on fatty acid composition and content of four commercial fish species from Krasnoyarsk Reservoir (Siberia, Russia). Fish. Res. 187, 178–187. https://doi.org/10.1016/j.fishres.2016.11.016.

Teece, M.A., Fogel, M.L., Dollhopf, M.E., Nealson, K.H., 1999. Isotopic fractionation associated with biosynthesis of fatty acids by a marine bacterium under oxic and anoxic conditions. Org. Geochem. 30, 1571–1579. https://doi.org/10.1016/S0146-6380(99)00108-4.

Thornton, S.F., McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. Estuar. Coast. Shelf Sci. 38, 219–233. https://doi.org/10.1006/ecss.1994.1015.

Toyoda, S., Suzuki, Y., Hattori, S., Yamada, K., Fujii, A., Yoshida, N., Kouno, R., Murayama, K., Shiomi, H., 2011. Isotopomer analysis of production and consumption mechanisms of N_2O and CH_4 in an advanced wastewater treatment system. Environ. Sci. Technol. 45, 917–922. https://doi.org/10.1021/es102985u.

Viso, A.-C., Marty, J.-C., 1993. Fatty acids from 28 marine microalgae. Phytochemistry 34, 1521–1533. https://doi.org/10.1016/S0031-9422(00)90839-2.

White, C.A., Nichols, P.D., Ross, D.J., Dempster, T., 2017. Dispersal and assimilation of an aquaculture waste subsidy in a low productivity coastal environment. Mar. Pollut. Bull. 120, 309–321. https://doi.org/10.1016/j.marpolbul.2017.05.042.

Yano, Y., Nakayama, A., Ishihara, K., Saito, H., 1998. Adaptive changes in membrane lipids of barophilic bacteria in response to changes in growth pressure. Appl. Environ. Microbiol. 64, 479–485.