

## Fatty acid alterations in the detritivorous *Prochilodus lineatus* promoted by opportunistic feeding on sewage discharges in the Río de la Plata estuary

E. D. SPERANZA\*†‡, M. COLOMBO\*§, L. M. TATONE\*†, N. CAPPELLETTI\*†, M. C. MIGOYA\*† AND J. C. COLOMBO\*§

\*Laboratorio de Química Ambiental y Biogeoquímica, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Av. Calchaquí 6200, Florencio Varela, Buenos Aires, 1888, Argentina, †Consejo Nacional de Investigaciones Científicas y Técnicas, Godoy Cruz 2290, C1425FQB C.A.B.A., Buenos Aires, Argentina and §Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, calle 10 y 526, La Plata, Buenos Aires, 1900, Argentina

(Received 13 September 2015, Accepted 30 June 2016)

Muscle fatty acid profiles and PCB contents of the detritivorous species *Prochilodus lineatus* and its diet (stomach contents, settling particles and sediments) were analysed from reference and polluted areas of the Paraná-Río de la Plata basin, to evaluate the alterations produced by opportunistic feeding on sewage discharges. Overall muscle fatty acid composition was dominated by saturated and monounsaturated 16 and 18 carbon (18 C-FA) components with reduced long-chain polyunsaturated fatty acids (LC-PUFA). Compared to sediments, settling particles and stomach contents were enriched in lipids and had a similar fatty acid composition. Opportunistic feeding on sewage detritus at Buenos Aires resulted in enhanced PCB and triglyceride accumulation, with higher proportions of 18 C-FA and lower proportions of 16:1 and LC-PUFA compared to fish from northern pristine reaches of the basin. Mid-Paraná showed intermediate values reflecting mixing of the North stock with migrating Buenos Aires *P. lineatus* identified by their lipid and contaminant profile. According to multivariate analyses, this geographical variation of fatty acid composition was strongly influenced by PCB concentration. *Prochilodus lineatus* assimilates the energy subsidy of sewage inputs through enhanced lipogenesis with dominant 18 C-FA and significant amounts of valuable LC-PUFA. This lipid alteration facilitates the bioaccumulation of PCBs which in turn may reinforce the adipogenic effect of sewage feeding.

© 2016 The Fisheries Society of the British Isles

Key words: detritivory; fatty acid composition; lipids; Paraná basin; PCB; pollution.

### INTRODUCTION

Detritivorous fishes play a key role in energy flux and material cycling of Neotropical hydrographic systems such as the Paraná-Río de la Plata basin (Bowen, 1983) which covers >3 million km<sup>2</sup> in Brazil, Bolivia, Paraguay, Uruguay and Argentina.

‡Author to whom correspondence should be addressed. Tel. +54 11 42758266; email: [esperanza@fcnym.unlp.edu.ar](mailto:esperanza@fcnym.unlp.edu.ar)

§Present address: University of British Columbia, Earth and Ocean Sciences Department, 6339 Stores Rd, Vancouver, BC, V6T 1Z4 Canada

The Paraná (3780 km) and Uruguay Rivers (1790 km) transport  $90 \times 10^6$  t of suspended solids per year into the Rio de la Plata estuary (Degens *et al.*, 1991) feeding a vast delta in front of Buenos Aires, a large city which concentrates one third of the total Argentinean population and is heavily polluted by crude urban-industrial effluent discharges (Colombo *et al.*, 2005a, b).

Sewage-derived organic matter has been recognized as an important energy subsidy for aquatic food webs, affecting multiple levels of biological organization (*e.g.* liver hypertrophy, fish production increase and community structure alterations), as well as a prevailing contamination pathway for detritus feeding organisms (deBruyn *et al.*, 2003; Porter & Janz, 2003). These effects are amplified further when highly specialized detritivorous fishes feed directly on anthropogenic organic matter (Colombo *et al.*, 2000). *Prochilodus lineatus* (Valenciennes 1837) has several morpho-physiological adaptations to selectively feed on organic detritus (sucker-like mouth, gillraker filtering grid, cardiac and muscularized pyloric stomach with pyloric caeca and intestinal mucosal folds; Bowen, 1983). Near the Buenos Aires sewer outfall, massive amounts of organic matter, hydrocarbons, polychlorinated biphenyls (PCB) and other persistent organic pollutants accumulate at the sediment surface and are efficiently assimilated by *P. lineatus* (Colombo *et al.*, 2007a, b, 2011) resulting in significant biochemical alterations compared to *P. lineatus* from northern sites of the basin, *i.e.* increased body mass and an unusually high muscle lipid content (Speranza & Colombo, 2009). Due to the high abundance, widespread distribution and ecological role as a main dietary item of medium and top predators of the basin, this migratory fish (100–1000 km south–north, trophic-reproductive movements) is one of the most important freshwater fisheries of South America (Sverlij *et al.*, 1993), representing a critical contamination pathway for freshwater ecosystems and human populations (Colombo *et al.*, 2000).

In this context, the study of lipid composition is relevant since lipids regulate the degree of bioaccumulation of hydrophobic pollutants and act as a protective storage compartment (Lassiter & Hallam, 1990; Kelly *et al.*, 2004). Conversely, organic pollutants can interfere with lipid metabolism, altering lipid content and the nutritional quality of fat, in particular the abundance of polyunsaturated fatty acids (Addison, 1982; Kainz & Fisk, 2009). Furthermore, lipid storage and dynamics are important attributes of fish health and population success since they are essential in the overall allocation of energy, *i.e.* lipid oxidation for metabolism, growth, reproduction and migration (Marshall Adams, 1999; Tocher, 2003). As major lipid constituents, fatty acids have been extensively used as biomarkers in fishes, since they regulate many physiological processes and their profile changes as a function of multiple environmental stressors (Marshall Adams, 1999). Polyunsaturated fatty acids are particularly important, since they play a key metabolic role (*e.g.* regulation of enzyme activity, modulation of membrane fluidity, differentiation of neural tissues, anti-inflammatory response and somatic growth) and some of them are essential fatty acids for many vertebrates (Valentine & Valentine, 2004; Parrish, 2009).

The objective of this study was to evaluate the physiological and ecological effects of opportunistic feeding, on sewage-derived organic matter, on lipid metabolism and fatty acid composition of a dominant detritivorous fish and its diet (sediments and settling particles) from various different locations on the Río de la Plata Basin.

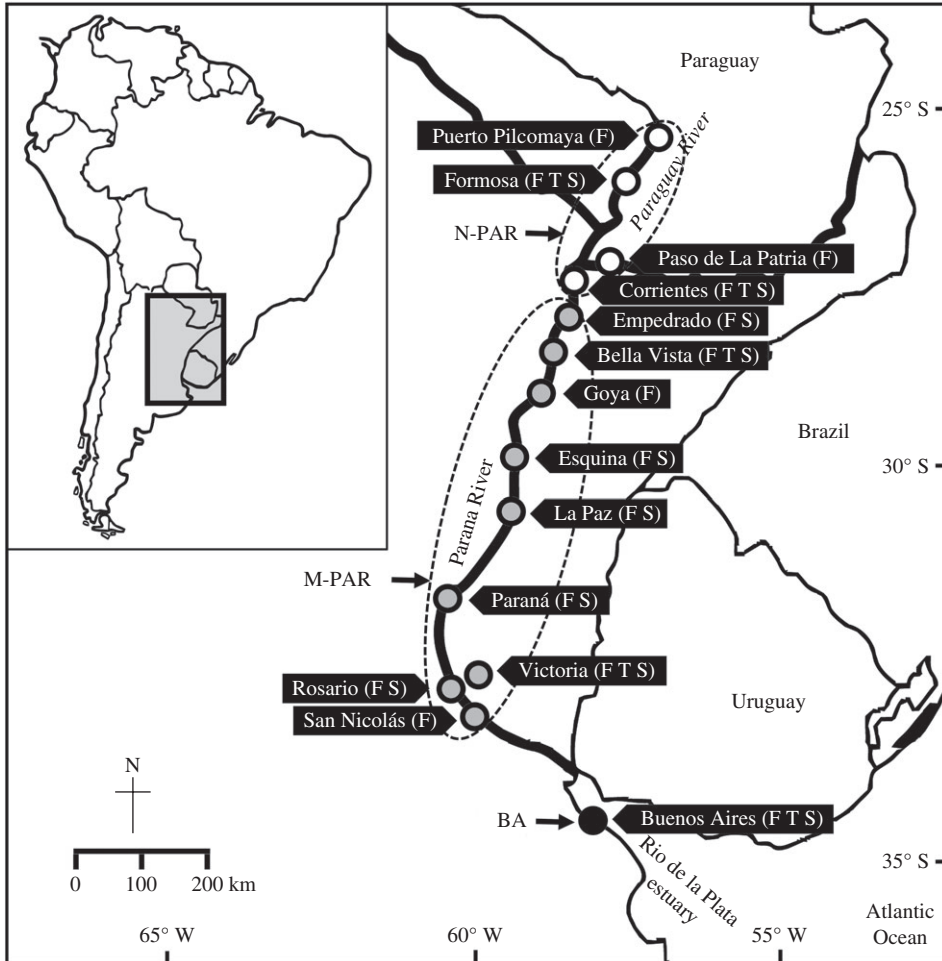


FIG. 1. Sampling stations of *Prochilodus lineatus* (F), sediments (S) and settling particles (T) in the three sectors of the Rio de la Plata basin: Buenos Aires (BA, ●), Mid-Paraná (M-PAR, ○) and North Paraná (N-PAR, ○).

## MATERIALS AND METHODS

### SAMPLING

*Prochilodus lineatus* was collected every 3 months between 2006 and 2012 by local fisherman near the main sewer outfall of Buenos Aires (BA) in the Río de la Plata estuary and at nine sites along the lower and mid-section of Paraná river (200–950 km upstream; M-PAR) and at four northern sites along the Paraná and Paraguay Rivers (>950 km upstream; N-PAR; Fig. 1). Individuals ( $n = 218$ ) were collected (78 at BA, 84 at M-PAR and 56 at N-PAR) and after measuring standard length ( $L_S$ ) and body mass, *P. lineatus* were opened along the ventral midline and livers were weighed and collected in plastic flasks, as well as cardiac stomach contents when available. A portion of dorsal muscle was excised and wrapped in aluminium foil. Samples were immediately frozen on dry ice, transported to the laboratory and stored at  $-20^\circ\text{C}$  until analysis. Superficial sediments were collected using a stainless steel Van-Veen grab sampler and settling particles with fixed sediment traps deployed at 1.5 m from the surface over 12–36 h at selected sites (Fig. 1).

## BIOCHEMICAL ANALYSES

Muscle lipids were extracted in a tissue homogenizer with chloroform:methanol (2:1 v/v; Folch *et al.*, 1957). In sediments, trap material and stomach contents, lipids were extracted ultrasonically with petroleum ether:dichloromethane (2:1). After gravimetric determination of lipid content, the extracts were stored at  $-20^{\circ}\text{C}$ .

Fatty acids were derivatized to their methyl esters with 1% methanolic sulphuric acid as a reagent (12 h at  $70^{\circ}\text{C}$  followed by extraction with petroleum ether; Christie, 1989). In sediments, settling particles and stomach contents, non-acylated lipids were eliminated by saponification with 1 M KOH in methanol before derivatization. Non-saponifiable compounds were extracted with petroleum ether–diethyl ether (4:1 v/v) followed by re-extraction of the acidified aqueous phase with petroleum ether. For fatty acid analysis of individual lipid classes, lipid extracts were separated by thin-layer chromatography using silica gel plates (Analtch; [www.ichromatography.com](http://www.ichromatography.com)) and hexane:diethyl ether:acetic acid (80:30:2 v/v) as the mobile phase, and the spots were identified after revealing the plate in iodine atmosphere, using a standard lipid mixture (diolein, cholesterol, oleic acid, triolein and cholesteryl-oleate; Sigma-Aldrich; [www.sigmaaldrich.com](http://www.sigmaaldrich.com)) run in parallel with the samples. The spots were then scraped into glass tubes, extracted with chloroform:methanol (2:1 v/v) and lipids were saponified and derivatized as described.

Fatty acids were analysed by high-resolution gas chromatography (KONIK 3000, Agilent 6890; [www.agilent.com](http://www.agilent.com)) with split–splitless injection ( $250^{\circ}\text{C}$ ), HP5-MS capillary columns programmed from  $65^{\circ}\text{C}$  (2 min) to  $195^{\circ}\text{C}$  (1 min) at  $12^{\circ}\text{C min}^{-1}$ , to  $260^{\circ}\text{C}$  (1 min) at  $4^{\circ}\text{C min}^{-1}$  and to  $300^{\circ}\text{C}$  (5 min) at  $5^{\circ}\text{C min}^{-1}$  and flame ionization detection ( $300^{\circ}\text{C}$ ). Carrier gas was hydrogen ( $1\text{ ml min}^{-1}$ ) and make-up gas was nitrogen ( $40\text{ ml min}^{-1}$ ). A mixture of 10–24 carbon fatty acid methyl ester standards (AccuStandard, Inc.; [www.accustandard.com](http://www.accustandard.com)) was used for quantification. Confirmation of fatty acid identities was performed by gas chromatography–mass spectrometry (Agilent 6850-5973N) with a similar temperature programme in full scan mode (electronic impact ionization at 70 eV, scan range: 50–550 amu). Fatty acid analyses were reproducible to  $\pm 0.9$ –18.0% and recoveries ranged between 87 and 109%.

For PCB analyses, muscle samples were extracted with acetone, dichloromethane and petroleum ether (1:2:2), concentrated under a nitrogen stream and treated with sulphuric acid. PCBs were separated by silica-gel chromatography, eluting the column with petroleum ether and dichloromethane (3:1), and quantified by high-resolution gas chromatography using  $30\text{ m} \times 0.25\text{ mm}$  DB5 capillary columns and electron capture (ECD) detection (Agilent 6890 and 7890). Quantification was performed with external standards of 41 PCBs (di to decachlorobiphenyls; C-QME-01, Quebec Ministry of Environment Congener Mix, AccuStandard, Inc.). Deuterated PCB 103 and 198 (Absolute Standards, Inc.; [www.absolutestandards.com](http://www.absolutestandards.com)) were added as internal standards. Method accuracy, evaluated through repeated analysis of a certified cod liver oil (SRM1588a, NIST, U.S.A.; [www.nist.gov](http://www.nist.gov)) ranged between 60 and 110%.

## STATISTICAL ANALYSIS

Statistical analyses were carried out using XLSTAT (Addinsoft SARL; [www.xlstat.com](http://www.xlstat.com)) and Statistica (Stat Soft, Inc.; [www.statsoft.com](http://www.statsoft.com)). Data were expressed as mean  $\pm$  s.d. For comparison among multiple samples, ANOVA followed by *post hoc* Tukey HSD test were used. A *t*-test was used to perform comparisons between two means as well as to evaluate the significance of correlation coefficients. Multivariate analysis was performed by principal component analysis of standardized data ( $x - Xy^{-1}$ , where  $X$  = mean and  $y$  = s.d.). Forward stepwise multiple regression ( $P$ -to-enter  $< 0.05$ ) was used to identify the variables that best accounted for the observed variation in fatty acid composition.

## RESULTS

### MUSCLE FATTY ACID COMPOSITION

*Prochilodus lineatus*  $L_S$  was  $44.0 \pm 5.6$  cm and weighed  $2308.9 \pm 1147.1$  g; the condition index ( $100 \times \text{body mass} \times L_S^{-3}$ ) exhibited significant geographical differences (ANOVA,  $F_{2,216} = 28.5$ ,  $P < 0.001$ ), with higher values at Buenos Aires

TABLE I. Lipid, triglyceride, fatty acid and PCB contents (mean  $\pm$  S.D.) of *Prochilodus lineatus* muscle from North (N-PAR) and Mid-Paraná (M-PAR) and Buenos Aires (BA), discriminating migratory *P. lineatus* collected in Mid-Paraná (BA MIG). Note similar values of BA and BA MIG

	N-PAR	M-PAR	BA	BA MIG
Lipids (% wet mass)	3.4 $\pm$ 3.2 <sup>C</sup>	9 $\pm$ 11 <sup>B</sup>	23 $\pm$ 15 <sup>A</sup>	29 $\pm$ 14 <sup>A</sup>
Triglycerides (mg g <sup>-1</sup> lipids)	523 $\pm$ 174 <sup>B</sup>	614 $\pm$ 208 <sup>B</sup>	884 $\pm$ 142 <sup>A</sup>	936 $\pm$ 50 <sup>A</sup>
SFA (mg g <sup>-1</sup> lipids)	224 $\pm$ 55 <sup>B</sup>	241 $\pm$ 65 <sup>B</sup>	288 $\pm$ 65 <sup>A</sup>	301 $\pm$ 38 <sup>A</sup>
MUFA (mg g <sup>-1</sup> lipids)	228 $\pm$ 79 <sup>B</sup>	252 $\pm$ 72 <sup>B</sup>	304 $\pm$ 77 <sup>A</sup>	331 $\pm$ 54 <sup>A</sup>
PUFA (mg g <sup>-1</sup> lipids)	181 $\pm$ 56 <sup>A</sup>	156 $\pm$ 59 <sup>A</sup>	181 $\pm$ 48 <sup>A</sup>	197 $\pm$ 49 <sup>A</sup>
18C-FA (mg g <sup>-1</sup> lipids)	245 $\pm$ 88 <sup>B</sup>	277 $\pm$ 114 <sup>B</sup>	427 $\pm$ 120 <sup>A</sup>	464 $\pm$ 121 <sup>A</sup>
LC-PUFA (mg g <sup>-1</sup> lipids)	123 $\pm$ 54 <sup>A</sup>	90 $\pm$ 44 <sup>B</sup>	63 $\pm$ 22 <sup>C</sup>	69 $\pm$ 17 <sup>C</sup>
PCB ( $\mu$ g g <sup>-1</sup> wet mass)*	0.62 $\pm$ 0.78 <sup>B</sup> (14 $\pm$ 11)	1.8 $\pm$ 3.8 <sup>B</sup> (11 $\pm$ 11)	5.9 $\pm$ 3.9 <sup>A</sup> (2.7 $\pm$ 1.7)	9.4 $\pm$ 4.6 <sup>A</sup> (2.3 $\pm$ 0.6)

Significant differences (Tukey HSD,  $P < 0.05$ ) are indicated by different superscript uppercase letters. 18C-FA, 18 carbon fatty acids; LC-PUFA, long-chain PUFA ( $\geq 20$  carbons); MUFA, monounsaturated fatty acids; PCB, polychlorinated biphenyls; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. \*The 6Cl:3-4Cl compositional ratio (hexachloro-PCB:tri- + tetrachloro-PCB) is shown in parentheses.

(3.0  $\pm$  0.5), followed by M-PAR and N-PAR (2.3  $\pm$  0.4 and 2.3  $\pm$  0.4, respectively; Tukey HSD,  $P < 0.01$ ). Total muscle lipids were highly variable (12.3  $\pm$  13.8% wet mass; Table I) and positively correlated with body mass (Pearson correlation,  $r = 0.78$ , d.f. = 216,  $P < 0.001$ ). Triglycerides were 68.5  $\pm$  23.3% of muscle lipids and were positively correlated with body mass and lipid content (Pearson correlation,  $r = 0.72$ , d.f. = 216,  $P < 0.001$  and  $r = 0.91$ , d.f. = 216,  $P < 0.001$ ). Muscle fatty acids averaged 688.2  $\pm$  178.2 mg g<sup>-1</sup> lipid with a high proportion of saturated (SFA) and monounsaturated (MUFA) fatty acids (37.6  $\pm$  4.0 and 38.8  $\pm$  5.9%, respectively), mainly 16:0, 18:1n-9, 16:1n-7 and 18:0 [Tables I and SI (Supporting Information) and Fig. 2(a)]. Polyunsaturated fatty acids (PUFA) represented 23.6  $\pm$  6.1%, with 18:2n-6 as the main component followed by variable proportions of 18:3n-3 and long chain PUFA ( $\geq 20$  carbons, LC-PUFA), *i.e.* 20:5n-3, 22:6n-3 and 20:4n-6. The fatty acid composition of polar phospholipids and neutral triglycerides showed distinct proportions of 18 carbon fatty acids (18C-FA, 36.1  $\pm$  6.2 v. 56.1  $\pm$  6.0% respectively, paired *t*-test,  $t = 11.2$ ,  $P < 0.001$ ) and LC-PUFA [18.0  $\pm$  6.9 v. 3.4  $\pm$  1.5% respectively, paired *t*-test,  $t = 5.7$ ,  $P < 0.001$ ; Table SII (Supporting Information) and Fig. 2(b)].

Superimposed over this general compositional pattern there were clear geographical variations, which were greatest between North Paraná and Buenos Aires. Muscle lipid content was three- to eight-fold higher at Buenos Aires, due to an enhanced triglyceride accumulation (Tukey HSD,  $P < 0.001$ ; Table I). Similarly, fatty acid composition of Buenos Aires *P. lineatus* was significantly enriched in 18C-FA (Tukey HSD,  $P < 0.001$ ), especially 18:1n-9 and 18:2n-6, and had lower proportions of 16:1n-7 and LC-PUFA (Tukey HSD,  $P < 0.001$ ), especially 20:4n-6, 20:5 and 22:6n-3. A group of 15 Mid-Paraná individuals (BA MIG) identified as migratory *P. lineatus* from Buenos Aires based on their length–mass relationship, biochemical composition and pollutant fingerprint (Speranza *et al.*, 2012) presented similar lipid, contaminant contents

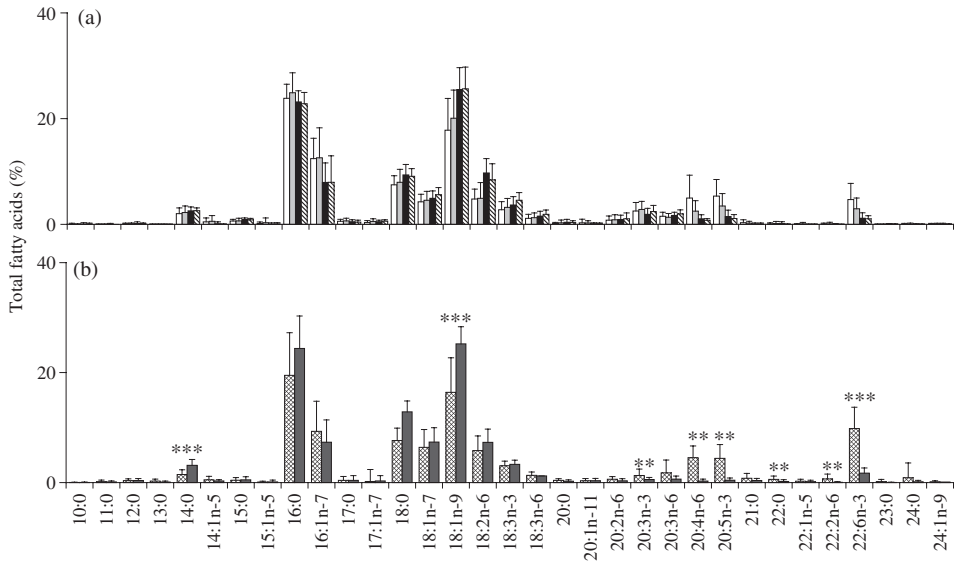


FIG. 2. (a) Muscle fatty acid composition of *Prochilodus lineatus* from North (N-PAR, □) and Mid (M-PAR, ▒) Paraná River and Buenos Aires (BA, ■), discriminating migratory *P. lineatus* collected in Mid Paraná (BA MIG, ▨). (b) Fatty acid composition of phospholipids (▨) and triglycerides (■) of *P. lineatus* muscle (significant differences are indicated with an asterisk: \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

and fatty acid profiles to Buenos Aires *P. lineatus* [Tables I and SI (Supporting Information)]. Excluding these *P. lineatus* from Mid-Paraná, the fatty acid composition became indistinguishable from North *P. lineatus* (18C-FA:  $38.8 \pm 7.9\%$ , LC-PUFA:  $16.0 \pm 4.7\%$ ).

These geographical differences resulted from different rates of change in fatty acid profiles as *P. lineatus* grew. Effectively, 18C-FA were directly related to body mass at Buenos Aires (Pearson correlation,  $r = 0.77$ , d.f. = 76,  $P < 0.001$ ), increasing 2.5 times ( $200 \pm 30$  to  $511 \pm 57$  mg g<sup>-1</sup> lipid) from <1 to >4 kg, while remaining fairly constant at North Paraná ( $180 \pm 17$  to  $212 \pm 46$  mg g<sup>-1</sup> lipid). LC-PUFAs also showed contrasting geographical patterns as *P. lineatus* grew; they were rather constant at Buenos Aires ( $56 \pm 22$  to  $64 \pm 13$  mg g<sup>-1</sup> lipid from <1 to >4 kg body mass) and were inversely related to body mass at North Paraná (Pearson correlation,  $r = -0.51$ , d.f. = 54,  $P < 0.001$ ), decreasing from  $155.0 \pm 21.0$  to  $78.5 \pm 12.4$  mg g<sup>-1</sup> lipid. Mid-Paraná *P. lineatus* showed an intermediate trend for both fatty acid series.

PCB reflected a similar pattern, with significant geographical differences (ANOVA,  $F_{2,215} = 26.7$ ,  $P < 0.001$ ). Total concentrations were 10 times higher in *P. lineatus* from Buenos Aires compared to North *P. lineatus* (Tukey HSD,  $P < 0.001$ ; Table I) with lower proportions of persistent hexachlorobiphenyls relative to more labile tri- and tetrachlorobiphenyls (6Cl:3-4Cl; Tukey HSD,  $P < 0.001$ ). As observed for the biochemical composition, M-PAR *P. lineatus* had intermediate PCB values, but after the exclusion of migratory *P. lineatus* from Buenos Aires the resulting values became similar to North *P. lineatus* ( $0.5 \pm 1.0$  µg g<sup>-1</sup> wet mass, 6Cl:3-4Cl:  $16.3 \pm 10.1$ ).

A collective evaluation by PCA of the fatty acids which contributed the most to the overall variability, i.e. 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 20:4n-6, 20:5n-3 and 22:6n-3,

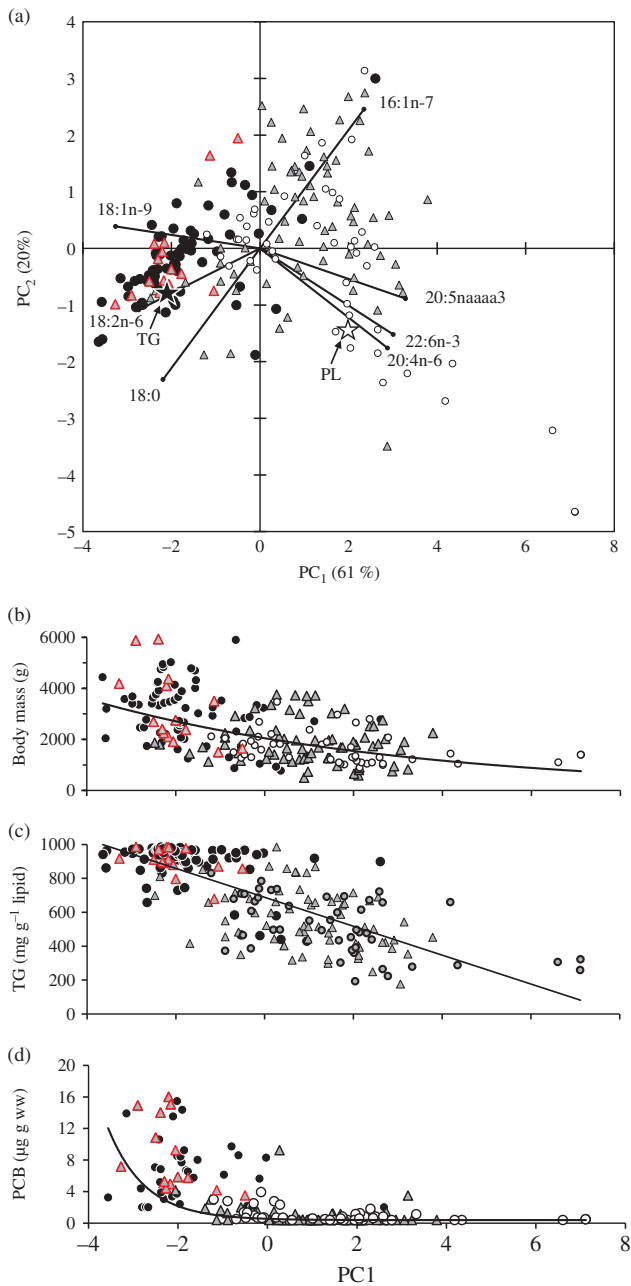


FIG. 3. (a) Principal component analysis (PCA) of fatty acid composition of *Prochilodus lineatus* from Buenos Aires (BA, ●), Mid-Paraná (M-PAR, Δ) and North Paraná (N-PAR, ○). Migratory individuals from Buenos Aires (▲) and the mean fatty acid composition of phospholipid (PL; ☆) and triglyceride (TG; ★) are indicated. The relationship of fatty acid composition (PC<sub>1</sub> values) with (b) body mass (c) muscle triglyceride content and (d) polychlorinated biphenyls (PCB) are shown. The curves were fitted by: (b)  $y = 2048.90e^{-0.14x}$  ( $r = -0.55$ ), (c)  $y = -85.22x + 688.41$  ( $r = -0.75$ ) and (d)  $y = 0.16e^{-1.21x}$  ( $r = -0.62$ ).

TABLE II. Stepwise forward multiple regression analysis of *Prochilodus lineatus* muscle fatty acid composition related to body mass, lipids, triglycerides (TG) and polychlorinated biphenyls (PCB). Significant adjusted correlation coefficients ( $r^2$ ,  $P < 0.05$ ) are shown in bold

	16:1 n-7	18:2 n-6	18:1 n-9	18:0	20:4 n-6	20:5 n-3	22:6 n-3
Body mass (g)	0.0002	0.006	0.0151	0.0337	0.00039	0.00290	.0012
Lipids (mg g <sup>-1</sup> wet mass)	0.0081	0.0010	0.0004	0.0017	0.0142	0.0213	<b>0.3235</b>
TG (mg g <sup>-1</sup> lipids)	0.0131	0.0024	<b>0.0564</b>	0.0008	<b>0.0792</b>	<b>0.0650</b>	<b>0.1269</b>
PCB (µg g <sup>-1</sup> wet mass)	<b>0.45</b>	<b>0.56</b>	<b>0.57</b>	<b>0.28</b>	<b>0.32</b>	<b>0.38</b>	0.01
Total $r^2$	0.48	0.57	0.64	0.32	0.41	0.47	0.46

TG, triglycerides; PCB, polychlorinated biphenyls.

is presented in Fig. 3(a). These fatty acids explained 81% of the total variability, mainly through PC<sub>1</sub>, which was loaded positively with LC-PUFA (20:5n-3, 22:6n-3 and 20:4n-6) and 16:1n-7 and negatively with 18C-FA (18:1n-9, 18:2n-6 and 18:0). The less significant PC<sub>2</sub> was mainly determined by 16:1n-7 (positive) and 18:0 (negative). Buenos Aires *P. lineatus* were discriminated on the left side from North Paraná *P. lineatus* on the right. *Prochilodus lineatus* from Mid-Paraná were widely dispersed, overlapping both with Buenos Aires and North-Paraná *P. lineatus*. Phospholipid and triglycerides were clearly discriminated by their fatty acid composition, *i.e.* phospholipids enriched in LC-PUFA at the right and triglycerides dominated by 18C-FA on the left close to BA *P. lineatus* and BA MIG individuals. The relationship of fatty acid composition with body mass, triglyceride content and PCB concentration is reflected by the inverse correlation of PC<sub>1</sub> values with these parameters [Pearson correlation,  $r = -0.55$ , d.f. = 201,  $P < 0.001$ ;  $r = -0.75$ , d.f. = 201,  $P < 0.001$  and  $r = -0.62$ , d.f. = 201,  $P < 0.001$ ; Fig. 3(b), (c), (d)].

The stepwise forward multiple regression analysis between body mass, lipid, triglyceride and PCB as independent variables and fatty acids (16:1n-7, 18:0, 18:1n-9, 18:2n-6, 20:4n-6, 20:5n-3 and 22:6n-3) explained 32–64% of fatty acid variability (Table II). The model revealed that PCBs were the most influential parameter on fatty acid profiles with the largest contribution to the multiple correlation coefficient (except 22:6n-3 chiefly dependent on lipids and triglycerides).

## FATTY ACID COMPOSITION OF SEDIMENTS, SETTLING PARTICLES AND STOMACH CONTENTS

Total lipids, triglycerides and total fatty acid showed significant differences among these compartments (ANOVA,  $F_{2,76} = 9.17$ ,  $P < 0.01$ ;  $F_{2,76} = 13.13$ ,  $P < 0.001$  and  $F_{2,76} = 7.85$ ,  $P < 0.01$ , respectively), with low values in sediments and a two to six times increase in settling particles and stomach contents which were more alike [lipids:  $1.1 \pm 1.3$ ,  $7.2 \pm 7.2$  and  $5.8 \pm 5.1$  mg g<sup>-1</sup>; triglycerides:  $29.7 \pm 18.3$ ,  $95.0 \pm 32.2$  and  $126.2 \pm 89.0$  mg g<sup>-1</sup> lipid; total fatty acid:  $168.0 \pm 110.4$ ,  $361.9 \pm 156.1$  and  $343.4 \pm 131.6$  mg g<sup>-1</sup> lipid, respectively, Tukey HSD,  $P < 0.01$ ; Tables III and SIII (Supporting Information)]. In general, the fatty acid composition was largely dominated by SFA and MUFA, with very low concentrations of LC-PUFA. Fatty acid profiles showed significant differences among all three compartments (ANOVA,  $P < 0.01$ ): basically increasing concentrations of MUFA and PUFA from sediments



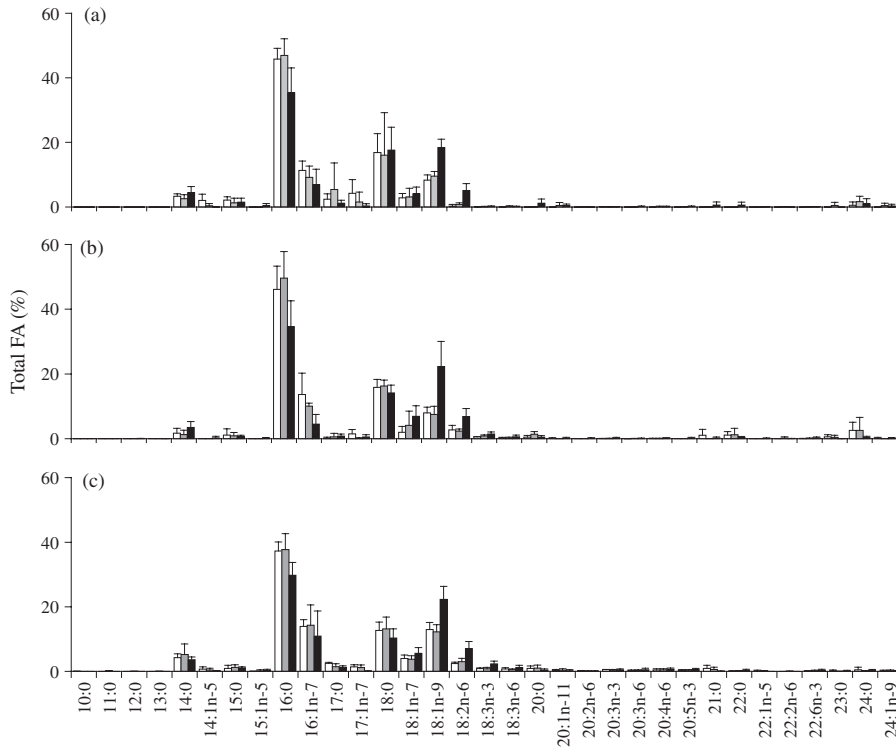


FIG. 4. Fatty acid composition of (a) sediments (black), (b) settling particles (grey) and (c) *Prochilodus lineatus* stomach contents (white) from North (□) and Mid-Paraná (▤) and Buenos Aires (■).

to settling particles and stomach contents (MUFA:  $50.5 \pm 39.3$ ,  $120.2 \pm 69.7$  and  $126.1 \pm 90.0$  mg g<sup>-1</sup> lipid, Tukey HSD,  $P < 0.01$ ; PUFA:  $8.0 \pm 9.3$ ,  $34.1 \pm 28.7$  and  $36.7 \pm 34.1$  mg g<sup>-1</sup> lipid, Tukey HSD,  $P < 0.01$ ). This difference was greatest for LC-PUFA which showed an eight to 16 fold increase from sediments to settling particles and stomach contents ( $0.54 \pm 0.87$  v.  $3.6 \pm 4.2$  and  $8.2 \pm 7.5$  mg g<sup>-1</sup> lipid, Tukey HSD,  $P < 0.001$ ).

As was found for the *P. lineatus*, sediments, settling particles and stomach contents exhibited geographical differences in terms of their lipid composition (ANOVA,  $P < 0.01$ ). Triglycerides and total fatty acid concentrations were two- to four-fold higher at Buenos Aires, compared to North and Mid-Paraná, in all three compartments (Tukey HSD,  $P > 0.01$ ; Table II). Fatty acid composition reflected this difference with significantly (Tukey HSD,  $P < 0.01$ ) higher proportions of 18C-FA (18:1n-9, 18:2n-6 and 18:3n-3) and lower values of 16:0 and 16:1n-7 at Buenos Aires [Table SIII (Supporting Information) and Fig. 4].

## DISCUSSION

The general fatty acid composition of *P. lineatus* muscle, dominated by 16 and 18 carbon saturated and monounsaturated fatty acids, agrees with previous reports (Andrade

TABLE III. Lipid, triglycerides and fatty acid composition of sediments, settling particles and *Prochilodus lineatus* stomach contents (mean  $\pm$  s.d.) from North and Mid-Paraná River (N-PAR and M-PAR, respectively) and Buenos Aires (BA)

	Sediments			Settling particles			Stomach content		
	N-PAR	M-PAR	BA	N-PAR	M-PAR	BA	N-PAR	M-PAR	BA
Lipids (mg g <sup>-1</sup> wet mass)	0.031 $\pm$ 0.042 <sup>B</sup>	0.18 $\pm$ 0.25 <sup>B</sup>	2.0 $\pm$ 1.1 <sup>A</sup>	0.25 $\pm$ 0.17 <sup>B</sup>	0.50 $\pm$ 0.26 <sup>B</sup>	10 $\pm$ 6.6 <sup>A</sup>	3.7 $\pm$ 1.2 <sup>A</sup>	3.9 $\pm$ 1.7 <sup>A</sup>	8.5 $\pm$ 6.8 <sup>A</sup>
TG (mg g <sup>-1</sup> lipids)	17 $\pm$ 7.3 <sup>B</sup>	17 $\pm$ 6.2 <sup>B</sup>	48 $\pm$ 14 <sup>A</sup>	52 $\pm$ 23 <sup>B</sup>	64 $\pm$ 7.5 <sup>B</sup>	110 $\pm$ 24 <sup>A</sup>	66 $\pm$ 40 <sup>B</sup>	63 $\pm$ 41 <sup>B</sup>	215 $\pm$ 58 <sup>A</sup>
SFA (mg g <sup>-1</sup> lipids)	55 $\pm$ 18 <sup>B</sup>	65 $\pm$ 32 <sup>B</sup>	154 $\pm$ 59 <sup>A</sup>	167 $\pm$ 17 <sup>AB</sup>	126 $\pm$ 13 <sup>B</sup>	236 $\pm$ 66 <sup>A</sup>	122 $\pm$ 47 <sup>AB</sup>	113 $\pm$ 65 <sup>B</sup>	264 $\pm$ 101 <sup>A</sup>
MUFA (mg g <sup>-1</sup> lipids)	22 $\pm$ 6.8 <sup>B</sup>	21 $\pm$ 10 <sup>B</sup>	76 $\pm$ 38 <sup>A</sup>	60 $\pm$ 14 <sup>B</sup>	37 $\pm$ 10 <sup>B</sup>	153 $\pm$ 59 <sup>A</sup>	65 $\pm$ 21 <sup>B</sup>	58 $\pm$ 25 <sup>B</sup>	208 $\pm$ 55 <sup>A</sup>
PUFA (mg g <sup>-1</sup> lipids)	0.33 $\pm$ 0.27 <sup>B</sup>	1.1 $\pm$ 0.89 <sup>B</sup>	14 $\pm$ 8.4 <sup>A</sup>	9.0 $\pm$ 5.0 <sup>A</sup>	6.7 $\pm$ 1.1 <sup>A</sup>	46 $\pm$ 27 <sup>A</sup>	15 $\pm$ 7.1 <sup>B</sup>	12 $\pm$ 3.4 <sup>B</sup>	76 $\pm$ 16 <sup>A</sup>
18C-FA (mg g <sup>-1</sup> lipids)	22.0 $\pm$ 8.3 <sup>B</sup>	24 $\pm$ 10 <sup>B</sup>	119 $\pm$ 40 <sup>A</sup>	71 $\pm$ 24 <sup>B</sup>	53 $\pm$ 11 <sup>B</sup>	229 $\pm$ 79 <sup>A</sup>	69 $\pm$ 25 <sup>B</sup>	56 $\pm$ 26 <sup>B</sup>	246 $\pm$ 86 <sup>A</sup>
LC-PUFA (mg g <sup>-1</sup> lipids)	0.042 $\pm$ 0.078 <sup>A</sup>	0.15 $\pm$ 0.30 <sup>A</sup>	0.93 $\pm$ 1.16 <sup>A</sup>	0.35 $\pm$ 0.39 <sup>A</sup>	0.85 $\pm$ 0.55 <sup>A</sup>	5.0 $\pm$ 4.4 <sup>A</sup>	4.1 $\pm$ 2.0 <sup>B</sup>	3.4 $\pm$ 1.7 <sup>B</sup>	15.0 $\pm$ 7.8 <sup>A</sup>

Significant differences (Tukey HSD,  $P < 0.05$ ) between sampling stations within each row are indicated by different superscript uppercase letters.

18C-FA, 18 carbons fatty acids; LC-PUFA, long-chain PUFA ( $\geq 20$  carbons); MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglycerides.

*et al.*, 1995; Bayo & Cordivola de Yuan, 1996; Brenner & Bernasconi, 1997) and is mostly determined by the balance between structural phospholipids from cell membranes (enriched in LC-PUFA for membrane fluidity regulation) and diet-controlled triglycerides (enriched in SFA and MUFA as energy depot; Maia *et al.*, 1994). This muscular fatty acid composition showed a six to 16 times enrichment in LC-PUFAs relative to sediments, settling particles and stomach contents dominated by saturated fatty acid (*i.e.* 16:0 and 18:0). This reflects the preferential assimilation and biosynthesis of PUFA in *P. lineatus* and the higher resistance to degradation of saturated fatty acids in settling particles and sediments (Meyers & Ishiwatari, 1993). The similarity of fatty acid composition between settling particles and stomach contents confirms the selective feeding of *P. lineatus* on the interfacial floc that is richer in organic matter than consolidated sediments.

Preferential intestinal absorption of dietary LC-PUFA (Sigurgisladottir *et al.*, 1992) could contribute to increased muscle LC-PUFAs, but these acids are very scarce in the diet suggesting that *P. lineatus* is able to synthesize them through elongation and desaturation of essential fatty acids 18:2n-6 and 18:3n-3 (Sargent *et al.*, 1995; Tocher, 2003). Thus, *P. lineatus* may constitute an important source of fatty acids to higher levels of the aquatic web, trophically upgrading PUFA that play a key metabolic role in aquatic ecosystems (*i.e.* 20:4n-6, 20:5n-3 and 22:6n-3; Henderson, 1996).

The marked geographical differences of muscle fatty acid profiles reflect the shift in *P. lineatus* diet from natural plant detritus in the North (Bayo & Cordivola de Yuan, 1996) to abundant anthropogenic matter at Buenos Aires. Consistently, sediments, settling particles and stomach contents displayed a similar spatial pattern with higher concentrations of total fatty acids enriched in 18C-FA including the relatively labile 18:2n-6 at Buenos Aires, due to massive contributions of fresh organic matter. The higher nutritional value of this anthropogenic detritus was also indicated by its higher carbohydrate and amino acid contents relative to Paraná samples (Speranza & Colombo, 2009). The abundance of easily assimilable lipids at Buenos Aires and the consequent increase of fat stores in *P. lineatus* facilitate the absorption of massive amounts of organic pollutants that are associated with settling particles in this area (Colombo *et al.*, 2007b, c). This is supported by the 10 times higher PCB concentration, with a fresher signature (lower 6Cl:3-4Cl) in Buenos Aires *P. lineatus*.

The geographical change of fatty acid profiles in *P. lineatus* muscle was clearly captured by the PCA, which discriminated Buenos Aires *P. lineatus* dominated by 18 carbon fatty acids, from North Paraná *P. lineatus* enriched with 16:1 and LC-PUFA. Mid-Paraná *P. lineatus* were widely distributed due to the presence of migrating Buenos Aires *P. lineatus* (BA MIG) clearly identified by their pollutant and lipid profiles. The key role of triglyceride and phospholipid relative abundance in determining the composition of fatty acids in fishes is reflected by their opposite position in the PCA and by the strong correlation between triglyceride concentration and PC<sub>1</sub> load. This geographical pattern reinforces the normal evolution of fatty acid profiles as the fish grows, *i.e.* lipid accumulation through triglyceride synthesis, as observed in the correlation of body mass and PC<sub>1</sub>. Remarkably, the PCB content also had a strong correlation with the PC<sub>1</sub> and was the best predictor of fatty acid composition according to a multiple regression model, suggesting an interference of the pollutants with lipid metabolism (*i.e.* accumulation of triglycerides enriched in 18C-FA). This has been demonstrated in rats *Rattus norvegicus* exposed to PCB, which showed an alteration of desaturase and elongase activities leading to an accumulation of 18:1n-9 and 18:2n-6 and a decrease

of PUFA (Matsusue *et al.*, 1997, 1999; Hennig *et al.*, 2005). Furthermore, there is growing evidence that organochlorines can act as obesogens, altering the expression of key genes of lipid metabolism and interfering with endocrine regulation (Grun & Blumberg, 2009; Ruzzin *et al.*, 2010). In this context, pollutants may exert a positive feed-back, reinforcing the nutritionally driven alteration of fatty acid profiles due to enhanced triglyceride accumulation in Buenos Aires *P. lineatus*.

Opportunistic feeding of *P. lineatus* on sewage-derived detritus resulted in energy assimilation through enhanced lipid–triglyceride accumulation with dominant 18C-FA and valuable LC-PUFA. This abnormal fat depot facilitates the bioaccumulation of hydrophobic PCBs which in turn may reinforce the adipogenic effect and fatty acid alterations of sewage feeding. The propagation of this energy subsidy and related contaminant load throughout the food web is facilitated by the large abundance, wide distribution and extensive upstream migrations of *P. lineatus* in this tropical-temperate ecosystem.

This work was funded by Research Grants to J.C.C. from the Argentinean Agency for Scientific Research Promotion (ANPCyT) and from the National University of La Plata (UNLP). E.D.S., N.C. and L.M.T. are members of the Research Career of the Argentinean National Research Council (CONICET). J.C.C. is member of the Research Career of the Buenos Aires Scientific Research Commission (CIC). Fieldwork in this study complied with current laws of the Argentine Republic, in which it was performed.

### Supporting Information

Supporting Information may be found in the online version of this paper:

TABLE SI. Fatty acid composition (% total fatty acids) of *Prochilodus lineatus* muscle from North and Mid-Paraná River (N-PAR and M-PAR, respectively) and Buenos Aires (BA), discriminating migratory *P. lineatus* collected in Mid-Paraná (BA MIG).

TABLE SII. Fatty acid composition (% total fatty acids) of *Prochilodus lineatus* muscle phospholipids (PL) and triglycerides (TG).

TABLE SIII. Fatty acid composition (% of total fatty acids) of *Prochilodus lineatus* stomach contents, settling particles and sediments from North and Mid-Paraná River (N-PAR and M-PAR, respectively) and Buenos Aires (BA).

### References

- Addison, R. F. (1982). Organochlorine compounds and marine lipids. *Progress in Lipid Research* **21**, 47–71.
- Andrade, A. D., Rubira, A. F., Matsuhita, M. & Souza, N. E. (1995).  $\omega$ 3 fatty acids in freshwater fish from South Brazil. *Journal of the American Oil Chemists' Society* **72**, 1207–1210.
- Bayo, V. & Cordiviola de Yuan, E. (1996). Food assimilation of a Neotropical riverine detritivorous fish, *Prochilodus lineatus*, studied by fatty acid composition (Pisces, Curimatidae). *Hydrobiologia* **330**, 81–88.
- Bowen, S. H. (1983). Detritivory in Neotropical fish communities. *Environmental Biology of Fishes* **9**, 137–144.
- Brenner, R. R. & Bernasconi, A. M. (1997). Aporte de ácidos grasos esenciales de las series n-6 y n-3 a la dieta humana por pescados comestibles del Río Paraná. *Medicina* **57**, 307–314.
- deBruyn, A. M. H., Marcogliese, D. J. & Rasmussen, J. B. (2003). The role of sewage in a large river food web. *Canadian Journal of Fisheries and Aquatic Sciences* **60**, 1332–1344.
- Christie, W. W. (1989). *Gas Chromatography and Lipids. A Practical Guide*. Ayr: The Oily Press.

- Colombo, J. C., Bilos, C., Remes Lenicov, M., Colautti, D., Landoni, P. & Brochu, C. (2000). Detritivorous fish contamination in the Río de la Plata estuary: a critical accumulation pathway in the cycle of anthropogenic compounds. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1139–1150.
- Colombo, J. C., Cappelletti, N., Barreda, A., Migoya, M. C. & Skorupka, C. (2005a). Vertical fluxes and accumulation of PCBs in coastal sediments of the Río de la Plata estuary, Argentina. *Chemosphere* **61**, 1345–1357.
- Colombo, J. C., Cappelletti, N., Lasci, J., Migoya, M. C., Speranza, E. & Skorupka, C. N. (2005b). Sources, vertical fluxes and accumulation of aliphatic hydrocarbons in coastal sediments of the Río de la Plata Estuary, Argentina. *Environmental Science and Technology* **39**, 8227–8234.
- Colombo, J. C., Cappelletti, N., Migoya, M. C. & Speranza, E. (2007a). Bioaccumulation of anthropogenic contaminants by detritivorous fish in the Río de la Plata Estuary: 1-aliphatic hydrocarbons. *Chemosphere* **68**, 2128–2135.
- Colombo, J. C., Cappelletti, N., Migoya, M. C. & Speranza, E. (2007b). Bioaccumulation of anthropogenic contaminants by detritivorous fish in the Río de la Plata Estuary: 2-polychlorinated biphenyls. *Chemosphere* **69**, 1253–1260.
- Colombo, J. C., Cappelletti, N., Speranza, E., Migoya, M. C., Lasci, M. J. & Skorupka, N. (2007c). Vertical fluxes and organic composition of settling material from the sewage impacted Buenos Aires coastal area, Argentina. *Organic Geochemistry* **38**, 1941–1952.
- Colombo, J. C., Cappelletti, N., Williamson, M., Migoya, M. C., Speranza, E., Sericano, J. & Muir, D. C. G. (2011). Risk ranking of multiple-POPs in detritivorous fish from the Río de la Plata. *Chemosphere* **83**, 882–889.
- Degens, E. T., Kempe, S. & Richey, J. E. (1991). Summary: biogeochemistry of major World rivers. In *Biogeochemistry of Major World Rivers* (Degens, E. T., Kempe, S. & Richey, J. E., eds), pp. 323–347. New York, NY: John Wiley & Sons.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.
- Grun, F. & Blumberg, B. (2009). Minireview: the case for obesogens. *Molecular Endocrinology* **23**, 1127–1134.
- Henderson, R. J. (1996). Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archives of Animal Nutrition* **49**, 5–22.
- Hennig, B., Reiterer, G., Toborek, M., Matveev, S. V., Daugherty, A., Smart, E. & Robertson, L. W. (2005). Dietary fat interacts with PCBs to induce changes in lipid metabolism in mice deficient in low-density lipoprotein receptor. *Environmental Health Perspectives* **113**, 83–87.
- Kainz, M. J. & Fisk, A. T. (2009). Integrating lipids and contaminants in aquatic ecology and ecotoxicology. In *Lipids in Aquatic Ecosystems* (Arts, M. T. & Brett, M. T., eds), pp. 93–114. New York, NY: Springer.
- Kelly, B. C., Gobas, F. A. P. C. & McLachlan, M. S. (2004). Intestinal absorption and biomagnification of organic contaminants in fish, wildlife, and humans. *Environmental Toxicology and Chemistry* **23**, 2324–2336.
- Lassiter, R. R. & Hallam, T. G. (1990). Survival of the fattest: implications for acute effects of lipophilic chemicals on aquatic populations. *Environmental Toxicology and Chemistry* **9**, 585–595.
- Maia, E. L., Rodriguez-Amaya, D. B. & Franco, M. R. B. (1994). Fatty acids of the total, neutral and phospholipids of the Brazilian freshwater fish *Prochilodus scrofa*. *Journal of Food Composition and Analysis* **7**, 240–251.
- Marshall Adams, S. (1999). Ecological role of lipids in the health and success of fish populations. In *Lipids in Freshwater Ecosystems* (Arts, M. T. & Wainman, B. C., eds), pp. 132–160. New York, NY: Springer.
- Matsusue, K., Ishii, Y., Ariyoshi, N. & Oguri, K. (1997). A highly toxic PCB produces unusual changes in the fatty acid composition of rat liver. *Toxicology Letters* **91**, 99–104.
- Matsusue, K., Ishii, Y., Ariyoshi, N. & Oguri, K. (1999). A highly toxic coplanar polychlorinated biphenyl compound suppresses  $\Delta 5$  and  $\Delta 6$  desaturase activities which play key roles in arachidonic acid synthesis in rat liver. *Chemical Research in Toxicology* **12**, 1158–1165.

- Meyers, P. A. & Ishiwatari, R. (1993). Lacustrine organic geochemistry – an overview of indicators of organic matter sources and diagenesis in lake sediments. *Organic Geochemistry* **20**, 867–900.
- Parrish, C. C. (2009). Essential fatty acids in aquatic food webs. In *Lipids in Aquatic Ecosystems* (Arts, M. T. & Brett, M. T., eds), pp. 309–326. New York, NY: Springer.
- Porter, C. M. & Janz, D. M. (2003). Treated municipal sewage discharge affects multiple levels of biological organization in fish. *Ecotoxicology and Environmental Safety* **54**, 199–206.
- Ruzzin, J., Petersen, R., Meugnier, E., Madsen, L., Lock, E. J., Lillefosse, H., Ma, T., Pesenti, S., Sonne, S. B., Marstrand, T. T., Malde, M. K., Du, Z. Y., Chavey, C., Fajas, L., Lundebye, A. K., Brand, C. L., Vidal, H., Kristiansen, K. & Froyland, L. (2010). Persistent organic pollutant exposure leads to insulin resistance syndrome. *Environmental Health Perspectives* **118**, 465–471.
- Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J. & Tocher, D. R. (1995). Requirement criteria for essential fatty acids. *Journal of Applied Ichthyology* **11**, 183–198.
- Sigurðsladottir, S., Lall, S. P., Parrish, C. C. & Ackman, R. G. (1992). Cholestane as a digestibility marker in the absorption of polyunsaturated fatty acid ethyl esters in Atlantic salmon. *Lipids* **27**, 418–424.
- Speranza, E. D. & Colombo, J. C. (2009). Biochemical composition of a dominant detritivorous fish (*Prochilodus lineatus*) along pollution gradients in the Paraná-Río de la Plata Basin. *Journal of Fish Biology* **74**, 1226–1244.
- Speranza, E. D., Cappelletti, N., Migoya, M. C., Tatone, L. M. & Colombo, J. C. (2012). Migratory behavior of a dominant detritivorous fish (*Prochilodus lineatus*) evaluated by multivariate biochemical and pollutant data in the Paraná- Río de la Plata Basin. *Journal of Fish Biology* **81**, 848–865.
- Sverlij, S. B., Espinach Ros, A. & Orti, G. (1993). Sinopsis de los datos biológicos y pesqueros del Sábalo *Prochilodus lineatus* (Valenciennes, 1847). *FAO Sinopsis sobre la Pesca* 154. Rome: FAO.
- Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* **11**, 107–184.
- Valentine, R. C. & Valentine, D. L. (2004). Omega-3 fatty acids in cellular membranes: a unified concept. *Progress in Lipid Research* **43**, 383–402.