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Migratory behaviour of a dominant detritivorous fish *Prochilodus lineatus* evaluated by multivariate biochemical and pollutant data

E. D. Speranza*†, N. Cappelletti*†, M. C. Migoya*†, L. M. Tatone*†
and J. C. Colombo*‡§

**Laboratorio de Química Ambiental y Biogeoquímica, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Av. Calchaquí Km. 23.5, Florencio Varela (1888), Buenos Aires, Argentina, †Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, C1033AAJ, Ciudad Autónoma de Buenos Aires, Argentina and ‡Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC) 526 e/ 10 y 11, La Plata (1900), Buenos Aires, Argentina*

This paper studies the migration pathways and ranges of a dominant detritivorous fish *Prochilodus lineatus* along pollution gradients in the Río de la Plata basin using multivariate analysis of biochemical and pollutant data. Biochemical composition (water, ash, lipids, proteins, carbohydrates, neutral lipids classes and fatty acids), aliphatic hydrocarbons (ALI), polychlorinated biphenyls (PCB), linear alkylbenzenes (LAB) and organochlorine pesticides (OClP) were determined in muscle samples of *P. lineatus* collected in Metropolitan Buenos Aires, the lower Paraná River (Paraná: 200–1000 km from Buenos Aires) and the middle Paraná and Paraguay Rivers (North: 1000–1400 km north). Biochemical variables and pollutants exhibited large variability [Lipids 1·1–89·5% wet mass; ALI 1·4–413; LABs not detectable (n.d.)–115·2; PCBs n.d.–27·9; OClPs n.d.–11·8 µg g^{-1} dry mass], due to the contrast of Buenos Aires with North fish. Fish from Buenos Aires were fatty (lipids 24·7 ± 12·3% wet mass), enriched in 18 carbon fatty acids and severely contaminated (ALI 152·4 ± 72·3; LABs 65·1 ± 26·4; PCBs 15·2 ± 6·8; OClPs 1·8 ± 1·9 µg g^{-1} dry mass mean ± s.d.). In contrast, fish from North were lean (4·1 ± 3·1% wet mass), enriched in long chain (>20 carbons) polyunsaturated fatty acids, with average one to two orders of magnitude lower pollutant levels (ALI 41·2 ± 51·9; PCBs 2·2 ± 3·5; LABs 8·8 ± 21·1; OClPs 0·67 ± 0·75 µg g^{-1} dry mass mean ± s.d.). Paraná showed intermediate values in all variables, denoting the mixing of different fish stocks. Based on principal component analysis, 14 outliers from 60 North and Paraná samples (representing 26 from 108 individual fish) were identified as pertaining to the Buenos Aires group with very similar lipid and pollutant levels. Data suggest that *P. lineatus* migrates a highly variable distance, exceeding 800–1000 km in multiple spatial and temporal overlapping ranges. Chemometric analysis of biochemical and pollutant data effectively discriminates fish according to the chemical signature acquired by detritus feeding in pristine and contaminated urban or industrial areas.

Key words: aliphatic hydrocarbons; fatty acids; polychlorinated biphenyls; chemometrics, lipids, Río de la Plata basin.

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§Author to whom correspondence should be addressed. Tel. +54 11 4275 8266; e-mail: laqab@intervar.com.ar

INTRODUCTION

The Río de la Plata basin is the second largest in South America, covering >3 million km^2 in Brazil, Bolivia, Paraguay, Uruguay and Argentina (Esteves *et al*., 2000). The main tributaries, the Paraná (3780 km) and Uruguay (1790 km) Rivers, carry 500–800 km^3 of fresh water to the Río de la Plata together with 90×10^6 t of suspended solids per year creating a shallow and turbid funnel-shaped estuary (Degens *et al*., 1991). This huge particulate-matter load feeds a vast delta in front of Buenos Aires city, which concentrates one third of the total Argentine population (*c*. 40 million) and most of its industrial capacity. This metropolitan area is heavily polluted with persistent organic pollutants, hydrocarbons and heavy metals, due to the direct discharge of urban (crude sewage) and industrial effluents (Colombo *et al*., 2005*a*, *b*, 2006; Tatone *et al*., 2009).

Sewage-derived organic matter has been recognized as an important energy subsidy for aquatic food webs which can lead to a fish production increase and a trophic structure alteration (deBruyn *et al*., 2003). These effects could be further amplified when highly specialized detritivorous fishes feed directly on anthropogenic organic matter. In South American aquatic systems this niche is occupied by Prochilodontidae, which play a critical ecological role regulating benthic matter settling, microbial community composition and particulate organic carbon flow (Taylor *et al*., 2006). In the Rıo de la Plata, the sábalo *Prochilodus lineatus* (Valenciennes 1837) dominates the fish community constituting >60% of the ichthyomass and is the principal capture item of freshwater fisheries (Bonetto *et al*., 1969; Sverlij *et al*., 1993). This strict detritivore presents several anatomical and physiological adaptations (sucker-like mouth with oral ridges, gillraker filtering structure, highly muscularized pyloric stomach and numerous pyloric caeca) for the efficient collection and digestion of detritus (Bowen, 1983). In the polluted Buenos Aires coastal area, these morpho-physiological adaptations allow *P. lineatus* to feed directly on flocculent sewage-industrial derived organic matter which leads to a rapid accumulation of fat and associated organic pollutants (Speranza & Colombo, 2009; Colombo *et al*., 2000).

Due to the ecological and economical importance of *P. lineatus*, its migratory behaviour was studied by tagging experiments for >40 years (Sverlij *et al*., 1993; Harvey & Carolsfeld, 2003) and, more recently in Brazil, by radiotelemetry (Godinho & Kynard, 2006; Hahn *et al*., 2007). Most of these studies, however, where based on local fisheries, covering limited spatial and temporal ranges. *Prochilodus lineatus* moves in large schools for hundreds of km (up to >1000 km) in reproductive-trophic, flood-controlled migrations (Agostinho *et al*., 2003). The migratory pattern is complicated by simultaneous longitudinal (north–south along the river mainstream) and lateral movements (between the channel and the extensive floodplains; Harvey & Carolsfeld, 2003). Furthermore, the reproductive cycle of *P. lineatus* is highly variable and gonad development is asynchronous, allowing the coexistence of fish with different gonadal stages in the same area (Sverlij *et al*., 1993;). In the south Río de la Plata basin fish perform an upstream reproductive migration in autumn (lower–middle Parana and Uruguay Rivers), and a trophic downstream migration in spring, after spawning. The timing of spawning is variable and is synchronized with the hydrological cycle. Eggs are released in the river main stream when the water level rises and drift passively to the floodplain where larvae and juveniles remain in sheltered, food-rich habitats until complete maturation (*i.e.* 1–2 years; Sverlij *et al*., 1993). Similar migratory patterns are observed between northern wetland areas of Pilcomayo, Paraguay, Parana and Uruguay Rivers (middle and upper sections) and the lower main stream river reaches from September to March (Bayley, 1973; Sverlij *et al.*, 1993; Resende, 2003; Zaniboni & Schulz, 2003). The migration of fish from severely polluted feeding grounds such as the Buenos Aires metropolitan coastal area facilitates the active transport of pollutants along the Paraná River (Colombo *et al.*, 2011).

Fish migrations were traditionally studied by tagging experiments (mark–recapture). These techniques benefit by low technical requirements and costs and allow tracking a potentially large number of individuals, but their main drawbacks are their low effectiveness and little temporal resolution (McKeown, 1984). More recently, radio and acoustic telemetric methods provided high resolution temporal and spatial information at individual level, but these techniques are relatively expensive, not suitable for a large number of individuals, and have reduced spatial range of detection which limits their application in large river-estuaries such as the Río de la Plata Basin (Lucas & Baras, 2001; Steig *et al.*, 2005). Biochemical methods are becoming increasingly useful in determining the extent of segregation of migratory populations, mainly through stable isotope and DNA analysis (Lucas & Baras, 2001).

In this paper a chemometric multivariate approach of a comprehensive set of biochemical and pollutant data was used to characterize different *P. lineatus* stocks in the lower-middle Río de la Plata Basin along a 1400 km pollution gradient as a complementary tool to characterize the migratory movements and relative habitat fidelity of this dominant detritus-feeding fish.

MATERIALS AND METHODS

A total of 176 fish were collected between 2002 and 2005 in two contrasting environments, quarterly at the sewer outfall area of Buenos Aires (BA; Fig. 1 and Table I) and yearly at 13 sites along the Paraná River, 200–1000 km away from Buenos Aires (PAR) and at four sites along the Paraná and Paraguay Rivers located >1000 km north from Buenos Aires (North, N). PAR and N fish were collected in winter, when their abundance is highest, before their upstream migration. Captures were made with gillnets by local fisherman. The fish were weighed (M_T) and measured (standard length, L_S), and a portion of dorsal muscle was excised and wrapped in aluminium foil. According to the length–age relationships based on scale and operculum analysis calculated by Cordiviola de Yuan (1971), the age of the fish sampled ranged from 3 (3300 mm) to 12 years (5800 mm). All the specimens were reproductively mature; the smallest specimens measured 3300 mm, well above the L_S of 2400–2800 mm reported for first maturity of this species (Agostinho *et al.*, 2003; Espinach Ros *et al.* 2012). Muscle samples were immediately frozen in dry ice, transported to the laboratory and stored at −20° C until analysis. For chemical analysis, depending on the abundance and homogeneity of fish at each site, several muscle samples were pooled (two to seven per pool) based on morphometric characteristics of the fish (L_S and M_T) resulting in a total of 44 pools and 40 individual muscle samples.

BIOCHEMICAL ANALYSIS

Water and ash content were determined gravimetrically after drying (120° C, 24 h) and calcination (500° C, 24 h) of 0·5–1·0 g of homogenized tissue. Proteins and carbohydrates were determined spectrophotometrically with Folin reagent (Lowry *et al.*, 1951) and phenol-sulphuric acid (Dubois *et al.*, 1956) after homogenization of *c.*0·5 g of fish tissue with an Elvejheim Potter with 0·05 M Tris-HCl buffer (Sigma–Aldrich; www.sigmaaldrich.com) at pH 7·0. Quantification was done using bovine serum albumin (Merck; www.merck.com)

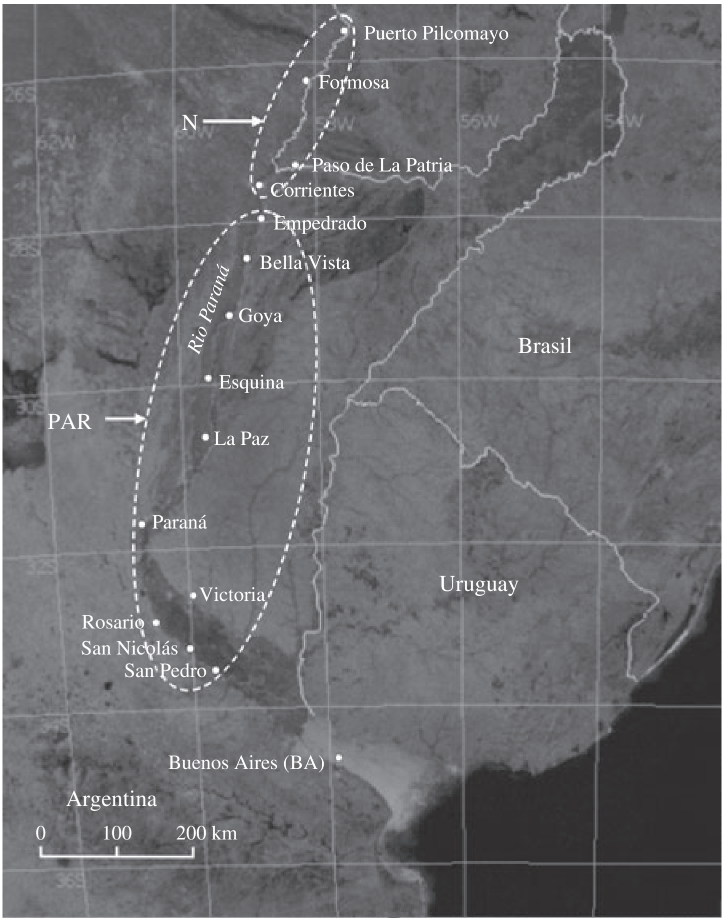

FIG. 1. Sampling stations of *Prochilodus lineatus* in the Río de la Plata Basin. Buenos Aires (BA), Paraná (PAR) and North (N) sites are indicated.

and dextrose (J. T. Baker; www.mallbaker.com) as standards. Total lipids were determined gravimetrically after extraction with chloroform:methanol (2:1v/v; Folch *et al*., 1957). Reproducibility of water, ash, lipid, protein and carbohydrate analyses was assessed by quintuplicate analyses of randomly selected samples; the relative standard deviation (RSD) ranged between 2·2 and 7·1%.

FATTY-ACID ANALYSIS

Individual fatty acids were determined as methyl ester derivatives (Christie, 1989) by gas liquid chromatography coupled to flame ionization detector (FID). Chromatographic analysis was carried out in a Konik 3000 gas chromatograph equipped with a 30 m × 0·25 mm HP5-MS capillary column (Agilent-J&W; www.agilent.com) and nitrogen as carrier gas. The temperature programme ramped from 65° C (2 min) to 195° C (1 min) at 12° C min^{-1}, to 260 °C (1 min) at 4° C min^{-1} and then at 300° C (5 min) at 5° C min^{-1}. Injector and detector were maintained at 250 and 300° C, respectively. Identification and quantification of individual fatty acids was achieved by comparing retention time data and response factors with a reference fatty acid methyl ester standard mixture (FAMQ-005, FAME Quantitative Standard Mix, AccuStandard, Inc.; www.accustandard.com), obtaining four-point, linear calibration curves.

TABLE I. Biochemical composition of *Prochilodus lineatus* muscle (values are mean ± S.D.)

	Sampling station (see Fig. 1)				
	BA	PAR	N	BA MIG	Total
Samples (individuals)*	23 (42)	29 (61)	18 (47)	14 (26)	84 (176)
L_S (mm)	471 ± 50	450 ± 56	425 ± 47	486 ± 46	450 ± 55
M_T (g)	3284 ± 1173	2328 ± 1084	1778 ± 685	3121 ± 1207	2465 ± 1176
I_C	3·01 ± 0·37^{a}	2·38 ± 0·42^{b}	2·25 ± 0·38^{b}	2·62 ± 0·49^{b}	2·53 ± 0·50
Water (%)	60·6 ± 11·1^{b}	69·5 ± 11·0^{a}	76·4 ± 3·9^{a}	58·8 ± 13·0^{b}	68·5 ± 11·3
Lipids (% wet mass)	24·7 ± 12·3^{a}	11·1 ± 13·4^{b}	4·1 ± 3·1^{b}	24·7 ± 15·8^{a}	13·3 ± 13·8
Proteins (% wet mass)	14·1 ± 2·5^{b}	17·1 ± 3·2^{a}	17·1 ± 2·7^{a}	14·4 ± 4·2^{b}	16·3 ± 3·1
Ash (% wet mass)	1·14 ± 0·33^{b}	1·39 ± 0·21^{a}	1·43 ± 0·094^{a}	1·17 ± 0·22^{b}	1·33 ± 0·26
Carbohydrates (% wet mass)	0·24 ± 0·08^{a}	0·18 ± 0·11^{a}	0·15 ± 0·12^{a}	0·16 ± 0·12^{a}	0·19 ± 0·11
DG (% neutral lipids)	0·44 ± 0·64^{a}	0·50 ± 0·49^{a}	0·45 ± 0·39^{a}	0·29 ± 0·33^{a}	0·49 ± 0·51
C (% neutral lipids)	0·8 ± 0·3^{b}	1·3 ± 1·1^{ab}	2·0 ± 2·0^{a}	0·8 ± 0·4^{b}	1·4 ± 1·3
FFA (% neutral lipids)	0·7 ± 1·5^{ab}	2·3 ± 3·5^{a}	2·2 ± 2·1^{a}	0·2 ± 0·6^{b}	1·9 ± 2·9
TG (% neutral lipids)	98·0 ± 2·5^{ab}	95·6 ± 4·5^{ab}	95·2 ± 4·5^{ab}	98·6 ± 1·2^{a}	96·2 ± 4·2
CE (% neutral lipids)	0·10 ± 0·28^{a}	0·11 ± 0·30^{a}	0·13 ± 0·34^{a}	0·08 ± 0·15^{a}	0·11 ± 0·31
18C FA (% total FA)	52·6 ± 4·6^{a}	41·4 ± 10·1^{b}	39·2 ± 9·4^{b}	54·6 ± 6·0^{a}	44·0 ± 10·2
LC-PUFA (% total FA)	8·5 ± 2·3^{c}	13·5 ± 5·1^{b}	18·2 ± 8·1^{a}	8·2 ± 2·1^{c}	13·2 ± 6·3

BA, Buenos Aires; PAR, Paraná; N, North; BA MIG, migratory specimens from Buenos Aires; L_S, standard length; $M_{T,}$ total body mass; I_C, condition index ($= M_T L_S^{-3}$); DG, diglycerides; C, cholesterol; FFA, free fatty acids; TG, triglycerides; CE, cholesteryl esthers; 18C FA, 18 carbon fatty acids; LC-PUFA, long chain (>20 carbons) polyunsaturated fatty acids. Significant differences ($P < 0·05$; Tukey's test) between sampling stations within each row are indicated by different superscript lower case letters.

*Samples analysed include several pools of two to seven individual fish of similar morphometric characteristics.

Fatty-acid composition analyses were reproducible to ±0·9–18·1% (quintuplicate analyses) and their recoveries ranged between 87 and 109% (oleic acid additions to muscle samples).

POLLUTANT ANALYSIS

Muscle samples for pollutant analysis were homogenized, mixed with pre-extracted sodium sulphate (1:3) and Soxhlet extracted with acetone dichloromethane and petroleum ether (1:2:2). After solvent evaporation under nitrogen stream, the extracts were treated with sulphuric acid and fractionated by silica-gel chromatography (F1: petroleum ether, F2: petroleum ether: dichloromethane 3:1). Individual aliphatic hydrocarbons (ALI), polychlorinated biphenyls (PCB), linear alkylbenzenes (LAB) and organochlorine pesticides (OClP) were quantified by high resolution gas chromatography using 30 m x 0·25 mm DB5 capillary columns and flame ionization (FID), electron capture (ECD) and mass spectrometry (MS) detection (Agilent 6890 and 7890 and 6850-5973N; www.chem.agilent.com). Quantification was performed by external standards of 31 individual aliphatic hydrocarbons (C10 to C38 n-alkanes plus isoprenoids pristane and phytane; DRH-008S-R1 Multi-State Hydrocarbon Window Defining Standard, AccuStandard Inc.; www.accustandard.com), 25 LABs (C10 to C14 congeners with 2–7 phenyl substitution; mixture provided by a local petrochemical company), 41 PCBs (di- to decachlorobiphenyls; C-QME-01, Quebec Ministry of Environment Congener Mix, AccuStandard Inc.; www.accustandard.com) and 20 individual organochlorine pesticides (M-680P Pesticide Mix, AccuStandard Inc.). Deuterated n-C16 and n-C24 alkanes and PCBs 103 and 198 (Absolute Standards Inc.; www.absolutestandards.com) were added as internal standards. Blanks included in every eighth sample batch gave negligible results. Method accuracy was evaluated through repeated analysis of an internal reference material prepared with homogenized *P. lineatus* from the Río de la Plata for ALI and a with certified cod liver oil (SRM1588a, NIST, USA; www.nist.gov) for PCBs and OClPs.

STATISTICAL ANALYSES

Statistical analyses were carried out using XLSTAT (Addinsoft SARL; www.xlstat.com). Data are expressed as mean ± S.D. The coefficient of variation (z) of the variables was calculated as $z = 100y\bar{x}^{-1}$,where $y =$ S.D. and $\bar{x} =$ mean. For comparison between multiple samples, ANOVA and the Tukey test were used. A significance level of $P < 0{\cdot}05$ was used, except otherwise indicated. Multivariate analyses included principal component analysis (PCA) of standardized data ($x - \bar{x}y^{-1}$)

RESULTS

BIOCHEMICAL AND FATTY-ACID COMPOSITION

Biochemical composition, comprising proximate composition, neutral lipid classes and total fatty acids is summarized in Table I and Figs 2 and 3. Overall, the main component of *P. lineatus* muscle is water (68·5 ± 11·3%, range: 37·8–82·0%), followed by a rather homogeneous protein proportion (16·3 ± 3·1% wet mass, range: 7·4–22·4%), a highly variable lipid content (13·3 ± 13·8% wet mass, range: 0·2–55·7%) and low ash and carbohydrates (1·3 ± 0·3% wet mass, range: 0·3–1·8% and 0·19 ± 0·11% wet mass, range: not detectable nd–0·40%). Water and lipid contents were inversely correlated ($r = -0{\cdot}96$) as were water and M_T ($r = -0{\cdot}80$) and lipid and M_T were correlated ($r = 0{\cdot}81$).

The lipid composition of *P. lineatus* muscle were dominated by neutral lipids (NL: 87·2 ± 14·8% of total lipids) which were mainly constituted by triglycerides (96·2 ± 4·2% NL), followed by low levels of free fatty acids and cholesterol (1·9 ± 2·9 and 1·4 ± 1·3% NL, respectively) and almost negligible diglycerides and cholesteryl

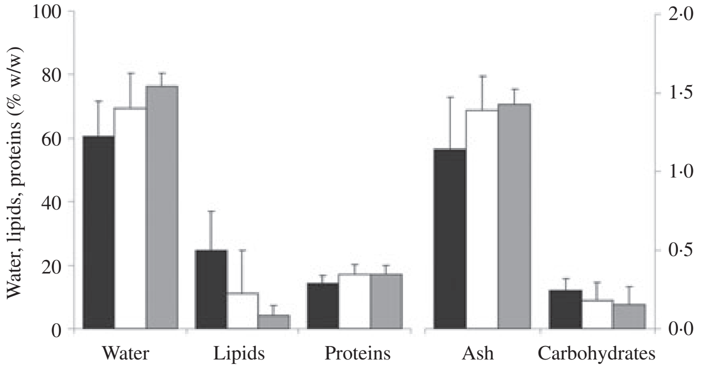

FIG. 2. General biochemical composition (water, lipids, proteins, ash and carbohydrates) of *Prochilodus lineatus* muscle from Buenos Aires (■), Paraná (□) and North (▨). Values are mean ± S.D.

esters (0·49 ± 0·51 and 0·11 ± 0·31% NL). Fatty-acid (FA) composition (Fig. 3) was dominated by 16 and 18 carbon components (36·0 ± 4·0 and 44·3 ± 9·9% of total FA, respectively) with 16:0 and 18:1n-9 as the main fatty acids. Polyunsaturated fatty acids (PUFA) were relatively low, with 18:2 as main component (6·8 ± 3·5% total FA), followed by longer chain 20 and 22 carbon PUFAs (LC-PUFAs: 13·2 ± 6·3% total FA).

The biochemical composition of *P. lineatus* muscle showed clear geographical differences which maximize between North and Buenos Aires specimens. North fish had higher contents of water (N 76·4 ± 3·9 *v*. 60·6 ± 11% BA, *P* < 0·001), proteins (N 17·1 ± 2·7 *v*. 14·1 ± 2·5% BA, wet mass, *P* < 0·01) and ash (N 1·43 ± 0·09 *v*. 1·14 ± 0·33% BA, wet mass, *P*< 0·001), and lower amounts of lipids relative to Buenos Aires fish (N 4·1 ± 3·1 *v*. 24·7 ± 12·3% BA, wet mass, *P*< 0·001). The fatty-acid composition also presented significant differences between N and BA fish, basically lower per cent 18 carbon fatty acids (N 39·2 ± 9·4 *v*. 52·6 ± 4·6% BA, total FA, *P* < 0·001) and enrichment in long chain-PUFAs in North fish (N 18·2 ± 8·1 *v*. 8·5 ± 2·3% BA, total FA, *P* < 0·001). The differences between Paraná

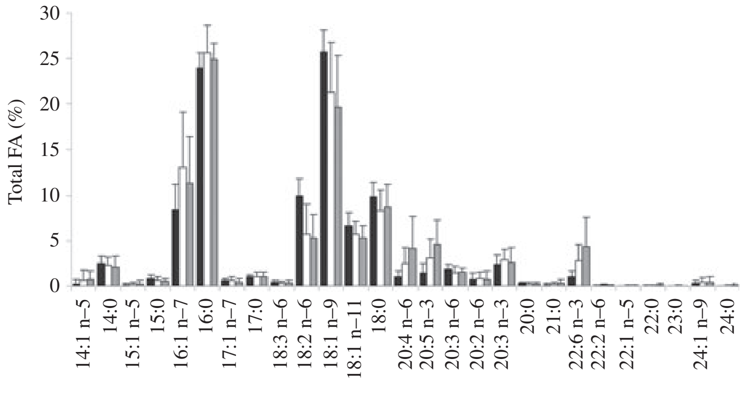

FIG. 3. Fatty-acid (FA) composition of *Prochilodus lineatus* muscle from Buenos Aires (■), Paraná (□) and North (▨). Values are mean + S.D.

and Buenos Aires fish are less pronounced, but still significant in terms of higher content of water (69·5 ± 11·0%, $P < 0·01$), proteins (17·1 ± 3·2% wet mass, $P < 0·001$) and ash (1·39 ± 0·21% wet mass, $P < 0·001$), lower amounts of lipids (11·1 ± 13·4% wet mass, $P < 0·001$), reduced levels of 18C FA (41·4 ± 10·1% total FA, $P < 0·001$) and higher LC-PUFAs (13·5 ± 5·1% total FA; $P < 0·001$).

POLLUTANT CONCENTRATIONS

Pollutant data are summarized in Table II. Overall, *P. lineatus* presented a wide range of contaminant concentrations in their muscle, from background-pristine values to highly polluted figures. Aliphatic hydrocarbons were the most abundant compounds (1·4 to 413·0 $\mu g\ g^{-1}$ dry mass; mean ± S.D.: 95·1 ± 101·4), followed by LABs (nd-115·2 and 26·4 ± 32·9 $\mu g\ g^{-1}$ dry mass), PCBs (nd-27·9 and 7·4 ± 8·6 $\mu g\ g^{-1}$ dry mass), and OClPs (nd-11·8 and 1·4 ± 2·0 $\mu g\ g^{-1}$ dry mass).

Pollutant concentrations exhibited large spatial variability (Fig. 4). Aliphatic hydrocarbons were significantly lower ($P < 0·01$) in North fish (41·2 ± 51·9 $\mu g\ g^{-1}$ dry mass) which presented prevailing biogenic sources (algae-derived C15 + C17 n-alkanes: 60·9%), relative to Buenos Aires fish with four times higher values (152·4 ± 72·3 $\mu g\ g^{-1}$ dry mass,) and a clear petrogenic signature (isoprenoids + < C20 n-alkanes: 75·2%). PCB differences between North and Buenos Aires fish are even more marked and significant ($P < 0·001$) with lowest concentrations (2·2 ± 3·5 $\mu g\ g^{-1}$ dry mass), enriched in persistent hexachlorobiphenyls relative to tri-tetrachlorobipheyls (6Cl:3-4Cl ratio: 14·5 ± 10·9) in North fish, and seven-times higher values in Buenos Aires fish (15·2 ± 6·8 $\mu g\ g^{-1}$) with a stronger signal of lower chlorinated congeners (6Cl:3-4Cl ratio: 2·7 ± 1·7). Similarly, the concentrations of the detergent-precursors and sewage tracers LABs, were seven-times lower in North fish, similar to PCBs (8·8 ± 21·1 *v.* 65·1 ± 26·4 $\mu g\ g^{-1}$ dry mass in Buenos Aires, $P < 0·001$). In contrast, OClP averages were only 2–3 times lower in the North (0·7 ± 0·7 *v.* 1·8 ± 1·9 $\mu g\ g^{-1}$, $P < 0·05$). This different abundance of contaminants results in significantly higher OClP:PCB ratios in the North relative to Buenos Aires fish (0·55 ± 0·36 *v.* 0·10 ± 0·07; $P < 0·001$). As observed for the biochemical composition, Paraná fish had intermediate values, significantly different ($P < 0·05–0·001$) from Buenos Aires but not from North fish (ALI 86·7 ± 116·1; PCBs 5·5 ± 8·1; LABs 12·8 ± 21·0; OClPs 1·5 ± 2·3 $\mu g\ g^{-1}$ dry mass).

MULTIVARIATE ANALYSIS

In order simultaneously to evaluate all variables and samples, identify the variables explaining most of the variance and classify the samples, principal component analysis (PCA) was performed for each type of variable and for the whole data set. The first PCA was executed with the general biochemical composition taking the proportions of water, lipid, proteins and ashe (carbohydrates were negligible) and the condition index ($I_C = M_T L_S^{-3}$) as a morphometric variable [Fig. 5(a)]. The first two principal components (PC) explain 86% of the total variability; 75% PC1 determined by lipids and I_C (positive) and water and proteins (negative), and 11% PC2, positively loaded with proteins and negatively with ash. Fish samples were poorly discriminated in this PCA (all around the centre of the biplot). North fish and most of Paraná, however, were segregated to the left side and almost all the Buenos Aires

TABLE II. Pollutant concentrations and compositional ratios in *Prochilodus lineatus* muscle (values are mean ± S.D.)

	Sampling site (see Fig. 1)				
	BA	PAR	N	BA MIG	Total
PCB (μg g^{-1} dry mass)	15·2 ± 6·8^{a}	5·5 ± 8·1^{b}	2·2 ± 3·5^{b}	15·6 ± 7·2^{a}	7·4 ± 8·6
ALI (μg g^{-1} dry mass)	152·4 ± 72·3^{ab}	86·7 ± 116·1^{bc}	41·2 ± 51·9^{c}	227·0 ± 113·6^{a}	95·1 ± 101·4
LAB (μg g^{-1} dry mass)	65·1 ± 26·4^{a}	12·8 ± 21·0^{c}	8·8 ± 21·1^{c}	42·4 ± 24·3^{b}	26·4 ± 32·9
OClP (μg g^{-1} dry mass)	1·8 ± 1·9^{ab}	1·5 ± 2·3^{b}	0·7 ± 0·7^{b}	3·6 ± 3·2^{a}	1·4 ± 2·0
Biogenic (% ALI)	24·8 ± 4·7^{b}	60·0 ± 32·0^{a}	60·9 ± 29·7^{a}	23·4 ± 6·4^{b}	50·4 ± 30·9
6Cl:3-4Cl PCBs	2·7 ± 1·7^{b}	13·4 ± 10·8^{a}	14·5 ± 10·9^{a}	2·7 ± 0·8^{b}	10·7 ± 10·4
OClP:PCB	0·10 ± 0·07^{b}	0·54 ± 0·38^{a}	0·55 ± 0·36^{a}	0·18 ± 0·11^{b}	0·42 ± 0·38

PCB, polychlorinated biphenyls; ALI, aliphatic hydrocarbons; LAB, linear alkylbenzenes; OclP, organochlorinated pesticides; Biogenic, biogenic hydrocarbons (C15 + C17 n-alkanes); 6Cl:3-4Cl PCBs, hexachloro-PCBs:(tri- + tetrachloro-PCBs). Values with different superscript lower-case letters within each row indicate significant differences ($P < 0·05$) between sampling stations.

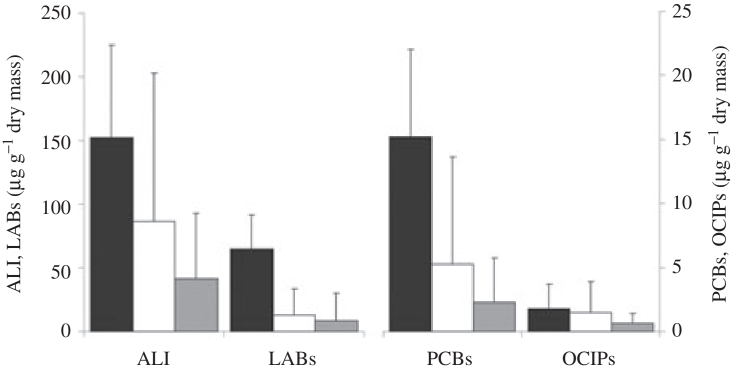

FIG. 4. Average pollutant concentrations (see Table II) of *Prochilodus lineatus* muscle in Buenos Aires (■), Paraná (□) and North (▨). Values are mean + s.d.

fish to the right. Six samples (10 individual fish) collected in the Paraná had high lipid content and I_C values, plot in the Buenos Aires scatter.

A second PCA carried out with the concentrations of the most abundant 13 fatty acids explained a lower variance [61%; Fig. 5(b)]. PC1 (43%) was determined by 18 carbon fatty acids (18:1n-9, 18:2n-6 and 18:0; positive) and long chain-PUFAs (22:6n-3, 20:4n-6 and 20:5n-3; negative). PC2 (18%) was positively loaded with LC-PUFAs and 16 carbon fatty acids and negatively with 14:1n-5. This fatty-acid PCA produced a better segregation of the samples with a similar pattern as the general biochemical composition; North fish on the left (higher LC-PUFAs) and Buenos Aires fish on the right (higher 18C FA). In this case, a total of nine samples, corresponding to 19 specimens collected in the Paraná were plotted closer to Buenos Aires, including the six observed in the previous PCA.

A third PCA was performed with the concentrations of ALI, PCBs, LABs and OClPs, and three ratios: biogenic:petrogenic aliphatic, 6Cl:3-4Cl PCBs and OClPs: PCBs [Fig. 5(c)]. This PCA was more discriminative than the previous ones and explained a higher percentage of total variability (82%), basically through PC1 (69%) determined by the highest concentrations (positive) and pollutant ratios (negative). Sample segregation was better than in the other PCA, with North fish clearly separated on the left and Buenos Aires fish on the right, and now a total of 14 samples (26 individual fish) collected in the Paraná and North (including previous outliers) plotting in the Buenos Aires scatter with very high pollutant concentrations.

To summarize, a final PCA was carried out with all variables, biochemicals, fatty acids and pollutants simultaneously [Fig. 5(d)]. This PCA explained 74% of total data variability, mainly through PC1 (64%) loaded with lipids, 18C FAs, and pollutant concentrations (positive), and water, protein and ash contents, LC-PUFA concentration and pollutant ratios (negative). A continuum V shaped transition was observed with North and Paraná specimens to the left (PC1 negative) and all Buenos Aires fish, together with Paraná and North outliers to the right (PC1 positive). The similarity between these outliers and Buenos Aires fish was confirmed by a Tukey analysis ($P < 0{\cdot}05$; Tables I and II; migratory specimens from Buenos Aires). One Buenos Aires outlier represented by a small, extremely lean specimen plotting close to Paraná was also identified.

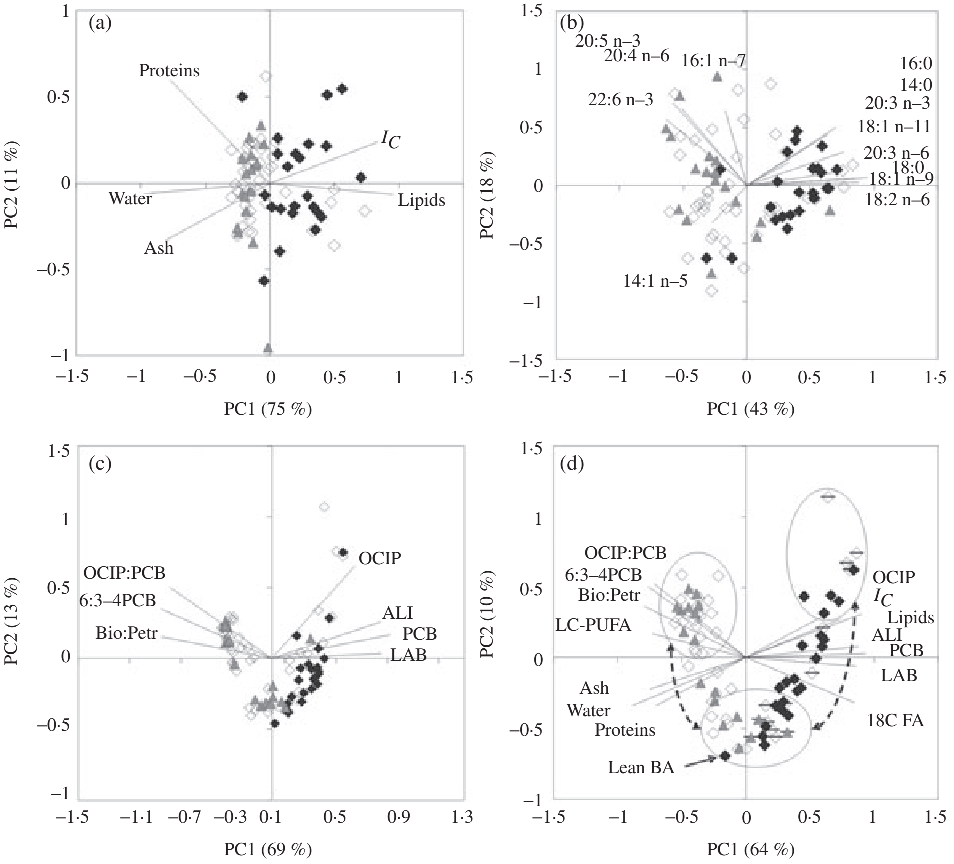

FIG. 5. Principal component analysis (PCA) of (a) general biochemical composition, (b) fatty-acid composition, (c) pollutant concentrations and ratios and (d) final global PCA of Buenos Aires (◆), Paraná (◇) and North (▲) *Prochilodus lineatus*. Migratory specimens from Buenos Aires and outlier specimens are indicated by a horizontal bar (—). I_C, condition index ($I_C = M_T L_S^{-3}$); 18C FA, 18 carbons fatty acids; LC-PUFA, long chain (>20 carbons) polyunsaturated fatty acids; PCB, polychlorinated biphenyls; ALI, aliphatic hydrocarbons; LAB, linear alkylbenzenes; OClP, organochlorinated pesticides; Bio:Petr, biogenic:petrogenic hydrocarbon ratio [(C15 + C17 n-alkanes):(>20 carbons + isoprenoids)]; 6:3-4 PCBs: hexachloro-PCBs: (tri- + tetrachloro-PCBs).

In order to identify spatially the origin of the fish along the North–South gradient and the distribution range of North and Paraná outliers, the load of each sample in PC1 of the last more integrative PCA was plotted against distance of the capture site from Buenos Aires (Fig. 6). Buenos Aires fish with high PC1 loads at distance zero included the lean Buenos Aires outlier (at the lower end) with similar PC1 load (-0·16) as North and Paraná *P. lineatus*. North and Paraná outliers were distributed over a wide spatial range; three (or five individual fish) corresponded to the 300–500 km segment, eight outliers (14 fish) were in the 800–1000 km range, and three (six fish) > 1000–1200 km. The zero value for the PC1 load (dotted line in Fig. 6) clearly separated Buenos Aires fish from those from Paraná and the North.

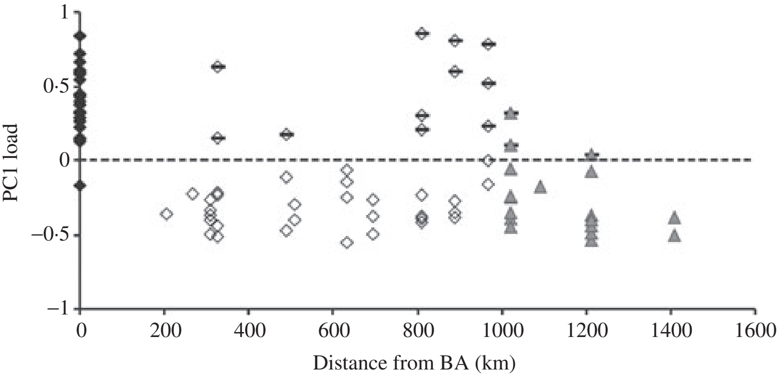

FIG. 6. PC1 load of principal component analysis [see Fig. 5(d)] of *Prochilodus lineatus* from Paraná (◇) and North (▲) against distance of the capture site from Buenos Aires (BA) (◆). Migratory specimens from Buenos Aires and outliers specimens are indicated by a horizontal bar (▁).

DISCUSSION

In general, the biochemical composition of *P. lineatus* characterized by high lipid contents with prevailing saturated and monounsaturated 16 and 18 carbon fatty acids and low LC-PUFAs (22:6 n-3, 20:5 n-3 and 20:4 n-6) agrees with previous reports (Brenner *et al*, 1961; Bayo & Maitre, 1983; Brenner & Bernasconi, 1997), reflecting the lower abundance of LC-PUFAs in freshwater fishes compared to marine species (Morris & Culkin, 1989). This difference is due to the contrasting diets, which in marine animals includes a substantial proportion of LC-PUFA-rich plankton, consumed directly or by trophic-web transfer (Sargent *et al.*, 1995). The scarcity of dietary LC-PUFAs in fresh waters is further intensified in detritus feeding fish as *P. lineatus*, forcing the animals to synthesize (Bayo & Maitre, 1983) these physiologically relevant PUFAs involved in the regulation of membrane fluidity and trans-membrane enzyme activity (Bell *et al.*, 1986). The metabolically more efficient route to do this is through *de novo* synthesis of 18 carbon fatty acids, which predominate in the fish, and elongation and unsaturation of dietary 18:2 and 18:3 PUFAs [essential fatty acids present in algae and terrestrial plant detritus (Henderson, 1996)].

The North–South spatial differences observed in the biochemical composition of *P. lineatus* muscles reflect the contrasting sources of organic matter, which include basically vegetal detritus in the North, and anthropogenic organic matter in Buenos Aires. The moderately low lipid contents of North fish (4·1 ± 3·1% wet mass) agrees with previous data reported for *P. lineatus* from the upper–middle Paraná and middle Uruguay Rivers (Bayo & Maitre, 1983; Angelini & Seigneur, 1992; Matsuhita & de Souza, 1994), and contrast with the unusually high muscular lipid contents of Buenos Aires fish (24·7 ± 12·3% w/w) and their high I_C as observed in the general biochemical composition PCA [Fig. 5(a)]. The consumption of abundant and easily assimilable anthropogenic organic matter, enriched in carbohydrates, fatty acids and contaminants at Buenos Aires, compared with organically poor vegetal detritus in the North, causes a massive lipid accumulation which results in an enhanced body mass gain (*i.e.* higher I_C; Speranza, 2008; Speranza & Colombo, 2009). In addition, the high contaminant load at Buenos Aires may induce an alteration of lipid metabolism resulting in high lipid accumulation and change of fatty-acid profiles (Dillon & Engler, 1988; Geyer *et al.*, 1994). Organochlorines have been recently recognized as true obesogens, *i.e.* compounds altering lipid homeostasis and promoting obesity, in rats and humans (Grun & Blumberg, 2009).

As observed in the fatty-acid PCA [Fig. 5(b)], the fatty-acid profile enriched in 18C fatty acids and impoverished in LC-PUFAs of *P. lineatus* show marked changes between Buenos Aires (higher in 18 carbons) and North fish (enriched in 16:1 n-7 and LC-PUFAs). This difference probably reflects the more intense *de novo* synthesis of 18 carbon fatty acids from the abundant anthropogenic organic matter at Buenos Aires prevailing over the dietary accumulation of LC-PUFA (Henderson, 1996; Speranza, 2008). The latter is comparatively more important in the less polluted North environment where algae and higher plant inputs prevail as organic matter sources (Bayo & Cordiviola de Yuan, 1992). In addition to this dietary factor, the interference of pollutants on fatty-acid profiles at Buenos Aires cannot be ruled out since previous reports for rats injected intraperitoneally with PCBs and OClPs indicated an alteration fatty-acid desaturase and elongase enzimatic activities leading to enrichment in 18:1 n-9 and reduction of LC-PUFAs (Darsie *et al.*, 1975; Matsusue *et al.* 1997, 1999).

The highly variable biochemical composition of Paraná fish, which is intermediate between Buenos Aires and the North, reflects the mixing of different *P. lineatus* stocks in the middle Paraná during migrations. Paraná and North specimens plotting in the Buenos Aires scatter of the biochemical composition PCAs [Fig. 5(a), (b)] are thus interpreted as migratory fish from Buenos Aires. The high lipid content of these migratory specimens indicates that, in contrast to the lipid depletion observed in migratory salmonids (Groot *et al.*, 1995), the energetic demand of *P. lineatus* migrations is not covered by muscle lipids but principally by the abundant visceral fat (Bayley, 1973).

The high pollutant concentrations in *P. lineatus* from Buenos Aires confirm the high degree of contamination of this fish whose pollutant levels are among the highest reported in the scientific literature (Colombo *et al.*, 2007*a*, *b*, 2011). This is due to the specific adaptations to detritus feeding of *P. lineatus,* which lead to an optimized ingestion and assimilation of organic carbon and associated persistent pollutants from urban or industrial discharges. Thus, *P. lineatus* bioaccumulate large amounts of hydrocarbons (ALI = $152{\cdot}4 \pm 72{\cdot}3$ μg g^{-1} dry mass), PCBs ($15{\cdot}2 \pm 6{\cdot}8$ μg g^{-1} dry mass), LABs ($65{\cdot}1 \pm 26{\cdot}4$ μg g^{-1} dry mass) and OClPs ($1{\cdot}8 \pm 1{\cdot}9$ μg g^{-1} dry mass) from rich-anthropogenic detritus in an area receiving huge vertical fluxes of pollutants from urban or industrial sources (ALI: 21 ± 23 mg cm^{-2} year^{-1}; PCBs: $1{\cdot}6 \pm 1{\cdot}8$ μg cm^{-2} year^{-1}; LABs: $0{\cdot}42 \pm 0{\cdot}62$ mg cm^{-2} year^{-1}; Colombo *et al.*, 2007*c*). The abundance of coprostanol in *P. lineatus* muscles originated by reduction of cholesterol in human intestine and released with faeces, further confirms the importance of sewage-derived organic matter in the diet of Buenos Aires fish (Speranza, 2008).

The Buenos Aires–North differences in pollutant concentrations are more pronounced than those observed for the biochemical composition of fish as shown by the better spatial discrimination obtained in the corresponding PCA [Fig. 5(c)]. The difference is maximized for PCBs and LABs whose concentrations are seven times higher in Buenos Aires fish than North fish indicating a reduced exposition in more pristine areas. The next more contrasting pollutants are hydrocarbons (four times higher in Buenos Aires fish) which include both, natural biogenic components (*i.e.* phytoplankton) prevailing in North fish, and fossil fuel inputs dominating at Buenos Aires. The pollutant showing the lowest North–Buenos Aires contrast are OClPs (only three times higher in Buenos Aires fish), reflecting their proportionally more intense use in agricultural areas compared to prevailing urban or industrial sources at Buenos Aires.

The composition of hydrocarbons and PCBs provides a complementary indication of the different quality and anthropogenic contribution to the diet of *P. lineatus*. Effectively, compositional ratios confirm that at Buenos Aires, *P. lineatus* optimize feeding strategy by consuming anthropogenic organic matter close to main outfalls. The ratio between biogenic and petrogenic hydrocarbons shows very low values at Buenos Aires (0·25 compared with 0·61 in the North) reflecting a chronic long-term exposure to crude effluent discharges (Colombo *et al.*, 2007*a*). The higher proportion of lower chlorinated PCB congeners in this fish (lower 6 Cl:3-4Cl ratios) support fresh inputs of urban or industrial sources. In contrast, the biogenic hydrocarbon signature in fish from the North (algae- derived n-C15 and n-C17) and the predominance of highly chlorinated PCBs (higher 6 Cl:3-4Cl ratio) confirm consumption of natural vegetal detritus and a low exposition to a degraded pollutant signal (background profile).

The interpretation of Paraná and North outlier specimens as migratory fish from Buenos Aires is supported by pollutant data. These individuals share not only a consistent biochemical composition differentiated from North specimens but principally a high pollutant load and different compositional ratios, as shown by the pollutant PCA [Fig. 5(c)]. As mentioned earlier, consumption of visceral fat for the migratory effort preserves muscle lipids thus minimizing the increase of lipophilic pollutants in this tissue and the possible associated toxicity enhancement, as has been observed in salmonids (deBruyn *et al.*, 2004; Hansson *et al.*, 2009; Arkoosh *et al.*, 2011).

The last PCA [Fig. 5(d)] integrating biochemical composition and pollutant data provides interesting results to evaluate the migratory movements of *P. lineatus*. The leanest and least polluted fish from Paraná and North in the upper-left region of the biplot are separated from the fattiest and heavily polluted Buenos Aires fish at the upper-right region, by a V-shape transition in the lower-centre sector. This reflects a gradual transition or overlapping of *P. lineatus* populations migrating along a 1000–1500 km range with contrasting food and contaminant sources. The dominant and highly migratory Prochilodontidae species from South America may effectively form a single panmictic population throughout vast drainage areas (Sivasundar *et al.* 2001). Genetic analyses of *P. lineatus* show a high gene flow, denoting a continuous mixing or population overlapping (Revaldaves *et al.*, 1997; Ramella *et al.*, 2006).

The present results effectively show that North and Paraná fish cannot be distinguished by biochemical and pollutant chemometric data (similar PC1 loads), suggesting a continuous mixing under similar environmental conditions. In contrast, the biochemical and pollutant profile of *P. lineatus* from Buenos Aires is significantly different (large PC1 distance) supporting a high degree of habitat fidelity to Buenos Aires where the fish find an abundant and highly energetic food supply. The apparent far north reach of Buenos Aires migrating fish is around 1000–1200 km, but with a reduced frequency (three from 14 samples). An apparent gathering of migrating specimens occurs at *c*. 800–1000 km which include the highest frequency of Paraná outliers (eight from 14 samples), whereas the lowest migration range of 200–500 km includes only three Buenos Aires samples (Fig. 6). These results although displaying a similar wide range of migration distances as previous tagging experiments (<50–1100 km), suggest longer travel displacements since the highest Buenos Aires migratory frequency is in the 800–1000 km range whereas tagging data are centred around 450–500 km (Sverlij *et al*., 1993). In summary, chemometric analysis of biochemical and pollutant data of a dominant detritivorous fish feeding in pristine and contaminated urban or industrial areas is a useful tool to discriminate Buenos Aires *P. lineatus* (fatty, enriched in 18C FA and heavily polluted) from Paraná and North fish (lean, enriched in LC-PUFA with background contaminant levels). The approach also permitted the identification of migratory specimens from Buenos Aires along a variable distance (up to 1200 km upstream), reflecting the multiple spatial overlapping of *P. lineatus* stocks in the basin.

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